Ignorance is not all bliss:

Investigating the added benefit of cannabidiol in anxiety disorder treatment

Caroline Kwee

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Onwetendheid is niet altijd een zegen: Onderzoek naar de toegevoegde waarde van cannabidiol in de behandeling van angststoornissen

(met een samenvatting in het Nederlands)

Proefschrift

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Caroline Marianna Bernadette Kwee
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Promotoren
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Chapter 1

General introduction

1.1 Anxiety disorders - Scope of the problem

A large number of individuals develop an anxiety disorder during their life [Box 1]. In the United States (US) lifetime prevalence was estimated to be 36% (Kessler et al., 2007). In Europe, anxiety disorders are the most prevalent of neuropsychiatric disorders (Wittchen et al., 2011). They are associated with disability across major activity domains (Hendriks et al., 2014) and in women, they have been repeatedly placed in the top 30 of leading causes of health loss (GBD 2017 DALYs and HALE Collaborators, 2018). Work-loss days attributed to anxiety disorders are equivalent to prevalent physical disorders, such as heart disease, rheumatism and diseases of the digestive system (Alonso et al., 2007; Buist-Bouwman et al., 2005). Being afraid often comes with bodily symptoms such as a racing heart, sweaty palms, shortness of breath, to name a few. These symptoms, which are gathered under the umbrella term 'anxiety arousal', as well as avoidance behavior are associated with functional impairment (Hendriks et al., 2014). Avoidance behavior is a maladaptive coping style that is central to anxiety disorders. For example, agoraphobia is defined by avoidance of anxiety-provoking situations, and can go as far as the patient not leaving their home anymore. Avoidance can also be more subtle, such as sitting down to avoid fainting in panic disorder. This latter form of avoidance falls under the umbrella term 'safety behavior', which is anxiety-driven behavior to prevent feared outcomes that are unlikely to happen (Helbig-Lang and Petermann, 2010). Avoidance behavior is predictive of anxiety disorder chronicity, more than anxiety arousal (Hendriks et al., 2013).

The onset of anxiety disorders is mostly in childhood, adolescence or young adulthood (Kessler et al., 2005a) and most anxiety disorders in adults are preceded by childhood/ adolescent anxiety disorders (Pine et al., 1998). However, anxiety disorders are not a disease of young people, but often evolve to become a chronic problem throughout adulthood. Even after the disorder has remitted, the level of functioning in important life domains is generally lower in adults with an anxiety disorder than in healthy adults (Iancu et al., 2014). And even after initially successful treatment, patients struggle with high recurrence rates after remission of an anxiety disorder (Scholten et al., 2016).

Box 1 - Diagnosis of anxiety disorders

As all mental disorders, anxiety disorders are diagnosed by means of a clinical interview. The criteria on which this diagnosis is based can be found in a classification manual. The authoritative Diagnostic and Statistical Manual of Mental Disorders (DSM) is being used internationally (Möller, 2018). The DSM-IV has been used for diagnosis of participants in our clinical trial (see section 1.7 Thesis aims and outline). Anxiety disorders recognized in the DSM-IV are panic disorder, generalized anxiety disorder (GAD), posttraumatic stress disorder (PTSD), obsessive-compulsive disorder (OCD), social phobia, specific phobia, and agoraphobia. In the DSM-IV, panic disorder can be diagnosed with or without agoraphobia. Most patients with panic disorder develop agoraphobic avoidance (Bandelow et al., 1996). Subjectively reported anxiety alone is not sufficient for classification of any of these disorders and physiological symptom of anxiety (e.g. palpitations, sweating, heart rate increase), along with anxious cognitions and avoidance behavior are found as well. Finally, the fear, anxiety or avoidance behavior should be causing significant distress or functional impairment (American Psychiatric Association, 1994).

To be diagnosed with an anxiety disorder, the symptoms should not be better accounted for by substance use or by another mental disorder or medical condition (American Psychiatric Association, 1994). Comorbid other mental disorders are common (Alonso et al., 2004; Kessler et al., 2005b; Lamers et al., 2011), and these comorbidity rates, together with substantial genetic correlations between mental disorders, especially within anxiety disorders (Smoller et al., 2019) and heterogeneous pathophysiological processes within diagnostic categories (King et al., 2019) challenge the biological validity of the categorical approach of the DSM. Yet, given the long history of research into (the treatment of) mental disorders based on DSM diagnoses, and the clinical utility they have obtained, the DSM approach is currently still highly valuable.

1.2 What causes anxiety to become a disabling rather than adaptive emotion?

For more than a century, Pavlovian fear conditioning paradigms have been utilized to study the pathogenesis of anxiety disorders (Beckers et al., 2013). From the 1980's onwards, fear conditioning paradigms have become increasingly popular to study aversive learning and memory in mammals (LeDoux, 2014). In these paradigms an environmental stimulus or context (CS+)

is repeatedly paired with an aversive event (unconditioned stimulus, US). Learning about the CS+ - US association can lead to a state of fear when encountering the CS+ [Box 2]. Such acquisition of fear (see Figure 1) is not necessarily inappropriate or pathological (Beckers et al., 2013). In fact, the capacity to learn about the meaning of the CS+ (that signals danger) implicates that when encountering a threat, neural circuits are activated that can increase the chance of survival (LeDoux, 2014).

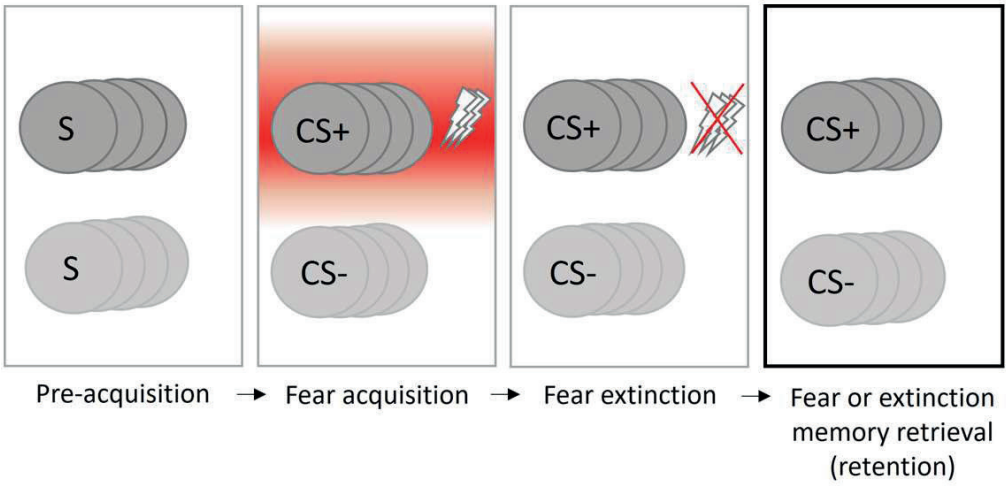

Figure 1. Fear acquisition and extinction in a differential cued fear conditioning experiment.

Note: Prior to fear acquisition, the to be conditioned stimuli (at this point simply stimuli (S)) do not yet predict an aversive event (the US; in animal and human studies, this often is electric shock). During acquisition, the US (the aversive/ danger stimulus) is repeatedly paired with the neutral stimulus (the CS+). Only upon CS+ presentations during fear acquisition danger is actually present (red color); the other trials and experimental phases are safe as no US reinforcement occurs. During extinction, no US reinforcement takes place anymore. The CS- stimulus is never paired with the US. During a fear or extinction memory retrieval (also called retention) phase, the CSs are presented again, without US reinforcement. The experimental context can be the same as in preceding phases or changed (thick-lined box).

Box 2 - Fear or anxiety: What's in a name?

The nouns 'dread', 'fright', 'fear' and 'anxiety' are used in daily speech to express (more or less) the same type of psychological distress. In the scientific literature this state is subdivided based on certain criteria, that being proximity of danger, and certainty about something dangerous occurring. Fear typically refers to the short-lived panic emotion during imminent and relatively unavoidable danger in the vicinity of a person, and its subsequent fight, freeze and flight responses; anxiety to the longer-lasting anxious state elicited by more distal and avoidable danger (Davis et al., 2010). Evidence from rodent studies suggest that distinct brain areas are involved in fear and anxiety, although they are not entirely independent (Davis et al., 2010; Walker et al., 2009). This subdivision notwithstanding, fear and anxiety are frequently used interchangeably, also in the scientific literature.

Heightened subjective and physiological emotional responses to anxiety-eliciting situations in particular appear to cause the biggest burden in patients with anxiety disorders (Grupe and Nitschke, 2013). In addition, individuals' responses to anxiety-eliciting situations, described as 'weak situations' because aversive outcomes can only be weakly predicted by the information at hand, are suitable to distinguish between patients with anxiety disorders and healthy individuals, because of the differences between the two group with respect to the intensity of their psychophysiological responses (Lissek et al., 2006). Further, in general, experiments that are anxiety- rather than fear eliciting are more sensitive to known anxiety-reducing drugs (Davis et al., 2010). At first glance therefore, fear conditioning paradigms do not seem optimal to study (the treatment of) pathological anxiety. However, procedural variants such as low vs high US reinforcement rate, conditioning to a context vs a cue and omission or inclusion of verbal instructions can affect imminence and ambiguity of threat, and thereby create a situation that is either more anxiety-, or more fear eliciting (Davis et al., 2010; Lonsdorf et al., 2017).

Patients with anxiety disorders experience fear and anxiety not only in dangerous situations, but also during daily routine tasks and activities (e.g., grocery shopping, traveling, work, social gatherings). Additionally, or alternatively, they are not able to perform these without attempts to attain safety, that interfere with these tasks and activities. Anxiety and safety behaviors contribute to the misperception of threat by patients with anxiety disorders (e.g., Arntz et al., 1995; Salkovskis et al., 1991, 1996; Wells et al., 2016). Conversely, accurate assessments of safety and danger would be needed to keep fear and anxiety at bay or in check (Lohr et al., 2007). This requires learning what is not dangerous (e.g., grocery shopping) or

learning what is not dangerous anymore (e.g., grocery shopping after once being assaulted in a supermarket). In fear conditioning paradigms such opportunities to learn about safety can be provided by a stimulus that is never paired with the US, the CS- (see Figure 1), or by an always safe context. Other possibilities are stimuli that signal US nonoccurrence in the presence of the CS+ or inform the individual about opportunities to control the US (Lohr et al., 2007). Further, repeated non-occurrence of the US presents a learning opportunity that the CS+ is not dangerous anymore. This process is called fear extinction (see Figure 1). The now dominant inhibitory model of fear extinction states that, rather than destruction of fear memories, extinction learning involves new learning about the contextuality of these memories. After fear extinction, the extinction context is learnt to be safe, conceptualized by the formation of an inhibitory CS-noUS association in memory (Bouton, 1993; Bouton et al., 2006).

Convergent evidence from experimental studies in humans exists that a failure to learn what is not dangerous (Baas et al., 2008, 2013; Chan and Lovibond, 1996; Duits et al., 2015; 2021; Leen et al., 2021; Lissek et al., 2009; Telch et al., 1994), or what is not dangerous anymore (Duits et al., 2015; 2021; Leen et al., 2021) is associated with high state anxiety in students (Baas et al., 2008; Chan and Lovibond, 1996), with high state and/or trait anxiety in healthy participants (Baas et al., 2008, 2013; Leen et al., 2021) and with being diagnosed with an anxiety disorder (Duits et al., 2015; 2021; Lissek et al., 2009; Telch et al., 1994). In Figure 1, this could be shown with red color not only for US reinforced trials, but also for other trials and experimental phases that are in fact safe. Prospectively, an inability to distinguish between safety and danger cues during fear conditioning predicted subsequent avoidance behavior in healthy subjects (Grillon, 2002). Moreover, poor extinction learning predicted PTSD symptom severity in Dutch soldiers after a four-month deployment to Afghanistan (Lommen et al., 2013). In summary, the available evidence suggests that deficits in learning about safety and danger characterize at least some patients with an anxiety disorder and may even play a role in the etiology.

1.3 First-line treatment options for patients with anxiety disorders

Current clinical guidelines recommend either psychotherapy or pharmacotherapy for individuals with a marked functional impairment, for whom self-help, support from an experience expert or education about the anxiety disorder alone are insufficient (Bandelow et al., 2022; van Balkom et al., 2013; NICE, 2006, 2013, 2018, 2019). In the most recent Dutch guidelines psychotherapy in the form of cognitive behavioral therapy (CBT; see below) is recommended over pharmacological interventions because of observed superior long-term effectiveness. Measured

at 12-months follow-up, CBT still outperformed control conditions in generalized anxiety disorder, social anxiety disorder, and posttraumatic stress disorder (van Dis et al., 2020). For panic disorder with or without agoraphobia however, CBT and control conditions (including care as usual, relaxation, psychoeducation, pill placebo, supportive therapy, or waiting list) did equally well at follow-up (van Dis et al., 2020). Long-term outcomes for specific phobia and obsessive-compulsive disorder were unavailable for meta-analysis. Evidence of long-term effectiveness of first-line pharmacological treatments is lacking. What is known, is that discontinuing pharmacological treatment increases the odds of relapse more than threefold (Batelaan et al., 2017).

In line with guideline recommendations, in one study patients had more positive views of CBT compared to pharmacotherapy (Deacon and Abramowitz, 2005). Notably, this difference only existed for patients who were not already taking medication.

1.3.1 First-line psychotherapeutic treatment

Current guidelines recommend CBT as the first treatment option for anxiety disorders (Bandelow et al., 2022; van Balkom et al., 2013; NICE, 2006, 2013, 2018, 2019). The cognitive theory of emotional disorders (Beck, 1976) states that patients with emotional disorders share specific cognitions. In anxiety disorders, these cognitions entail overpredictions of threat and future harm (Beck et al., 1987; Beck and Emery, 1985). Traditional second generation CBT is built upon the premise that modifying these expectancies is required for symptom change (Hayes et al., 2006). Next to cognitive therapy to modify dysfunctional cognitions, CBT also includes behavioral therapeutic techniques. Exposure to feared situations while refraining from safety behaviors should be a central element of treatment if phobic avoidance is an important aspect of the disease presentation (Bandelow et al., 2017; van Balkom et al., 2013). Already a century ago, it was discovered that repeated confrontation with phobic objects led to substantial decrease in fear towards these objects (Jones, 1924). Analogous to fear extinction in fear conditioning paradigms, the symptom relief brought about by exposure therapy can be mechanistically explained by inhibitory learning and retrieval of a CS-noUS association (Craske et al., 2012).

An alternative interpretation of clinical change during exposure therapy places emphasis on patients' consciously experienced violation of harm expectancies during exposures (Craske et al., 2018). This fits with the classical conditioning theory of Rescorla and Wagner (1972), that predicts that the difference between expected and actual outcome should be large for optimal learning. Experimental studies suggested that disconfirmation of threat beliefs can provide a sufficient explanation for the therapeutic effect of exposure therapy (e.g., Salkovskis et al., 1999). However, other work showed that cognitive change does not necessarily predict

symptom change, but that cognitions as well as symptoms change during CBT due to a common factor (Burns and Spangler, 2001). In conclusion, cognitive change seems to be not the sole mediator of symptom reduction during CBT.

Regardless of the mechanism of change, CBT has been extensively studied in randomized controlled trials (RCTs) and found to be effective at posttreatment (for (reviews of) meta-analyses, see Butler et al., 2006; Hofmann et al., 2012; Hofmann & Smits, 2008; van Dis et al., 2020). Despite these positive findings, CBT including exposure elements is only delivered to a minority of patients with anxiety disorders (Powers and Deacon, 2013), which may be partly due to therapists' concerns about exposure therapy (Olatunji et al., 2009). Drawbacks of exposure-based therapy are the required time and effort, and temporary symptom exacerbation in some patients (Foa et al., 2002; Moritz et al., 2015). Moreover, not every patient responds to exposure therapy, and risk of relapse is high (see section 1.4).

1.3.2 First-line pharmacotherapeutic treatment

Based on high quality evidence regarding efficacy for treating generalized anxiety disorder, panic disorder with agoraphobia, social anxiety disorder, posttraumatic stress disorder and obsessive-compulsive disorder, selective serotonin reuptake inhibitors (SSRIs) and selective serotonin-norepinephrine reuptake inhibitors (SNRIs) are now considered the first-line pharmacological treatments for these disorders in European countries and the USA (Bandelow et al., 2022; Nezgovorova et al., 2022; Sartori and Singewald, 2019; Williams et al., 2022).

Unfortunately, serotonergic antidepressants often have side effects such as sexual dysfunction and weight gain (Masand et al., 2002; Vaswani et al., 2003). When patients wish to discontinue using these medications, which is a logical step to take if treatment response is insufficient (see section 1.4), long tapering periods may be necessary to mitigate withdrawal symptoms (Horowitz and Taylor, 2019).

1.4 Non-reponse and relapse after first-line treatments

Response to currently available treatment varies substantially between individual patients with anxiety disorders. Responder rates between 40 and 70% have been reported for both psychotherapy and pharmacotherapy (Bradley et al., 2005; Eddy et al., 2004; Williams et al., 2022), which leaves an unacceptable number of patients with substantial residual symptoms. Further, relapse after having recovered from an anxiety disorder is common, and affects more than half of patients (Bruce et al., 2005; Penninx et al., 2011; Scholten et al., 2016).

After nonresponse to treatment, a holistic reassessment of the individual, their environment and social circumstances is needed by specialist mental health services (NICE, 2019). Environmental stressors such as the frequent use of psychoactive substances and lack of social support should be addressed when a patient remains symptomatic (Roy-Byrne, 2015). More treatment hours (for OCS; NICE, 2006), or concentrated treatment within a short period of time (for panic disorder/agoraphobia; NICE, 2019) can be attempted. There is increasing evidence indicating high success rates of concentrated exposure therapy in anxiety disorders (Hansen et al., 2018, 2019; Iversen et al., 2022; Pittig et al., 2021) and faster treatment gains than standard, temporally spaced exposure therapy (Pittig et al., 2021).

Combination of treatment modalities has been recommended when response to one treatment modality is insufficient (Bandelow et al., 2022; NICE, 2006, 2013, 2019; van Balkom et al., 2013). There is evidence suggesting that patients prefer the combination of psychotherapy and pharmacological approaches, over monotherapy (McHugh et al., 2013). In addition, in terms of efficacy, a meta-analysis showed large effect sizes of combined treatment for panic disorder/agoraphobia, generalized anxiety disorder and social anxiety disorder (Bandelow et al., 2015). Other meta-analyses indicated that for panic disorder (Bandelow et al., 2007; Cuijpers et al., 2014; Furukawa et al., 2008; Hofmann et al., 2009), obsessive-compulsive disorder (Cuijpers et al., 2014) and for generalized anxiety disorder (Hofmann et al., 2009) combination treatment was superior to pharmacotherapy alone (Bandelow et al., 2007; Furukawa et al., 2008) or CBT alone (Hofmann et al., 2009). However, during the acute treatment phase, dropouts due to side effects were more frequent with combined treatment compared to psychotherapy alone (Furukawa et al., 2008) and after the acute treatment phase the added value of combining treatments disappeared (Furukawa et al., 2008; Hofmann et al., 2009).

When first-line psychotherapy and pharmacotherapy have both been attempted, a next step would be to consider alternative or augmented treatments. There are several alternative medication options to SSRIs and SNRIs. If a patient does not respond well to first-line pharmacological treatment, monoamine oxidase inhibitors (MAOIs) and tricyclic antidepressants (TCAs), that both affect monoamine neurotransmission, can be prescribed (Bandelow et al., 2022; Sartori and Singewald, 2019). Another option entails administering benzodiazepines (BDZs), that affect GABA neurotransmission and have the advantage of reducing anxiety symptoms acutely, i.e., there is only a negligible latency period for the therapeutic effect to set in (Sartori and Singewald, 2019). There is a reluctancy towards BDZ prescription because of the high abuse potential (Bandelow et al., 2022), that, however, may not be completely justified (O'Brien, 2005). In Europe and the USA these second-line drugs have been approved for treatment of specific anxiety disorders, for other anxiety disorders, prescription is off-label (Sartori and Singewald,

2019). Considering the higher frequency and/or severity of side effects in comparison to SSRIs and SNRIs (Bandelow et al., 2022; Sartori and Singewald, 2019), the most recent Dutch clinical guidelines recommend to prescribe MAOIs, TCAs and BDZs only to treatment refractory patients.

Several psychotherapeutic alternatives to CBT have been incorporated in clinical guidelines. For example, applied relaxation for GAD (NICE, 2019), eye movement desensitization and reprocessing (EMDR) for PTSD (NICE, 2018), and psychodynamic treatment for social anxiety disorder (NICE, 2013). In addition, so called third wave cognitive behavioral interventions such as acceptance and commitment therapy (ACT) show effects on anxiety outcomes comparable to those of 'traditional' second generation CBT (Glombiewski et al., 2021; Haller et al., 2021). These interventions focus on changing the function of cognitive events and the patient's relation to them, rather than changing cognitions directly (Hayes et al., 2006). Some of these psychotherapeutic alternatives have lower efficacy than CBT (NICE, 2013; Bandelow et al., 2017), and even when therapeutic effects are comparable, not all patients profit (Arch et al., 2012; Bradley et al., 2005; Schottenbauer et al., 2008).

Unfortunately, despite the existence of multiple treatment options, many patients remain treatment refractory, even after being treated in specialized mental health care centers according to treatment guidelines (van Dijk et al., 2015).

1.5 Anxiolytic drug developments

Two decades ago the last drugs for treating anxiety symptoms (more specifically those of generalized anxiety disorder) were approved in Europe and/or the US [Box 3]. These drugs were duloxetine (a SNRI) and pregabalin (a calcium channel blocker with glutamatergic effects; Griebel and Holmes, 2013; Sartori and Singewald, 2019). Novel pharmacological targets such as the latter have been explored as alternatives to 'traditional' drugs for anxiety that affect monoamine neurotransmitters such as serotonin and norepinephrine. Different drug classes also differentially affect activity in brain area's involved in pathological anxiety. This variation in neuropharmacological mechanisms and sites of action should be further investigated (Davidson and Gabos-Grecu, 2020), because the underlying pathology is not the same for every patient (King et al., 2019). In addition, efficacy of drugs for reducing anxiety cannot be predicted by DSM classifications, whose biological validity has been challenged (see Box 1). This has led to the more transdiagnostic approaches for drug development (Davidson and Gabos-Grecu, 2020).

Another promising avenue for treatment refractory patients encompasses development of drugs that act synergistically with psychotherapy. Importantly, it has been argued that deficits

in learning and retrieval of inhibitory associations that signal "no danger" may contribute to nonresponse to exposure therapy in some patients (Craske et al. 2018). Preliminary empirical evidence exists that such deficits may have predictive value for treatment nonresponse (Duits et al., 2021). Therefore, several strategies have been proposed to optimize inhibitory learning during exposure therapy. The first strategy can be implemented by the therapist and/or patient, such as ascertaining that the patient refrains from safety behaviors during exposure, or the use of retrieval cues after therapy (Craske et al. 2018). The second strategy entails so called 'cognitive enhancers', drugs aimed to strengthen the learning process.

Box 3 - Animal research in drug development

Before proceeding to clinical trials, efficacy and safety of candidate drugs for treating anxiety are first screened in preclinical animal research. Most of these drugs are not approved for treating patients despite promising findings in animal research, mainly due to poor efficacy, safety concerns or because of financial reasons (Mandrioli and Mercolini, 2015). In addition, reaching the site of action in humans can be problematic due to the fact that the blood-brain barrier is impermeable to certain classes of systemically administered drugs (e.g., peptides such as oxytocin; Mandrioli and Mercolini, 2015).

In anxiety research, the fear conditioning paradigm, that can be employed in humans as well as non-human animals, is very suitable to measure drug effects on anxiety-like behavior. While fear conditioning is aimed at eliciting defensive responses (LeDoux, 2014), in other frequently employed anxiety tests conflict is brought upon the animal between the need to defend oneself and other motivational needs (La-Vu et al., 2020). This preclinical research has been criticized because the translation of efficacy in animals to known effective drugs in patients seems poor and/or inconsistent (Griebel and Holmes, 2013). Explanations for these shortcomings of animal research are interlaboratory differences in test conditions and procedures that, for example, affect arousal in animals (Crestani et al., 2000), and further the almost exclusive use of male animals and the select focus on acute drug effects. Some have argued that the use of animals that show frequent anxiety-like behaviors over longer periods of time (compared to only when elicited in an anxiety test) may improve the validity of anxiolytic drug screening and its translation to humans with anxiety disorders (Ennaceur, 2014). These efforts notwithstanding, the ability of anxiety tests to predict anxiolytic drug in animals effects remains far from optimal. However, despite the difficulties with interspecies translation, research in non-human animals is still considered an indispensable translational step between in vitro and in silico research and research in other living beings: humans (Scannell et al., 2012).

One such compound is d-cycloserine (DCS), a partial agonist of the glutamatergic N-methyl-D-aspartate (NMDA) receptor (Sartori and Singewald, 2019). Research in rodents has shown that DCS enhances fear extinction by acquisition and/or consolidation of this new, inhibitory learning (Richardson et al., 2004; Walker et al., 2002). Because fear extinction is the hypothesized mechanism of improvement during exposure therapy (Craske et al., 2014), one would expect that these preclinical findings would translate to clinical applications. Initially, the effects of applying DCS as an adjunct to exposure therapy sessions in clinical samples seemed promising. For example, compared to placebo treatment, DCS application resulted in greater improvement in PTSD (Difede et al., 2014) and acrophobia symptoms (Ressler et al, 2004). In addition, these benefits were long-lasting, up to 6 months after exposure therapy termination. However, more recent work that synthesized the data of all high quality studies in patients suggest that DCS augmentation of exposure-based CBT has only a modest benefit, as the advantage of DCS over placebo was small at most (Mataix-Cols et al., 2017). More recent research efforts have therefore been aimed at establishing tailored clinical applications of DCS, for example by customizing administration timing (e.g., Hofmeijer-Sevink et al., 2017; Smits et al., 2020), without convincing effects.

1.6 Cannabinoid medicines

1.6.1 Cannabinoid type 1 receptor activation as an extinction-enhancing strategy

Cannabis-based products are permitted in many countries across the globe for medical and research purposes (Fleisch and Woodbridge, 2022). Research on cannabis products led to the discovery of cannabinoid receptors and endogenous cannabinoid ligands ('endocannabinoids', [Box 4]). Converging lines of evidence point to mediation of fear extinction by endocannabinoid neurotransmission.

Most of the research evidence exists for involvement of the following subregions in the brain in fear extinction: the basal and lateral nucleus of the amygdala (basolateral amygdala, BLA), the ventral and/or dorsal hippocampus and the infralimbic cortex (a region in the rodent brain that corresponds to the human ventromedial prefrontal cortex; Myers and Davis, 2007; Tovote et al., 2015). In humans, CB1 receptors are densely expressed in these brain structures: they have been localized in the amygdala, hippocampus and associated regions including the prefrontal cortex (Glass et al., 1997).

Marsicano et al. (2002) were the first to show the central function of CB1 receptors in fear

extinction in mice. They demonstrated that fear extinction to a tone was impaired after genetically or pharmacologically blocking of CB1 receptors. In addition, they found that re-exposure to the tone after de novo fear acquisition led to increased endocannabinoid (AEA and 2-AG) levels in the BLA. Subsequent experiments with a CB1 antagonist administered before fear extinction in mice (Suzuki et al., 2004) and in rats (Pamplona et al., 2006) employing fear conditioning to a context (rather than a cue, such as a tone) showed long-term detrimental effects on freezing behavior (measured $\geq$ 24 h after extinction). Taken together, these findings point to mediation of fear extinction by cannabinoid neurotransmission.

Research into the role of the ECS in extinction learning in humans discovered that polymorphisms in major cannabinoid genes were associated with poor extinction in healthy individuals (e.g., Dincheva et al., 2015; Heitland et al., 2012; Mayo et al., 2018) and more recently, also in patients with PTSD (Ney et al., 2021). Although these findings are preliminary, this demonstrates that the ECS is involved in extinction learning in humans and that makes CB1 receptor activation a potential strategy for enhancing extinction.

Box 4 - Cannabis compounds and the endocannabinoid system

Cannabis has many constituents, of which Δ9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD) are well-known. Delta-9-THC (also referred to with THC) exerts partial agonist effects at the cannabinoid receptors (Zagzoog et al., 2020). This brings about psychotomimetic effects (feeling "high"; Dalton et al., 1976; Karniol et al., 1974), which limits its suitability for therapeutic application. The second cannabis constituent cannabidiol (CBD), in contrast, has low activity at the cannabinoid receptors, but has many other molecular targets, including other G-protein coupled receptors, ionotropic receptors and intracellular transporters of endocannabinoids (Mlost et al., 2020; Zagzoog et al., 2020). CBD at varying dosages does not induce psychotic symptoms or other side effects associated with THC and is in fact protective against some of the unwanted effects of THC, such as increased anxiety (Karniol et al., 1974; Zuardi 1982).

Research on the cannabis constituent THC and on cannabimimetic compounds have led to the discovery of the endogenous cannabinoid system (ECS) and the various receptors involved. After discovery of the cannabinoid receptors types 1 and 2, endogenous cannabinoids (endocannabinoids) N-arachidonoylethanolamide (AEA; Devane et al. 1992) and 2-arachidonoylglycerol (2-AG; Mechoulam et al. 1995; Sugiura et al. 1995) were unraveled. N-arachidonoylethanolamide is also named "anandamide", which is derived from the Sanskrit word "ananda", freely translated as "bliss" (Devane et al., 1992). Cannabinoid type 2 (CB2) receptors were originally believed to be exclusively present in peripheral tissues, which appeared to be a misconception, as they were also found in the brain, with enhanced expression in neuroinflammatory disorders (Roche and Finn, 2010). Nevertheless, the role of cannabinoid type 1 (CB1) receptors as a possible treatment target for anxiety disorders has been studied far more extensively than that of CB2 receptors. CB1 receptors ubiquitously occur in the central nervous system (Lutz, 2020). Activation of CB1 receptors by cannabis- or cannabimimetic compounds or by endocannabinoids inhibits neurotransmission throughout the adult human brain (Pertwee, 1997).

1.6.2 Strategies to stimulate cannabinoid type 1 receptor signaling

There are at least two strategies to stimulate CB1 receptor signaling by administration of external compounds. The first strategy entails agonists with high affinity at the CB1 receptor, such as THC (Zagzoog et al., 2020). Unfortunately, THC can confer psychotomimetic effects (Dalton et al., 1976; Karniol et al., 1974) and tolerance to beneficial effects of THC containing products

has been observed in humans with repeated administration (e.g. Cuttler et al., 2020). The therapeutic potential of this first strategy is therefore limited.

The second strategy to stimulate CB1 receptor signaling in a more targeted way is to increase endogenous anandamide (AEA) levels by pharmacological inhibition of fatty acid amide hydrolase (FAAH), the catabolic enzyme of AEA. CBD is a weak inhibitor of FAAH (Bisogno et al., 2001; Mlost et al., 2020), nevertheless, repeated CBD administration can increase AEA levels, as was measured in mouse hippocampal tissue (Campos et al., 2013) and in human serum (Leweke et al., 2012). In mice, prolonged elevations of AEA did not lead to receptor desensitization and tolerance to potential beneficial effects, in contrast to prolonged elevations of endogenous 2-arachidonoylglycerol (2-AG; Ignatowska-Jankowska et al., 2014; Schlosburg et al., 2010). This second strategy to stimulate CB1 receptor signaling may therefore be more suitable for the aim of fear extinction enhancement in humans, and was employed for this thesis.

1.6.3 Added value of cannabidiol in treating anxiety disorders

Despite the fact that the medicinal use of cannabis dates back to before the beginning of our era (Crocq, 2020), that CBD has been marketed as a dietary supplement that may alleviate anxiety, and the demand for such products is high, insufficient scientific evidence has been available for efficacy of cannabinoid medicines in patients with anxiety disorders (Black et al., 2019). Therefore, cannabinoid medicines have not been incorporated in US and European guidelines for treating anxiety symptoms (Häuser et al., 2018; NASEM, 2017; NICE, 2021).

CBD is known as a cannabinoid with relatively few adverse effects (Bergamaschi, 2011; Chesney et al., 2020). Because of this property, CBD would be suitable for controlled therapeutic use, especially for those patients who are prone to the side effects of traditional medication for anxiety. Only very few studies have investigated the therapeutic effects of CBD on anxiety symptoms (Bolsoni et al., 2022; Crippa et al., 2021; Gournay et al., 2023; Masataka, 2019). At the time this PhD project started, CBD had not been investigated as an augmentation strategy for exposure therapy. We considered it a worthwhile endeavor to test the added benefit of CBD in treating anxiety disorders, considering that:

- Anxiety disorders come with a high personal and societal burden (section 1.1);
- Some patients with anxiety disorders have difficulties with fear extinction (section 1.2);
- Exposure therapy, the first-line psychotherapeutic treatment for anxiety disorders, seems to share an underlying mechanism of change with fear extinction (section 1.3);
- Many patients are refractory to currently available treatments (section 1.4);

- A promising avenue for treatment refractory patients are drugs that act synergistically with psychotherapy (section 1.5);
- Preclinical evidence suggests that enhancement of CB1 receptor signaling could enhance fear extinction. This could be achieved by administration of CBD (sections 1.6.1-1.6.2).

Central to this thesis is the investigation of CBD administration as an augmentation strategy preceding exposure therapy. The main aim was to study the added benefit of CBD in treating anxiety disorders, for currently treatment refractory patients. Subsidiary aims are outlined in section 7: Thesis aims and outline.

1.7 Thesis aims and outline

The chapters of this thesis build up from systematic literature reviews of past research into CBD and anxiety (**Chapters 2 and 3**) and current clinical research with CBD in patients with anxiety disorders (**Chapters 4, 5 and 6**) to a study aimed at predicting exposure treatment success (**Chapter 7**). The data of the studies described in **Chapter 5, 6 and 7** were collected in the context of a multicenter randomized controlled trial (RCT; study protocol in **Chapter 4**) to investigate the application of CBD as an augmentation strategy for exposure therapy.

The first aim of this thesis was to systematically review and meta-analyze the research into anxiety reducing effects of ECS manipulations that has been published so far. We focused specifically on compounds that were developed to increase CB1 receptor activation by enhancing AEA levels, including CBD. This comprehensive quantitative summary of the mainly preclinical literature provided an indication of clinical efficacy of endocannabinoid enhancing compounds. We employed a systematic literature search with prespecified eligibility criteria. The heterogeneity in procedures of included studies allowed for identifying potential moderators of drug effects. By narrowing down the circumstances under which pharmacological AEA enhancement is anxiety-reducing, these moderators could be pivotal in the development of cannabinoid medicines for treating anxiety disorders. We conducted separate meta-analyses for tests of conditioned versus unconditioned anxiety, to dissect acute anxiolytic effects from effects on fear extinction and related learning mechanisms. This systematic review and meta-analysis into anxiolytic effects of endocannabinoid enhancing compounds is presented as **Chapter 2** of this thesis.

The second aim of this thesis was to predict the therapeutic dose window for anxiety-reducing effects of CBD in humans based on preclinical models. The absence of established dosing guidelines impedes successful translation to clinical applications. To address this

omission in the literature, we analyzed all pharmacodynamic (PD) results that concerned anxiety-reduction by CBD from the broader systematic review described in Chapter 1, as per protocol. For this study an additional systematic search was executed into pharmacokinetic (PK) data after systemic CBD administration. From included studies safety outcomes were extracted, when available. We then used a tool to integrate data from an Investigator's Brochure (IB) or to obtain an overview of published preclinical and clinical literature, the IB-de-risk tool (van Gerven and Cohen, 2018), to synthesize these three types of data (PD, PK and safety). This approach for data synthesis of preclinical and clinical data and the obtained semiquantitative color-coded overview are presented **Chapter 3**.

The third aim of this thesis was to investigate whether CBD administered prior to exposure therapy sessions would enhance the effect of exposure therapy in treatment refractory patients with social anxiety disorder and patients with panic disorder with agoraphobia. These disorders are among the main phobic disorders, for which an exposure-based treatment is clinically indicated. Study medication (300 mg CBD or placebo) was administered orally preceding 8 weekly exposure therapy sessions. We examined whether CBD augmentation would lead to faster, stronger and/or more enduring improvement on clinical outcomes compared to placebo augmented exposure therapy. We also explored enhancement of within-session fear extinction by CBD and measured CBD plasma levels. This investigation of CBD as an augmentation strategy for exposure therapy is reported in **Chapter 5**.

The fourth aim was to test enhancement of fear re-extinction by CBD. We were also interested in another potential effect of CBD with high clinical relevance: attenuation of fear memory expression. To answer our research questions a fear conditioning task was administered in the context of our multicenter RCT. The effects of a single dose of 300 mg CBD vs. placebo in this fear conditioning task are described in **Chapter 6**. We used a differential (including CS+ and CS- stimuli) cued fear conditioning paradigm to study these memory and learning processes, and measured fear memory expression under increasing levels of threat imminence. Multiple subjective and physiological indices of fear were used, considering that anxiolytic drugs can affect these output systems differentially.

The fifth aim of this thesis was to predict who would remain treatment refractory, by characterizing patients prior to the start of exposure therapy using the results of a fear conditioning task. We used part of the fear conditioning task, administered prior to the start of the CBD or placebo augmented exposure therapy and not augmented by study medication. This part consisted of a pre-acquisition, fear acquisition and a short extinction phase. Latent fear extinction and safety learning trajectories were identified, measured with subjective fear indices taken after each phase. After assignment of all patients to the latent trajectories, we examined the predict-

ive value of individual differences in fear extinction and safety learning for exposure treatment response, as described in **Chapter 7**.

1.8 List of abbreviations

AEA	N-arachidonoylethanolamide or anandamide
BDZ	benzodiazepine
BLA	basolateral amygdala
CBD	cannabidiol
CBT	cognitive behavioral therapy
CB1	cannabinoid type 1
CB2	cannabinoid type 2
CS+	a stimulus that elicits a conditioned response after repeated pairings with an unconditioned stimulus
CS-	a stimulus that is never paired with an unconditioned stimulus
DCS	d-cycloserine
DSM	Diagnostic and Statistical Manual of Mental Disorders
ECS	endogenous cannabinoid system
EMDR	eye movement desensitization and reprocessing
FAAH	fatty acid amide hydrolase
FDA	Food and Drug Administration
GAD	generalized anxiety disorder
IB	Investigator's Brochure
MAOI	monoamine oxidase inhibitor
NMDA	N-methyl-D-aspartate
OCD	obsessive-compulsive disorder
PD	pharmacodynamic
PK	pharmacokinetic
PTSD	posttraumatic stress disorder
SNRI	selective serotonin-norepinephrine reuptake inhibitor
SSRI	Selective serotonin reuptake inhibitor
THC	tetrahydrocannabinol
TCA	tricyclic antidepressant
US	unconditioned stimulus
2-AG	2-arachidonoylglycerol
Δ9-THC	Δ9-tetrahydrocannabinol

1.9 References

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Part I

Systematic literature reviews of past research into cannabidiol and anxiety

Chapter 2

Anxiolytic effects of endocannabinoid enhancing compounds: A systematic review and meta-analysis

Caroline M.B. Kwee
Nadia A. Leen
Rian C. van der Kamp
Caspar J. van Lissa
Danielle C. Cath
Lucianne Groenink
Johanna M.P. Baas

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JB and DC obtained funding for this study from the Helmholtz Institute and GGZ Drenthe. All authors contributed to the design of the study. CK, LG and JB were responsible for the study protocol. CK, NL and RvdK screened and selected studies, CK, NL and collaborators IdV and AM extracted data, and CK and NL graded the quality of the evidence, under the supervision of LG and JB. CvL analyzed the data. CK wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Abstract

The endocannabinoid system is a promising candidate for anxiolytic therapy, but translation to the clinic has been lagging. We meta-analyzed the evidence for anxiety-reduction by compounds that facilitate endocannabinoid signaling in humans and animals. To identify areas of specific potential, effects of moderators were assessed. Literature was searched in Pubmed and Embase up to May 2021. A placebo/vehicle-control group was required and in human studies, randomization. We excluded studies that co-administered other substances. Risk of bias was assessed with SYRCLE's RoB tool and Cochrane RoB 2.0. We conducted three-level random effects meta-analyses and explored sources of heterogeneity using Bayesian regularized meta-regression (BRMA). The systematic review yielded 134 studies. We analyzed 120 studies (114 animal, 6 human) that investigated cannabidiol (CBD, 61), URB597 (39), PF-3845 (6) and AM404 (14). Pooled effects on conditioned and unconditioned anxiety in animals (with the exception of URB597 on unconditioned anxiety) and on experimentally induced anxiety in humans favored the investigational drug over placebo/vehicle. Publication year was negatively associated with effects of CBD on unconditioned anxiety. Compared to approach avoidance tests, tests of repetitive-compulsive behavior were associated with larger effects of CBD and URB597, and the social interaction test with smaller effects of URB597. Larger effects of CBD on unconditioned anxiety were observed when anxiety pre-existed.Studies reported few side effects at therapeutic doses. The evidence quality was low with indications of publication bias. More clinical trials are needed to translate the overall positive results to clinical applications.

2.1 Introduction

Cannabis has long been considered to have therapeutic potential (Cohen, 1978). Research on the cannabis constituent Δ9-THC and cannabimimetic compounds led to the discovery of cannabinoid receptors and, subsequently, of endogenous cannabinoids N-arachidonoylethanolamide (AEA; anandamide; Bisogno et al., 2001; Mlost et al., 2020) and 2-arachidonoylglycerol (2-AG; Devane et al., 1992; Mechoulam et al., 1998; Sugiura et al., 1995). Early studies with cannabidiol (CBD), a second major constituent of cannabis, demonstrated anxiolytic properties in animals (Guimarães et al., 1990; 1994; Onaivi et al., 1990) and humans (Zuardi et al., 1993).

In subsequent years, preclinical data in rodents accumulated suggesting that disruptions in endocannabinoid tone in brain regions including the amygdala, hippocampus and prefrontal cortex contribute to anxiety-like behavior induced by acute or repeated stress (for narrative reviews see Gorzalka et al., 2008; Hill et al., 2010; Morena et al., 2016; Patel et al., 2008). Several experiments in rodents used fear extinction (e.g., Chhatwal et al., 2005; Ganon-Elazar and Akirav, 2009; Marsicano et al., 2002), a widely used translational model for learning that takes place during exposure therapy (Craske et al., 2018). It was shown that endocannabinoid signaling in the amygdala and hippocampus mediates the stress and glucocorticoid-induced enhancement of fear extinction and fear memory consolidation, and impairment of fear memory retrieval (Morena et al., 2016). The clinical potential of this approach has spurred more mechanistic investigations in the endocannabinoid system (ECS) as a candidate target for anxiolytic drug development.

CBD is a prominent constituent of cannabis with a complex pharmacology, including as a mechanism of action inhibition of fatty acid amide hydrolase (FAAH), the primary metabolic enzyme of AEA. Although CBD's inhibition of FAAH is relatively weak (Bisogno et al., 2001; Mlost et al., 2020), subchronic CBD administration increased AEA levels in mouse hippocampal tissue (Campos et al., 2013) and in serum of patients with acute schizophrenia (Leweke et al., 2012).

In contrast to direct CB1R agonists such as Δ9-tetrahydrocannabinol (Δ9-THC), CBD does not induce psychomotor impairment (Dalton et al., 1976) or psychotomimetic effects (Dalton et al., 1976; Karniol et al., 1974).Further, CBD does not induce a change in heart rate (Dalton et al., 1976; Karniol et al., 1974), and seems to attenuate the anxiogenic effect of Δ9-THC in healthy volunteers (Karniol et al., 1974; Zuardi et al., 1982). These data suggest that CBD may indirectly exert CB1R mediated therapeutic actions, while circumventing unwanted side effects.

To overcome the lack of target selectivity of CBD (Bisogno et al., 2001; Mlost et al., 2020)

and aiming to optimize a fear extinction enhancing effect, several classes of more selective inhibitors of FAAH have been developed. The *O*-aryl carbamate URB597 turned out to be a potent and irreversible inhibitor of FAAH (Kathuria et al., 2003). The transport inhibitor AM404 selectively attenuates breakdown of AEA (Bortolato et al., 2006) by inhibition of intracellular fatty acid binding proteins (FABS; Deutsch, 2016; Kaczocha et al., 2012). The irreversible FAAH inhibitor PF-3845 is more potent, more selective, and has a longer duration of action than URB597 (Ahn et al., 2009). URB597, PF-3845 and inhibitor of AEA cellular uptake AM404 are prototypical examples of the many compounds that were developed to increase CB1R activation by enhancing endocannabinoid levels (Paredes-Ruiz et al., 2021).

To the best of our knowledge, numerous narrative (Griebel and Holmes, 2013; Lutz et al., 2015; Morena et al., 2016) but no systematic review on preclinical research into anxiolytic effects of ECS manipulations has been published so far. One systematic review of animal studies of ECS manipulations including CBD, with a primary focus on inflammation and neurogenesis, included five studies that reported variable effects on anxiety outcomes (Giacobbe et al., 2021).

A previous systematic review and meta-analysis summarized the limited available evidence from controlled studies conducted in human patients suffering from anxiety disorders, which included only two randomized controlled studies in patients (Black et al., 2019). This meta-analysis demonstrated no benefit of single doses of CBD (up to 600 mg) over placebo (Black et al., 2019). These preliminary findings in humans raise questions about the often discussed potential of pharmacological enhancement of AEA levels for treating anxiety symptoms. Clearly, there is a need for a systematic review and meta-analysis of the large body of mainly preclinical literature on this topic. This literature can provide an indication of clinical efficacy but is especially suitable for identifying potential moderators of clinical effects given the diversity in anxiety models used in these studies (Griebel and Holmes, 2013; Vesterinen et al., 2014).

The primary aim of the present systematic review and meta-analysis was to investigate anxiolytic effects of inhibitors of FAAH and AEA transport, by synthesizing all evidence from animal, human, preclinical and clinical studies. Behavioral, physiological, and subjective effects were investigated. In addition, theoretically relevant moderators and sources of heterogeneity of drug effects were explored. Part of the current literature examines acute anxiolytic effects, but a more recent approach is to develop treatments that aim to work synergistically with psychotherapeutic approaches by supporting adaptive learning, particularly fear extinction (cf., Davis et al., 2006). As discussed above, modulators of brain endocannabinoid levels have been shown to exert an effect on fear extinction and related learning mechanisms (for narrative reviews see Lafenêtre et al., 2007; Morena et al., 2016; Ruehle et al., 2012) and attempts have been made to translate these findings to potential use in psychotherapy (Kwee et al., 2022a). We there-

fore conducted separate meta-analyses for tests of conditioned versus unconditioned anxiety (Rodgers and Dalvi, 1997). Additionally, we explored whether drugs affected different aspects of fear conditioning and extinction, and investigated factors that are likely to moderate drug effects: 1) variables related to the drug regimen (single vs (sub)chronic administration, acute vs delayed effects); 2) species (Haller et al., 2007; Kwee et al, 2022b; 3) the pre-existing anxiety condition of the animal or human individual (Bach, 2022; Sams-Dodd, 2006); 4) type of anxiety test (Sams-Dodd, 2006); 5) sex differences with respect to the effects of AEA modulators, in light of the association between oestradiol and CB1 receptor density in amygdala and prefrontal cortex (Castelli et al., 2014); 6) publication year (Shrout and Rodgers, 2018).

For our secondary research aim we summarized any information that was available in included studies on drug safety and tolerability. Several reviews are available for CBD (Chesney et al., 2020; Huestis et al., 2019; Iffland and Grotenhermen, 2017; Kwee et al., 2022b). Previous preclinical research shows divergent results with respect to safety and tolerability of FAAH inhibitors (Panlilio et al., 2016). We therefore evaluated adverse effects in included studies on a drug-by-drug basis.

2.2 Experimental procedures

This review was preregistered with PROSPERO (CRD42021236572) and conducted in line with Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines (see Supplemental Tables 1 and 2).

2.2.1 Search strategy

Studies were searched in the electronic databases PubMed and Embase using both free text and underlying terms (MeSH and Emtree, respectively) up to 19-05-2021. The search was aimed at evidence on modulation of fear expression, anxiety symptoms and fear memory or extinction learning, by AEA hydrolysis and transport inhibitors in humans and non-human mammals (see Supplemental Table 3). Only peer-reviewed articles were included. No restrictions were placed on publication year or language. Preregistered but as of yet unpublished studies were searched in the EU Clinical Trials Register, the Australian and New Zealand Clinical Trials Registry, Animal Study Registry (German centre for the Protection of Laboratory Animals), ClinicalTrials.gov and Preclinicaltrials.eu, in order to get an indication of potential positive results bias.

2.2.2 In- and exclusion criteria

Table 1 lists in- and exclusion criteria for the selection of studies.

Table 1. Study in- and exclusion criteria

	Participants	Interventions	Comparison	Outcomes
Included				
	1. Healthy or anxious phenotype 2. Adult 3. Mammal	1. FAAH inhibitor or AEA transport inhibitor	1. Randomized placebo-controlled design	1. Fear expression,fear or extinction memory, extinction learning or anxiety disorder symptoms 2. Outcome domain behavioral physiological, or subjective 3. Data type continuous
Excluded				
	1. Chronic users of cannabis compounds	1. Compounds with catabolic pathways for AEA other than FAAH hydrolysis 2. Dual FAAH/ monoacylglycerol lipase inhibitors (Fowler, 2021) 3. Intracerebral/ intracerebroventricular/intravenous administration 4. Coadministration of other substances[b] 5. Time between drug administration and anxiety assay $\geq$ 24 h	1. Studies without control group 2. Non-randomization (studies in humans only)[a]	1. Acquisition of fear

Note: AEA: anandamide; FAAH: fatty acid amide hydrolase.

[a] The use of randomization is usually not reported in animal research (Muhlhausler et al., 2013) and it had not been empirically demonstrated whether the use of randomization would influence outcomes. Therefore, in animals we considered vehicle-controlled experiments without information about randomization and explicitly non-randomized but placebo/vehicle-controlled studies to be eligible as well.

[b] In humans, studies that allowed stable concomitant anxiolytic and/or antidepressant medication were included.

2.2.3 Study screening and selection

Titles and abstracts of articles retrieved using the search strategy were independently screened by a first (CK) and second reviewer (NL or RvdK) to identify studies that appeared to meet the inclusion criteria. They then independently screened the full text of these studies for eligibility. Disagreements were resolved through discussion, when no consensus was reached a third (LG) or fourth reviewer (JB) was consulted.

2.2.4 Data extraction

According to the PICO framework (Schardt et al., 2007) we recorded the details of the populations, interventions (including concomitant medication in human studies), and outcomes. The comparison group was always placebo/vehicle.

2.2.4.1 Primary research aim

For our first research aim of drug effects on anxiety outcomes within behavioral, physiological, and subjective outcome domains (see Supplemental Table 4), parameters of interest were means (Ms) and standard deviations (SDs) of the anxiety outcome in vehicle/placebo and active drug conditions. We used these parameters to calculate Hedge's g, an effect size that corrects for bias resulting from small sample sizes (Hedges, 1981). Higher scores on the effect size indicate an anxiolytic drug effect. Effect sizes were reverse-coded if higher values indicated less anxiety than lower values. Decision rules in case of unreported data, or multiple outcome measures or experimental drug-placebo comparisons are described in the Supplemental material, Section 1.2. If parameters were not fully reported we estimated them from graphs in the paper or requested the information from the authors.

We extracted theoretically relevant moderators dose, type of anxiety test, selected outcome parameter, publication year, information on frequency of drug administration and timing of effect measurement, pre-existing anxiety condition, sex, and species (ten moderators in total), of which the first three were selected as theoretically most relevant for exploratory follow-up analysis. To standardize 'dosages' across species human equivalent dose (HED) was calculated by using allometric scaling factors (Center for Drug Evaluation and Research, 2015). This dose-normalization approach is common in systematic overviews of preclinical study results across different species (van Gerven and Cohen, 2018). Our semi-quantitative analyses on the relation between CBD dose and anxiety-reducing effects tentatively suggest an inverted U-shaped dose-response curve (Kwee et al., 2022b), modeled here with a quadratic trend for dose/HED.

2.2.4.2 Secondary research aim

The terms 'harm', 'adverse', 'side', 'unwanted', 'undesirable', 'safe*', 'toler*' were searched in included articles.

2.2.4.3 Procedure

The majority of the data were extracted by CK, the remainder by a second reviewer (NL or one of the collaborators on the project. When one of the authors was in doubt about (categorization of) the data to be extracted, the issue was resolved through discussion (with a third (LG) and fourth reviewer (JB) when necessary). Generally, the outcomes extracted by the first and second reviewer matched (see Supplemental material, Section 1.3 for more information).

2.2.5 Data analysis

Meta-analyses were performed using R packages metafor (Viechtbauer, 2010) and pema (van Lissa and van Erp, 2021, Preprint). All models were three-level random effects models. A three-level random effects model accounts for three sources of variance: sampling error of the observed effect size (which is treated as known), within-experiment variance of true experiment-specific effect sizes, and variance of true experiment-specific effect sizes across experiments. Effect sizes from different papers were always categorized as independent; effect sizes from the same paper only if it was explicitly stated that effects were tested in independent experiments and/or independent sets of study subjects.

We conducted separate analyses per drug (within the class of AEA enhancing drugs), for unconditioned and conditioned anxiety in animals and experimentally induced anxiety in humans. Effect sizes per comparison and overall pooled effect size per meta-analysis were visualized in forest plots (Supplementary Figs. 2-9).

Statistical heterogeneity was assessed using τ^2(a measure of between-study variance) and I^2 (percent of variability in effect sizes not caused by sampling error; Higgins and Thompson, 2002; Vesterinen et al., 2014). We conducted sensitivity analyses to examine whether substantiated conclusions would change by excluding studies with high risk of bias or atypical route of drug administration.

For categorical moderators, we used dummy coding, treating the largest category of each variable as the reference category. We standardized continuous predictors only and not dummy variables. This may have given dummy variables a slight advantage, leading them to become significant sooner than continuous ones.

The number of effect sizes was small relative to the number of moderators. This introduces risks of model-nonidentification, overfitting, and multicollinearity (van Lissa, 2020). A novel technique called Bayesian regularized meta-regression (BRMA) overcomes these risks by imposing a regularizing horseshoe prior to shrink the regression coefficients of irrelevant moderators towards zero (van Lissa and van Erp, 2021, Preprint). Thus, we used BRMA in all moderator analyses to select moderators that are important in predicting the effect size. The resulting regression coefficients are negatively biased by design, but simulation studies show that the estimate of residual heterogeneity τ^2 is relatively unbiased (van Lissa and van Erp, 2021, Preprint). Supplementary classic meta-regression with the maximum likelihood approach (Supplemental Tables 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, 40, 42) indeed evidenced model non-convergence and high variance inflation factors (VIF) confirmed the expected problems caused by the high ratio of moderators to effect sizes.

We decided a priori to only perform the planned quantitative syntheses for each meta-analysis (separate per drug and conditioned/unconditioned/experimentally induced anxiety for humans and animals) if the number of included effect sizes in the meta-analysis exceeded the number of moderator variables + 1, which we considered the minimum for model identification. In addition to planned moderator analyses which included all moderators, we conducted exploratory moderator analyses on potential interactions of drug dose with a smaller number of key moderators.

To interpret these interaction effects, see plots with posterior predictive distribution of drug effects per moderator category, conditional upon the observed effects (Fig. 3).

The Workflow for Open Reproducible Code in Science (van Lissa et al., 2021) was used to make analyses reproducible. A reproducible repository with all analysis codes and data are available at (doi:10.5281/zenodo.7829148).

2.2.6 Assessment of the quality of evidence

Assessment of the quality of the meta-analytic evidence with the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) approach (Schünemann and Santesso, 2010) was done by CK and checked by NL. GRADE criteria and are summarized in the Supplemental material, Section 1.4.

2.3 Results

2.3.1 Included studies and characteristics

A PRISMA flowchart is shown in Fig. 1. Study characteristics of included studies are summarized in Table 2. The majority of included studies (n=114 out of a total of n=120 studies; 95%) were conducted in non-human mammals. Only n=6 studies (5%) were conducted in humans. Types of anxiety tests in included studies are provided in Supplemental Table 7.

Table 2. Summary characteristics of included studies

Population	
Publication year	1990-2021
Species	44% mouse, 50% rat, 5% human, 2% other
Pre-existing anxiety condition	in 17% of studies
Sample size per study*	88 (109)
Sample size per effect	20 (6)
Sex	90% male
Intervention	
Drug	52% CBD, 32% URB597, 11% AM404, 5% PF-3845
HED*60	90.08 (143.65)
Administration route	90% i.p., 10% oral
Frequency of administration	68% single dose
Timing of effect measurement	82% acute drug effects
Outcome	
Type of anxiety	71% unconditioned
Type of anxiety test	See Supplemental Table 7
Selected outcomes for tests of conditioned anxiety	See Supplemental Table 8 and Supplemental figure 1

Note: Numbers are mean (SD) or as otherwise stated.
* Sample sizes per tested effect can be found in the data files doi:10.5281/zenodo.7829148.

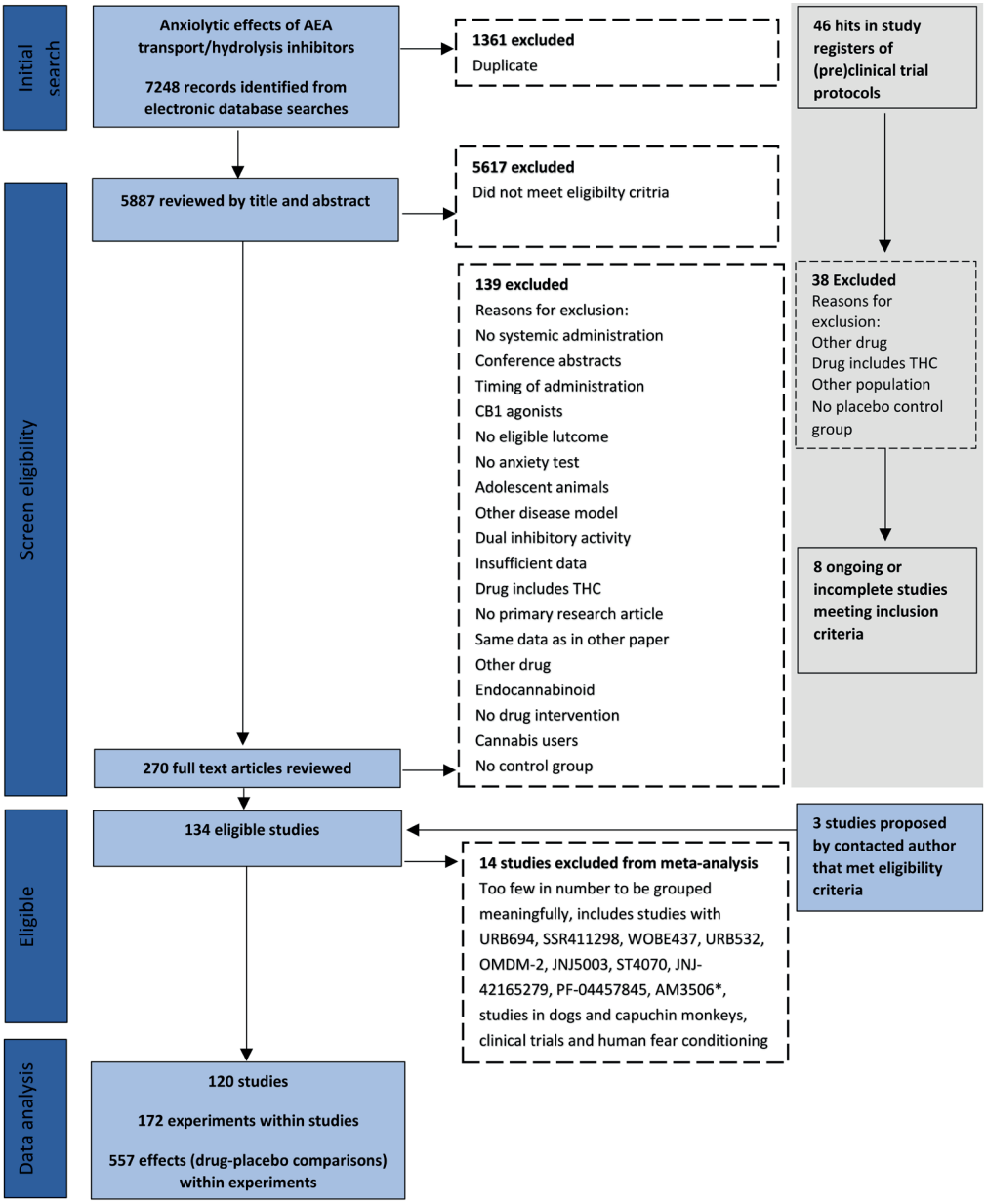


Figure 1. PRISMA flowchart.

Note: References of eligible studies are listed in Supplemental Table 5. Supplemental Table 6 describes ongoing or incomplete studies that meet inclusion criteria.

In Supplemental Table 8 the distribution is shown of outcomes in tests of conditioned anxiety, selected from the studies according to a-priori definitions (see Supplemental Fig. 1 for details). Outcomes were categorized as effects on fear memory reconsolidation when the drug was administered after memory retrieval, and as effects on extinction consolidation when administered after an extinction learning phase (before extinction retention was tested).

2.3.2 Effects of FAAH and AEA transport inhibitors on anxiety

2.3.2.1 Overall summary of findings regarding drug effects

Across meta-analyses, the pooled effect size estimates indicated a lower level of anxiety after treatment with the investigational drug than after placebo/vehicle treatment (Fig. 2 and Table 3). This was true for all combinations of drug types and types of anxiety for humans and non-human mammals except one, the effect of URB597 on unconditioned anxiety in animals. The size of these drug effects was moderate-to-large. Note that CBD was the only compound for which sufficient studies in humans were available to analyze meta-analytically. For PF-3845 only studies with tests of unconditioned anxiety in animals were available. The illustrations of effect sizes of all studies from which the pooled effect sizes were derived can be found in Supplemental Figs. 2-9.

For most analyses, both within- and between-experiment variance were significant, which indicates heterogeneity between effect sizes both within and across experiments (see Sections 3.2.2 and 3.2.5 for results of moderator analyses).

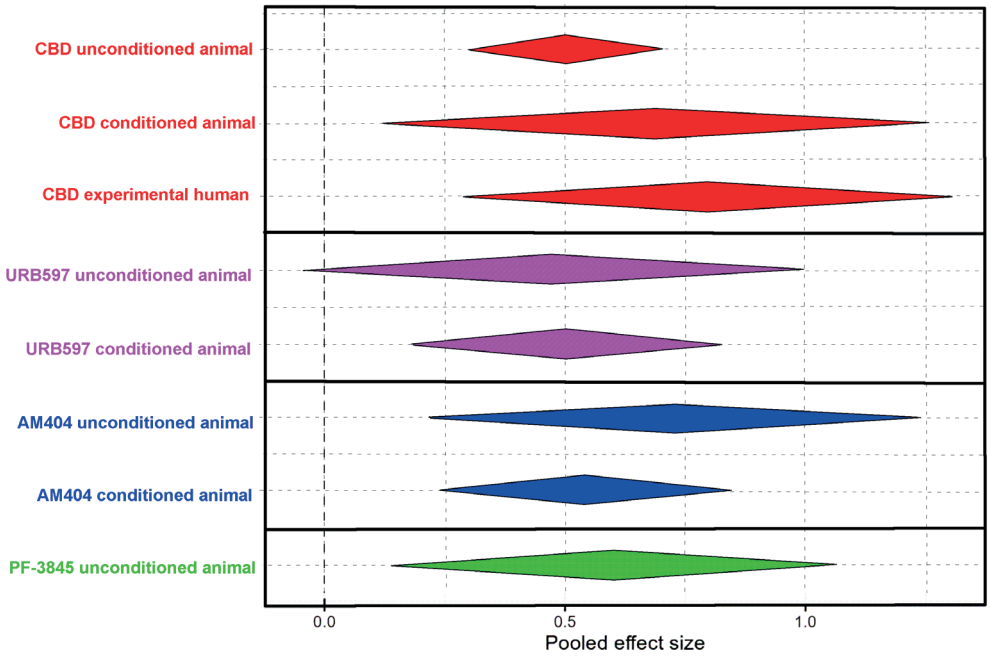


Figure 2. Pooled effects per drug for unconditioned and conditioned anxiety in animals and experimentally induced anxiety in humans.

Note: Diamonds illustrate point estimates plus 95% confidence intervals for each meta-analysis, see Table 3 for further details. Negative values indicate effects in favor of the placebo group; positive values indicate effects in favor of the experimental group that received the drug. Supplementary Figures 2-9 provide forest plots of the distributions of observed effect sizes.

Table 3. Summary of findings of anxiolytic effects of FAAH and AEA transport inhibitors

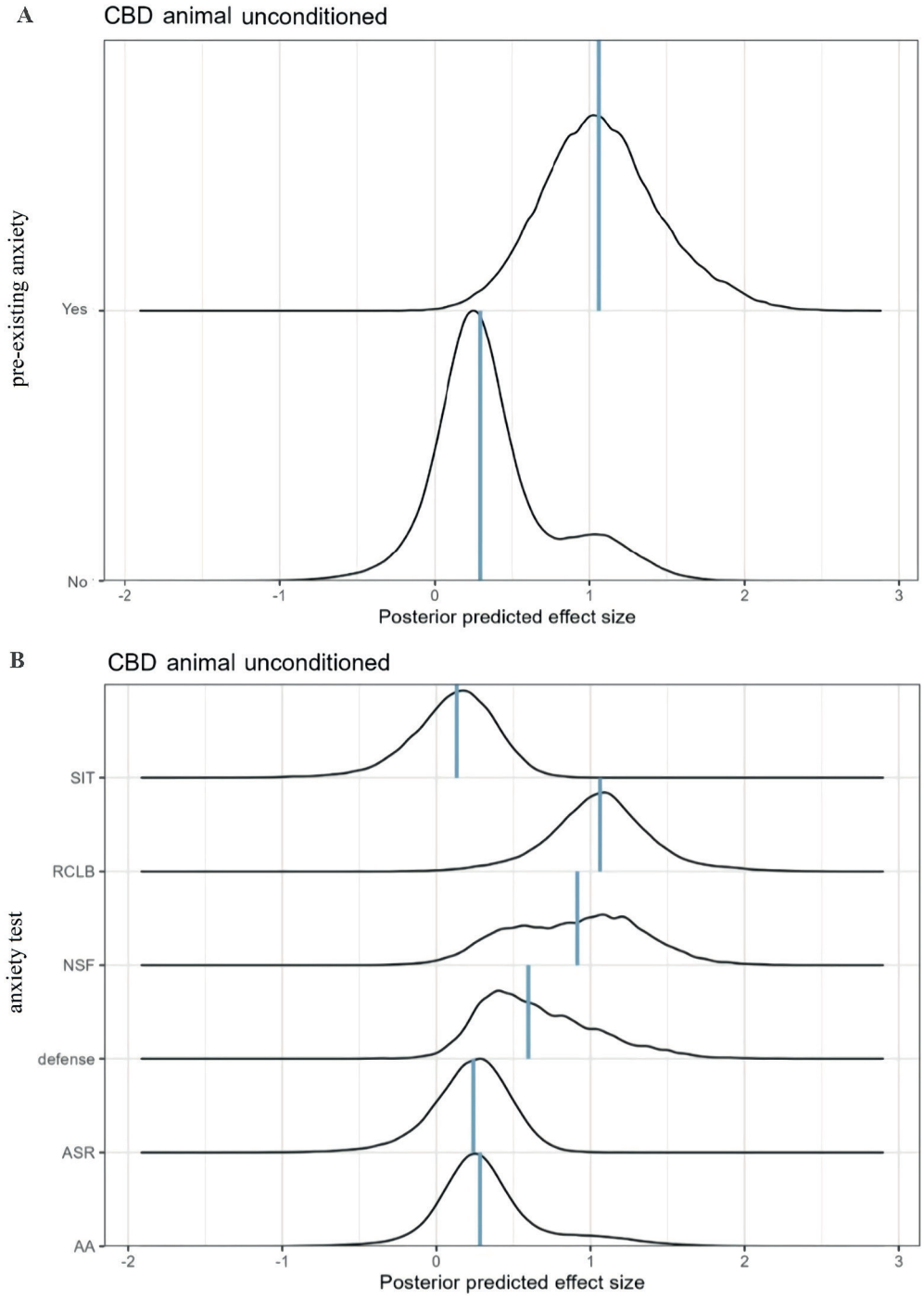
Type of anxiety	Drug	Participants (experiments)	Hedge's G [95%CI]	σ_w^2, σ_b^2 [95%CI]	I_w^2, I_b^2	Favors	QoE
Unconditioned in animals	Cannabidiol	4859 (61)	0.50 [0.29, 0.70]*	0.20 [0.12, 0.31] 0.44 [0.23, 0.82]	23.49 52.23	Cannabidiol	Low
	URB597	2153 (50)	0.47 [-0.06, 1.00]	0.12 [0.01, 0.31]* 3.31 [2.13, 5.35]	3.29 89.18	Neither	Low
	AM404	743 (12)	0.73 [0.21, 1.24]*	0.62 [0.27, 1.32]* 0.46 [0.08, 1.74]*	46.02 34.04	AM404	Low
	PF-3845	726.5 (7)	0.60 [0.13, 1.07]*	0.15 [0.00, 0.47]* 0.28 [0.01, 1.57]*	23.80 45.30	PF-3845	Low
Conditioned in animals	Cannabidiol	1125 (16)	0.68 [0.11, 1.26]*	<0.01 [<0.01, 0.10]* 1.22 [0.50, 3.17]	< 0.01 83.57	Cannabidiol	Low
	URB597	787 (13)	0.50 [0.17, 0.83]*	0.01 [0.00, 0.23]* 0.23 [0.04, 0.83]	2.46 49.31	URB597	Low
	AM404	351 (7)	0.54 [0.24, 0.85]*	0.14 [0.00, 0.66] <0.01 [<0.01, 0.70]	47.40 <0.01	AM404	Low
Experimental in humans	Cannabidiol	442 (6)	0.79 [0.28, 1.31]*	<0.01 [<0.01,0.25]* 0.28 [0.03, 2.07]	0.09 60.62	Cannabidiol	Moderate

Note: QoE: Quality of evidence; σ_w^2, σ_b^2, I_w^2, I_b^2: heterogeneity statistics.
* p .05.

2.3.2.2 Planned moderator analyses

Moderator analyses with theoretically relevant moderators were conducted to identify sources of heterogeneity of drug effects and to generate hypotheses on which circumstances and for whom the tested drugs could be beneficial. Supplemental Table 9 presents the applicable moderators per meta-analysis. Relevant predictors selected with BRMA are listed in the Supplemental Tables 10, 12, 14, 15, 17, 19, 21, 23. In the text below, only moderator effects whose 95% credible interval excluded zero are discussed. This interval contains the population effect size with 95% probability and is the Bayesian counterpart of statistical significance.

Publication year, presence or absence of a pre-existing anxiety condition, and anxiety test moderated CBD effects on unconditioned anxiety. Effects of CBD were larger in the presence of pre-existing anxiety (Fig. 3, panel A) and in tests of repetitive compulsive-like behavior (RCLB) than in approach avoidance tests (Fig. 3, panel B). Conversely, the effects of CBD were smaller in more recent compared to older publications. In URB597, anxiety test moderated drug effects on unconditioned anxiety. The social interaction test was associated with smaller anxiolytic effects compared to approach avoidance tests (Fig. 3, panel C).


A
CBD animal unconditioned
pre-existing anxiety
Yes
No
-2
-1
0
1
2
3
Posterior predicted effect size
B
CBD animal unconditioned
anxiety test
SIT
RCLB
NSF
defense
ASR
AA
-2
-1
0
1
2
3
Posterior predicted effect size

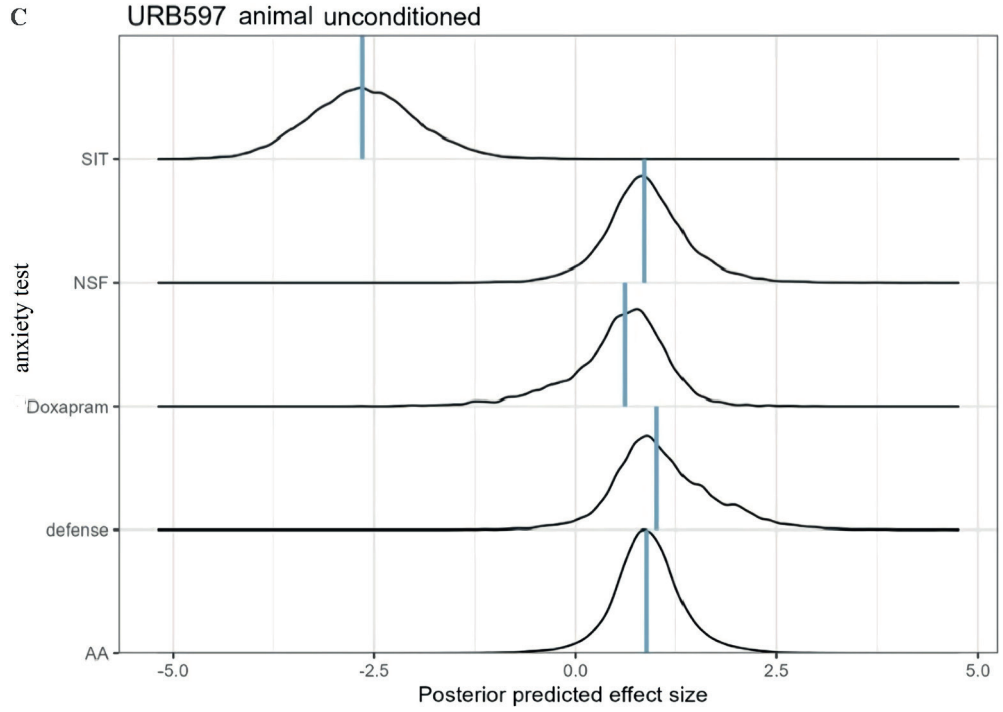


Figure 3. Plots of posterior predictive distributions of effect sizes for the levels of categorical moderator variables whose 95% credible interval excluded zero.

Note: Break-down is presented of the different levels of moderators of unconditioned anxiety in animals: pre-existing anxiety condition (Panel A, CBD) and anxiety test (Panel B, CBD; Panel C, URB597). Blue lines represent median effect sizes. CBD: Cannabidiol; SIT : social interaction test; RCLB: repetitive compulsive-like behavior; NSF: novelty suppressed feeding; ASR: acoustic startle response; AA: approach avoidance. Please note that all anxiety tests investigated per drug are plotted.

2.3.2.3 Quality of evidence

Assessments of the quality of evidence using the GRADE approach (Schünemann and Santesso, 2010) are summarized in Supplemental Table 25. Risk of bias assessments for anxiety outcomes for individual studies are provided in Supplemental Fig. 10. Our ratings of quality of the body of evidence were low for all combinations of drug (CBD, URB597, AM404, PF-3845) in unconditioned and conditioned anxiety in animals and experimentally induced anxiety in humans. Quality of evidence was impacted negatively by:

1) **Unclear to high risk of bias for reported effects**. Risk of bias was considered serious across all animal studies due to underreporting of this information, and competing financial interests. Risk of bias was also considered serious for the effect of CBD on experimentally induced

anxiety in humans, as 3 out of 6 studies were assessed as high risk of bias because of 1) increased mental sedation in the CBD condition and, as a potential consequence, unsuccessful blinding (Crippa et al., 2004); 2) highly variable CBD plasma concentrations (4.7 (7) and 17 (29) ng/mL 1 and 2 h after administration (Fusar-Poli et al., 2009), that led to concerns about failures in implementing the intervention; 3) unclear bias due to missing outcome data and concerns about selective outcome reporting (Zuardi et al., 1993).

2) **Publication bias** which was (very) strongly suspected for all drugs and types of anxiety. Visual inspection of funnel plots (see Supplemental Fig.s 11-18) and significant results (ps $\leq$.02) on Egger's test for funnel plot asymmetry indicated an overrepresentation of publications with large and beneficial compared to smaller or adverse drug effects across smaller studies, relative to a more balanced mix of findings across larger studies (Peters et al., 2008);

3) **Significant unexplained heterogeneity.** High heterogeneity in our included animal studies renders interpretation of an overall effect rather difficult (Vesterinen et al., 2014);

4) **Indirect evidence** by the use of healthy subjects and no pre-existing anxiety in most preclinical studies, which may lower the level of face and predictive validity (Bach, 2022; Sams-Dodd, 2006), the use of conventional rather than ethological measures of anxiety (e.g., Carobrez and Bertoglio, 2005), and test conditions that were not always optimized to measure anxiolytic effects (Seillier and Giuffrida, 2017);

5) **Imprecision** of URB597 effects on unconditioned anxiety, indicated by a large range of drug effects, from anxiolytic to anxiety increasing;

The moderate to large overall effect sizes, despite the fact that within many studies (52%) different drug doses were tested, led to quality of evidence upgrades.

2.3.2.4 Sensitivity analyses

The robustness of the findings regarding our primary research aim was evaluated in sensitivity analyses (see Supplemental Table 26 for excluded effects). Results of the sensitivity analyses are available in supplementary online material (doi:10.5281/zenodo.7829148). After excluding studies with a high risk of bias, the pooled effect of CBD on human experimentally induced anxiety became smaller and non-significant, Hedge's g [95% CI] = 0.50 [-0.05, 1.05], p = 0.07. The pooled effect of URB597 on unconditioned anxiety became significant, Hedge's g [95% CI] = 0.55 [0.11, 1.00], p = 0.01, but direction and magnitude of the effect were unaltered. For the other compounds and types of anxiety, direction, magnitude, and significance of pooled effects remained unchanged in the sensitivity analyses. The moderators identified as having a non-zero effect with BRMA in the planned moderator analyses (Section 3.2.2) remained the same in the sensitivity analyses. This indicates that the meta-analytic findings are largely robust to

excluding studies assessed as high risk of bias or otherwise strongly affecting the overall results.

2.3.2.5 Exploratory moderator analyses

Exploratory moderator analyses were planned with a subset of theoretically most important study characteristics: anxiety test, drug dose (human equivalent dose (HED)*60 across drugs in included studies ranged between 0.05 and 900 mg) and type of outcome for tests of conditioned anxiety. See Supplemental Tables 27, 29, 31, 33, 35, 37, 39, 41 for all selected predictors with BRMA. Interaction effects between anxiety test and type of outcome, and dose and dose^2 (or HED and HED^2 for animal studies) were included in these models to explore dose-response relationships. The moderator analyses showed that tests of repetitive-compulsive behavior were associated with larger CBD effects and the social interaction test was associated with smaller URB597 effects compared to approach avoidance tests. Further, only effects of AM404 in tests of repetitive compulsive-like behavior were dependent on dose. Within the range of tested doses (HED 0.0081-1.62), higher HED was associated with larger drug effects (Supplemental Fig. 19).

2.3.3 Safety and tolerability of FAAH hydrolysis and AEA transport inhibitors

Harm-related information was a secondary outcome, and our literature search did not include terms related to safety and tolerability. Our qualitative summary of harm-related information from the included studies with harm-related objectives (n=17) is therefore non-systematic.

2.3.3.1 Safety and tolerability of CBD

Included studies employing CBD, in which side effects were either noted when mentioned spontaneously by human participants (Masataka, 2019) or were monitored as part of the study in humans (Fusar-Poli et al., 2009) or dogs (Morris et al., 2020), reported no significant adverse events. Self-rating of subjective states yielded no particularities (Crippa et al., 2004, 2011; Fusar-Poli et al., 2009) , except from increased mental sedation in healthy individuals with 400 mg CBD, 60 and 75 min after oral drug intake (Crippa et al., 2004), that was not observed in patients with social anxiety disorder (Crippa et al., 2011). This is in line with previous reviews (Chesney et al., 2020; Huestis et al., 2019; Iffland and Grotenhermen, 2017; Kwee et al., 2022b).

No undesirable effects of the drug on learning and memory were observed when repeatedly administered in mice (Myers et al., 2019; Schleicher et al., 2019) and rats (Kajero et al., 2020).

Differential effects of repeated CBD administration, including no effect on motor activity in mice (Schleicher et al., 2019; Todd et al., 2017) and rats (Kajero et al., 2020) and weight gain in

rats (Kajero et al., 2020) and dogs (Morris et al., 2020) underline the difficulties of interspecies translation.

2.3.3.2 Safety and tolerability of FAAH inhibitors

Sub-chronic treatment with irreversible FAAH inhibitors PF-04457845 (Mayo et al., 2020) and JNJ-42165279 (Paulus et al., 2021) in experimental studies with healthy human volunteers, and JNJ-42165279 in a clinical trial with patients with social anxiety disorder (Schmidt et al., 2021) yielded no serious adverse events.

Doses of PF-3845 sufficient to induce an anxiolytic effects in acute (Bedse et al., 2018; Duan et al., 2017) and chronically (Duan et al., 2017) stressed mice exerted no effect on working memory (Duan et al., 2017), locomotor activity, body temperature, and tests of learning and memory (Bedse et al., 2018).

Six weeks of treatment with the irreversible FAAH inhibitor URB597 unexpectedly led to chemical alterations in the cingulate cortex in mice (Lomazzo et al., 2017). The reversible FAAH inhibitor SSR411298 elicited in mice hyperlocomotion, hypothermia, antinociception, and catalepsy at doses higher than needed to produce an anxiolytic effect (Griebel et al., 2018).

2.3.3.3 Safety and tolerability of AEA transport inhibitors

The endocannabinoid transport inhibitor WOBE437 (Chicca et al., 2017) elicited in mice a full cannabinoid tetrad response at doses higher than needed to produce an anxiolytic effect.

2.3.3.4 Risk of bias for harm-related outcomes

All studies (n=17) with information on safety and tolerability were assessed as unclear risk of bias, see Supplemental material, section 2.7 for grading per criterion. Risk of bias for individual studies and summary risk of bias assessments are displayed in Supplemental Fig. 20.

2.4 Discussion

The endocannabinoid system has gathered a lot of interest in relation to its potential role in (the alleviation of) anxiety. The potential of pharmacological enhancement of AEA levels for treating anxiety symptoms has often been discussed. However, a comprehensive systematic review and meta-analysis into the effectiveness of this strategy, potential moderators, and side effects, had not yet been conducted, which was the aim of this paper.

2.4.1 Overall drug effects

Our results showed significant anxiety reduction across drugs for conditioned and unconditioned anxiety in rats, mice and Cricetidae, and for experimentally induced anxiety in humans, with moderate to large effect sizes (Hedge's g between 0.47-0.79) and anxiety-reducing effects with all compounds (CBD, URB597, AM404, PF-3845). The only exception to these positive meta-analytic results was a lack of significant effect of the selective and irreversible FAAH inhibitor URB597 on unconditioned anxiety in animals. These findings provide broad evidence for the often discussed potential of AEA augmentation for treating symptoms of anxiety and related disorders.

2.4.2 Moderators of drug effects

We identified several moderators of drug effects on anxiety outcomes, as expected given the large diversity in study procedures. As explained in the introduction, a theoretical distinction can be made between unconditioned and conditioned anxiety. For animal studies we conducted meta-analyses for both classes of anxiety for CBD, URB597 and AM404. For PF-3845, only tests of unconditioned anxiety were available. Overall, the meta-analytic analyses demonstrated evidence of beneficial effects of CBD, AM404, and PF-3845 on unconditioned anxiety and of CBD, URB597 and AM404 on conditioned anxiety.

Moderators analyses were conducted using Bayesian regularized meta-regression (BRMA, van Lissa and van Erp, 2021, Preprint). Firstly, we found drug effects of CBD and URB597 on unconditioned anxiety to be dependent on type of anxiety test. More than half (56%) of the effects on anxiety outcomes in this meta-analysis were measured using approach avoidance tests in animals. Interestingly, approach avoidance tests yielded relatively low effect sizes, and in comparison larger beneficial effects of CBD were found in tests of repetitive compulsive-like behavior. The marble burying test is an established and often used model of repetitive behavior (Thomas et al., 2009). Attenuating effects of CBD on marble burying are not likely a consequence of sedation. Motor functioning was not affected by CBD in included studies that measured both marble burying and motor activity (Casarotto et al., 2010; Murphy et al., 2017; Nardo et al., 2014).

The dose effect-relation for AM404 on repetitive compulsive-like behavior, identified in exploratory moderator analyses, strengthens the evidence for beneficial effects of AEA enhancement for this type of behavior. However, beneficial effects of CBD and AM404 on repetitive compulsive-like behavior have mostly been demonstrated in studies using the marble burying test. Single test results have limited predictive validity for drug effects in patients. These pre-

clinical findings therefore warrant more extensive testing in other models of repetitive behavior as well as in humans.

While URB597 was anxiolytic in other anxiety tests, our moderator analysis showed that overall, it decreased time in social interaction across studies (Matricon et al., 2016; Seillier et al., 2010, 2013, 2018).An explanation for this finding may be that the social interaction test is not aversive enough to detect beneficial URB597 effects on anxiety (Bambico et al., 2016; Haller et al., 2009). Some effects in the opposite direction may result from a curvilinear relation between amygdalar AEA levels and time in social interaction (Seillier et al., 2013). That is, normal physiological AEA levels in the amygdala during the test were associated with maximum time in social interaction, and URB597 could only improve interaction time in rats with pharmacologically reduced amygdalar AEA levels. Administration of URB597 to healthy animals increased AEA levels above the optimum and led to social withdrawal (Seillier et al., 2013, 2018).

Next to type of anxiety test, a second moderator with respect to the effects of CBD on unconditioned anxiety in animals was pre-existing anxiety condition, which increased effects compared to no such condition. Anxiety conditions were generated by exposure to a single stressor (Campos et al., 2012; Rock et al., 2017; Shallcross et al., 2019), or to chronic unpredictable stressors (Campos et al., 2013; Fogaça et al., 2018). All procedures had in common that they induced anxiogenic behavior by stress, compared to control animals. From the stress literature it is known that the ECS acts mediates stress effects on behavior (for a review, see Morena et al., 2016). Further, within single studies, anxiolytic effects of inhibitors of FAAH in rats seemed to depend on the stressfulness of experimental conditions (Haller et al., 2009; Song et al., 2016).

A third moderator of CBD effects on unconditioned anxiety was publication year. Our sample was characterized by a large range in publication years (1990-2021). Effects of CBD were smaller in more recent compared to older publications. This result is in line with a phenomenon called the decline effect: over time, the number of controlled studies increases and scientifically discovered effects tend to become smaller (Schooler, 2011).

No moderator effects related to different types of outcomes in conditioned anxiety tests in animals were identified. This may partly be due to the duration of drug effects that can overlap different phases unless they are carefully separated experimentally. No other moderators of drug effects on conditioned anxiety in animals, and of the effect of CBD on experimentally induced anxiety in humans were identified, whereas significant statistical heterogeneity suggests variation in effect sizes. Some categories in the moderator analyses included only few studies, and therefore these levels of moderator variables were relatively poorly represented.

2.4.3 Quality of the evidence

Notwithstanding our positive results, the quality of the evidence was assessed as low. Importantly, publication bias was strongly or very strongly suspected across all drug types and types of anxiety. To date no procedures are yet available to estimate the extent of this bias for multilevel meta-analysis. Nevertheless, we caution that the reported pooled effect sizes likely overestimate the true effect sizes. Furthermore, our findings provide only indirect evidence of clinical efficacy, since the vast majority of included studies (95%) was conducted in non-human mammals. Given the diversity in study procedures in preclinical research (Vesterinen et al., 2014), the available body of evidence is suitable for identifying potential moderators of clinical effects, while conclusions about overall clinical efficacy are premature.

Our sensitivity analyses demonstrated lack of robustness of our findings with respect to the effect of URB597 on unconditioned anxiety in animals and of CBD on experimentally induced anxiety in humans. We excluded studies based on our assessment of bias that was, in retrospect, rather stringent. For example, concerns about blinding success given sedative effects of CBD led to a high risk of bias rating in one human study, while blinding may have been unsuccessful in other studies as well. However, this remains obscure because blinding success was rarely assessed across studies. Yet, the results of these sensitivity analyses indicate that more high quality evidence is paramount to further substantiate our findings regarding beneficial effects of AEA augmentation for treating symptoms of anxiety and related disorders (Guyatt et al., 2008).

2.4.4 Safety and tolerability

We described data from the n=17 included papers with harm-related objectives, each with unclear risk of bias for harm-related outcomes. In most of these studies no functional or behavioral side-effects were reported that could be attributed to the drugs under study. Side effects typically induced by CB1 receptor agonists were reported in two studies with drugs that were not studied enough to warrant meta-analysis (SSR411298; Griebel et al., 2018 and WOBE437; Chicca et al., 2017). In line with the overall favorable picture that emerges from previous reviews (Chesney et al., 2020; Huestis et al., 2019; Iffland and Grotenhermen, 2017; Kwee et al., 2022b), the studies we reviewed reported no severe adverse events after CBD administration. A systematic investigation of relations between drug concentrations and desirable and undesirable drug effects is needed to elucidate whether undesirable effects also occur at doses needed for anxiolytic effects. As we argue in Kwee et al. (2022b) , more studies that also include integrated pharmacokinetic and anxiety assessments are needed to answer this question for repeated CBD dosing.

2.4.5 Limitations of the review

A primary critical note concerns the assumption that the effects of the studied compounds are associated with an increase in AEA levels. Most studies have relied on single dosing, whereas available evidence with CBD suggests significant increases in AEA levels after continuous dosing during several weeks (Leweke et al., 2012). Moreover, some compounds exert additional effects next to enhancement of AEA availability. Specifically, FAAH inhibitors do not only elevate AEA levels, but also elevate levels of oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) (Bortolato et al., 2006; Fegley et al., 2005; Schmidt et al., 2021. Nevertheless, AM404, an AEA transport inhibitor that does not affect PEA and OEA levels (Bortolato et al., 2006) also exerted beneficial effects on anxiety outcomes in our meta-analysis. This strengthens the assumption that the anxiolytic effects of the drugs under study are set about via pharmacological enhancement of AEA levels. For CBD, the mechanistic route for anxiety reduction is even less clear. Although CBD is a weak inhibitor of FAAH (Bisogno et al., 2001; Mlost et al., 2020) its action may also be partly explained by its binding to intracellular AEA transporters. In fact, 76 different molecular targets of CBD were identified, including ionotropic, non-cannabinoid targets (Mlost et al., 2020).

Several methodological limitations affect the generalizability of our results. First, the number of studies in our meta-analysis did not allow testing a plethora of moderator variables. That is, although BRMA limits overfitting, generalizability can still be low if the sample of studies is small and idiosyncratic (van Lissa and van Erp, 2021, Preprint). With this in mind, the data of different types of non-human mammals were analyzed together and we only investigated main effects of species. Although the 95% credible interval of species on itself included zero in our planned moderator analyses, an interaction between species and other variables, such as dose (Kwee et al., 2022b) cannot be excluded.

Second, our findings regarding safety and tolerability of tested compounds do not result from a systematic literature search and evaluation of these parameters. For a translation of wanted and undesirable drug effects in preclinical models to substantiated and safe dose selection for clinical trials we recommend using the IB-de-risk tool (van Gerven and Cohen, 2018) see, for example Cohen et al. (2022) and Kwee et al. (2022b) for a dose response analysis of CBD. Such a structured approach for dose-rationale, as well as FAAH inhibition assays and measurement of AEA plasma concentrations (e.g., Russo et al., 2007) are required to identify what constitutes unnecessarily high and perhaps unsafe dosing.

Third, literature was searched up to May 2021 and at that time, only two clinical trials with inhibitors of FAAH and AEA transport were published. The first randomized controlled trial re-

ported a positive effect of four weeks of 300 mg CBD in social anxiety disorder (n=37; Masataka, 2019), whereas the second observed no effect of 12 weeks of JNJ-42165279 (n=134; Schmidt et al., 2021). More recent publications including two clinical trials were not included in this meta-analysis. The first entailed an open-label study in which 300 mg oral CBD plus standard care (n=61) was compared to standard care alone (n=59) in frontline health care professionals working with patients with COVID-19 (Crippa et al., 2021). In this study, CBD induced anxiolytic effects. The second double-blind clinical, trial augmentation of eight therapist-assisted exposure in vivo sessions (weekly, outpatient) with 300 mg oral CBD yielded no differences in treatment outcome over time between CBD (n = 39) and placebo (n = 41; Kwee et al., 2022a).

2.4.6 Recommendations for future work

This promising field of research has room for improvement. More systematic reporting of methods and study design can aid in interpreting each other's work and assessment of research quality. A structured approach to reporting for human research has been available in the form of the CONSORT statement (Begg et al., 1996). Standards for reporting are now also available for animal research in the ARRIVE 2.0 guidelines (Percie du Sert et al., 2020). More uniformity across anxiety tests in the parameters that are studied may aid in synthesizing findings from multiple studies. The definitions for outcomes of tests of conditioned anxiety that we established for this meta-analysis (see Supplemental Fig. 1) may help specify (reporting of) endpoints in conditioned anxiety research.

In the past two decades FAAH inhibitors have been developed at a rapid pace. These compounds have greater selectivity than the 'old' FAAH inhibitor URB597, for example with respect to off-target carboxylesterases that may limit therapeutic applicability (Clapper et al., 2009; Hill et al., 2013). Keeping in mind the serious adverse events in the BIAL phase 1 trial (Kerbrat et al., 2016) and given the divergent results with respect to safety and tolerability of FAAH inhibitors (Panlilio et al., 2016), a structured approach for dose-rationale (e.g., Cohen et al., 2022; Kwee et al., 2022b) should be employed on a drug-by-drug basis before proceeding to first in-human trials.

2.4.7 Conclusions

This systematic review and meta-analysis provides extensive evidence for the beneficial effects of FAAH inhibitors and inhibitors of AEA transport in preclinical tests of anxiety. The beneficial drug effects on conditioned anxiety are especially relevant to clinical practice, because fear conditioning paradigms model the learning that takes place during psychotherapy. Furthermore,

a pre-existing anxiety condition in animals predicted larger effects of CBD on unconditioned anxiety. It is therefore tempting to conclude from our meta-analytic results that effective application in patients is feasible. However, the quality of the evidence was low and human studies are still scarce. Therefore, definitive conclusions will have to await more high quality evidence. The analyses we present here indicate that anxiety-reducing effects of the studied compounds can be demonstrated across-the-board but may also depend on the specific facets of anxiety that are studied. They suggest that anxious animals and repetitive behavior seem most susceptible to pharmacological AEA enhancement. An increased focus on the specific aspects of stress and anxiety that are under endocannabinoid control will narrow down potential clinical applications. At the same time, investigation of drug efficacy in patients remains paramount to allow the flow of information back and forth between preclinical and clinical research.

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Conflict of interest

The authors have no conflicting interests to declare.

Part of the supplemental materials of Chapter 2 is included in this thesis. We refer to the online supplemental materials (https://doi.org/10.1016/j.euroneuro.2023.04.001) for the following supplemental figures:
Supplemental figures 2–9, which provide forest plots of the distributions of observed effect sizes (pooled effects are provided in Fig. 2);

Supplemental figure 10, which provides risk of bias assessments for anxiety outcomes for individual studies (summary plots are provided in this thesis);
Supplemental figure 20, which provides risk of bias assessments for harm-related outcomes for individual studies (a summary plot is provided in this thesis).

A reproducible repository with all analysis codes and data are available at doi:10.5281/zenodo.7829148.

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2.S Supplemental Material

Contents

2.S.1 Supplemental Methods

2.S.1.1 Protocol amendments

1. Hedge's G was calculated instead of SMD, because the former statistic includes a correction for small sample size bias (Vesterinen et al., 2014);

2. "In order to provide sufficient statistical power for sub-group analyses we will perform a meta-analysis if we meet the criterion of including per outcome at least 10 independent experiments from 6 independent papers" could not be supported by literature and was therefore changed to: "In order to allow for model identification we will perform a

meta-analysis if the number of effect sizes per outcome exceeds the number of moderator variables + 1";

3. In order to standardize dose across species, human equivalent dose (HED) was calculated by using body surface area conversion factors[2] for all animal studies. This variable was used in subgroup analyses instead of Cmax, which is only sparsely measured in effect studies;

4. We stratified the meta-analyses by drug and conditioned or unconditioned anxiety in animals/experimentally induced anxiety in humans. Drug was added in order to render (human equivalent) dose a meaningful variable in all subsets of data;

5. Given the highly variable outcome parameters for tests of conditioned anxiety, we included this predictor in the moderator analyses. The subdivision in tests in which the aversive unconditioned stimulus is either avoidable or inevitable was dropped;

6. We added species to moderator analyses in non-human mammals, because multiple species were in these subsets of data;

7. We added publication year to the moderator analyses, assuming an increased likelihood of null findings being published over the years;

8. Separate predictor variables for subgroup analyses were construed for anxiety disorder or pre-existing anxiety (present / absent) and for type of anxiety test. Because these are distinct study characteristics they are best represented as such;

9. We conducted exploratory subgroup analyses with a limited number of theoretically important predictors, given concerns about insufficient power when comprehensively investigating sources of between-study variability;

10. We omitted some sensitivity analyses:

    - Excluding repeated dosing studies with 24 h or more between last drug administration and behavioral testing (the predictor timing of administration (test of acute effect; test of delayed effect) was added to the moderator analyses).

11. And added some sensitivity analyses:

    - Excluding statistical outliers;

- Excluding studies with route of administration in animals other than intraperitoneal. For the great majority of studies in animals, drugs were administered intraperitoneally and bioavailability may differ dependent on administration route;

- Excluding the Cricetidae family of rodents, because the FDA conversion factors to calculate HED are listed per species rather than families (Center for Drug Evaluation and Research, 2015).

2.S.1.2 Decision rules for data extraction

If M and SD were not reported, we requested the corresponding author to provide this information. In case no answer was received within two months and data were presented graphically, parameters were estimated using Plot Digitizer software (`http://plotdigitizer.sourceforge.net/`). If standard errors (SEs) rather than SDs were reported, we calculated SD = SE x sqrt(n).

One effect size per outcome domain was extracted, unless assessments of anticipatory anxiety (in humans) were available next to assessments during the anxiety induction well. In that case, we also collected results for this assessment. On outcomes of conditioned freezing multiple comparisons were reported over time during re-exposure(s) to the conditioned cue or context. For single exposures to the conditioned stimulus or context we opted to report on the first and last comparison in order to differentiate between conditioned fear expression and extinction learning (Almeida et al., 2013; Watt et al., 2020a). For multiple extinction sessions as f.i. in exposure therapy (Morena et al., 2018), we reported outcomes on the last endpoint.

We used the following decision rules when multiple outcome measures were available in articles. First, outcome measures in animal studies were prioritized based on a priori defined preferred outcome measures of anxiety which were established after discussion with author LG (see for a list per outcome domain Supplemental Table 4). We opted for the most conventional outcome measures (see, for example, Rodgers and Dalvi, 1997), to avoid unnecessary heterogeneity. If the preferred outcome measures were not indicated by the authors of a paper, we selected the most frequently reported outcome measure across studies employing the same anxiety test. If this outcome measure was not reported either, we selected the second most frequently reported outcome measure across studies employing the same anxiety test, et cetera. For clinical studies, we selected results for the primary endpoint as predefined by the authors.

2.S.1.3 Data collection process

The most important issue on which consensus needed to be reached entailed classifying drug effects as delayed versus acute. This was a discussion point for study designs aimed at measuring delayed drug effects, (e.g., drug administration in the evening, testing in the morning), but with less than 24 h between drug administration and testing (our predefined criterium for delayed drug effects). Timing in these studies was largely different from studies into acute drug effects, that mostly tested for effects at 1h after drug administration. We therefore decided to adapt our predefined criterium to better fit the employed procedures in included studies. We classified all effects in which testing took place the day after drug administration as delayed drug effects.

2.S.1.4 GRADE criteria

1. Risk of bias

   Included studies were assessed independently by two authors (CK and NL or one of the collaborators on the project) using version 2 of the Cochrane risk of bias tool (RoB 2.0; Sterne et al., 2019) for human studies. We used the Systematic Review Centre for Laboratory animal Experimentation's risk of bias tool (SYRCLE's RoB tool), an adapted version of the Cochrane RoB tool for animal intervention studies with a control group that takes into account specific aspects of bias that play a role in these studies (Hooijmans et al., 2014). Our assessment of bias in studies with harm-related objectives was based on the CONSORT (Consolidated Standards of Reporting Trials) extension on reporting of HARMS (Ioannidis et al., 2004). The Rob 2.0 tool has the option for judging each type of bias as 'low', 'some concerns', or 'high'. The development group for Cochrane RoB 2.0 recommends that a result should be judged as high risk of bias when some concerns exist for multiple types of bias at the same time (Higgins et al., 2019). We opted for the term 'unclear' rather than 'some concerns' or 'high concerns' for the risk of bias assessments for animal intervention studies and harm-related outcomes, because the elements that are considered to be important for internal validity and for judging bias for harm-related outcomes in animal studies do not have a strong empirical basis (Vollert et al., 2020);

2. Publication bias

   Publication bias was assessed by visual inspection of funnel plots to estimate whether small studies with small effect sizes are missing (Supplemental Figures 11-18; Peters et al., 2008) and by the Egger's test for degree of funnel plot asymmetry (Egger et al., 1997). Although no adequate test for assessing potential publication bias in multilevel meta-

analysis exists as of yet, this provide at least an indication;

3. Inconsistency of the evidence

   Inconsistency of the evidence was assessed by considering residual heterogeneity estimates in moderator analyses;

4. Indirectness of the evidence

   Indirectness of the evidence was assessed by considering the use of subjective outcome measures, disordered or at risk individuals, and ecological validity of anxiety assessments;

5. Imprecision

   Imprecision was suggested by the presence of both positive (beneficial) and negative drug effects;

   Other factors considered to assess the evidence quality were size of pooled effects and significant pooled effects despite testing across a wide range of doses.

2.S.2 Supplemental Results

Supplemental Table 1. PRISMA 2020 for Abstracts Checklist

Section and Topic	Item #	Checklist item	Reported (Yes/No)
TITLE			
Title	1	Identify the report as a systematic review.	Yes
BACKGROUND			
Objectives	2	Provide an explicit statement of the main objective(s) or question(s) the review addresses.	Yes
METHODS			
Eligibility criteria	3	Specify the inclusion and exclusion criteria for the review.	Yes
Information sources	4	Specify the information sources (e.g. databases, registers) used to identify studies and the date when each was last searched.	Yes
Risk of bias	5	Specify the methods used to assess risk of bias in the included studies.	Yes
Synthesis of results	6	Specify the methods used to present and synthesise results.	Yes
RESULTS			
Included studies	7	Give the total number of included studies and participants and summarise relevant characteristics of studies.	Yes
Synthesis of results	8	Present results for main outcomes, preferably indicating the number of included studies and participants for each. If meta-analysis was done, report the summary estimate and confidence/credible interval. If comparing groups, indicate the direction of the effect (i.e. which group is favoured).	Yes

Supplemental Table 1. PRISMA 2020 for Abstracts Checklist

Section and Topic	Item #	Checklist item	Reported (Yes/No)
DISCUSSION			
Limitations of evidence	9	Provide a brief summary of the limitations of the evidence included in the review (e.g. study risk of bias, inconsistency and imprecision).	Yes
Interpretation	10	Provide a general interpretation of the results and important implications.	Yes
OTHER			
Funding	11	Specify the primary source of funding for the review.	Yes
Registration	12	Provide the register name and registration number.	Yes

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

Supplemental Table 2. PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Reported (Yes/No)
TITLE			
Title	1	Identify the report as a systematic review.	p. 33
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	p. 34
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	p. 35
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	p. 36
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	p. 38
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	p.37
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Supplemental table 3
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	p. 40

Supplemental Table 2. PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Reported (Yes/No)
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	p. 40
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	p. 40 , Supplemental material, section 2.2
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	p. 40 , Supplemental material, section 2.2-2.3
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	p. 42 , Supplemental material, section 2.4
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	p. 40
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	NA
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	p. 40 , Supplemental material, section 2.2

Supplemental Table 2. PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Reported (Yes/No)
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	p. 41
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	p. 41
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	p. 41
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	p. 41 , Supplemental table 26
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	p. 42 , Supplemental material, section 2.4
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	p. 42 , Supplemental material, section 2.4
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Figure 1
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Figure 1
Study characteristics	17	Cite each included study and present its characteristics.	p. 45 , Supplemental Table 5-8
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	p. 50 , Supplemental Figure 10, Supplemental Figure 20

Supplemental Table 2. PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Reported (Yes/No)
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Supplemental Figures 2-9
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	Supplemental Table 7-8, p. 50 , 53
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	p. 47
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	p. 48 , 52
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	p. 51
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	p. 50
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	p. 50, Supplemental Table 25
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	p. 53
	23b	Discuss any limitations of the evidence included in the review.	p. 56
	23c	Discuss any limitations of the review processes used.	p. 57
	23d	Discuss implications of the results for practice, policy, and future research.	p. 58
OTHER INFORMATION			

Supplemental Table 2. PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Reported (Yes/No)
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	p. 37
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	p. 37
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	Supplemental material, section 2.1
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	p. 59
Competing interests	26	Declare any competing interests of review authors.	p. 59
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	doi:10.5281/zenodo.7829148

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

Supplemental Table 3.

Pubmed

1	"cbd" [All Fields]
2	"cannabidiol"[MeSH Terms] OR "cannabidiol"[All Fields] OR "cannabidiolic" [All Fields]
3	"cannabinoids"[MeSH Terms] OR "cannabinoids"[All Fields] OR "cannabinoid"[All Fields]
4	"FAAH"[All Fields]
5	"Fatty Acid Amide Hydrolase"[All Fields]
6	"anandamide"[Supplementary Concept] OR "anandamide"[All Fields] OR "anandamide s"[All Fields] OR "anandamides"[All Fields]
7	#1 OR #2 OR #3 OR #4 OR #5 OR #6
8	"fear"[MeSH Terms] OR "fear"[All Fields]
9	"phob*" [All Fields]
10	"anxi*" [All Fields]
11	"defens*"
12	#8 OR #9 OR #10 OR #11
13	#7 AND #12

Embase

1	cbd
2	cannabidiol'/exp OR 'cannabidiol'
3	cannabinoid'/exp OR 'cannabinoid'
4	'epidiolex'/exp OR epidiolex
5	faah
6	fatty acid amide hydrolase'/exp OR 'fatty acid amide hydrolase'
7	anandamide'/exp OR 'anandamide'
8	#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7
9	fear'/exp OR 'fear'
10	phob*
11	anxi*
12	defens*
13	#9 OR #10 OR #11 OR #12
14	#8 AND #13
15	case report'/de OR 'conference abstract'/de OR 'review'/de OR 'cross sectional study'/de
16	#14 AND 'case report'/de
17	#14 AND 'conference abstract'/de
18	#14 AND 'review'/de
19	#14 AND 'cross sectional study'/de
16	#14 NOT #15

Preclinical and clinical study registries
The search query (CBD OR cannabidiol OR cannabinoid OR FAAH OR "Fatty Acid Amide Hydrolase" OR anandamide) AND (fear OR phobic OR phobia OR anxiety OR anxious OR defense OR defensive) was used, if needed broken down to single search terms to match database specific search fields.

Supplemental Table 4.

Domain	Outcome	Anxiety test	Preferred outcome measure
Subjective experience	*State anxiety*	SPECT scanning	
		Simulated public speaking	Visual Analogue Mood Scale - Anxiety factor (items calm–excited, relaxed–tense, and tranquil–troubled)
	Social-evaluative anxiety	NA	Primary outcome measure as defined by authors (Fear of Negative Evaluation questionnaire; Liebowitz Social Anxiety Scale)
	Within-session fear extinction	Cued fear conditioning	Anxiety ratings CS+
Behavior	*Repetitive, compulsive-like behaviors (Angoa-Pérez, 2013; Moreno & Flores, 2012; Thomas, 2009)*		
	- Defensive burying	Marble burying test	Number of buried marbles
	- Polydipsia	Schedule-induced polydipsia	Consumed water volume
	- Nestlet shredding	Nestlet shredding test	Percentage of Nestlet shredded
	Within-session fear extinction	Contextual/cued fear conditioning	(percentage of) time freezing
	Extinction retention	Contextual/cued fear conditioning	(percentage of) time freezing
	Fear memory reconsolidation	Contextual fear conditioning	(percentage of) time freezing
	Extinction consolidation	Contextual fear conditioning	(percentage of) time freezing
	Extinction recall	Cued fear conditioning	(percentage of) time freezing

Supplemental Table 4.

Domain	Outcome	Anxiety test	Preferred outcome measure
	Fear during CS reexposure	Contextual/cued fear conditioning	(percentage of) time freezing
	Conditioned fear expression	Contextual/cued fear conditioning	(percentage of) time freezing
	Retention of safety learning	Inhibitory avoidance task	(percentage of) time freezing
	Approach behaviour during approach/ avoidance conflict (unconditioned approach behaviour and elicited aversion; Dulawa & Hen, 2005; Carobrez & Bertoglio, 2005; Prut & Belzung, 2003)		
	- Exploration of environment	Elevated plus maze	First choice: proportion/percentage of open arm entries Second choice: proportion/percentage of open arm time or distance traveled in open arms
		Elevated zero maze	Proportion/percentage of time in open quadrants

Supplemental Table 4.

Domain	Outcome	Anxiety test	Preferred outcome measure
		Light dark test	First choice: proportion/percentage of entries in light compartment Second choice: proportion/percentage of open arm time or distance traveled in light compartment
		Open field test, actitrack	(Percentage) of time in centre of arena, Percentage of distance traveled in centre of arena
		Novel object exploration test	Time spent near a novel object
	- Food consumption	Novelty suppressed feeding test	Latency to feed
	Suppression of punished responses		
	- Water consumption	Vogel conflict test	Number of punished licks
	Panic-like behaviour		
	- Defensive immobility (immobility followed by autonomic reactions, such as defecation, exophthalmia and/or micturition)	Prey versus predator paradigm	Frequency of defensive immobility outside the burrow proportional to time spent outside the burrow
	- Flight	Rat avoidance test	Number of avoidances

Supplemental Table 4.

Domain	Outcome	Anxiety test	Preferred outcome measure
	Social interaction	Social interaction test	First choice: Time in active social interaction Second choice: Total time in social interaction
	Tail posture	Exposure to noise (3 min 90 dB)	Time tail stiff
	Defecations	Open field test	Number of fecal boluses
Autonomic	*Sympathetic nervous system activation (Joyner et al., 2008; Kim et al., 2018; Rosebrock et al., 2017)*		
	- Heart rate	Exposure to noise (3 min 90 dB)	Heart rate
		Fear memory expression	
		Simulated public speaking	
	- Blood pressure	Simulated public speaking	Arterial systolic and/or diastolic pressure
	- Electrodermal activity	Simulated public speaking	Skin conductance level, fluctuations
		fMRI paradigm: Viewing fearful faces with different intensity of fearfulness	Skin conductance level, fluctuations, fluctuation latency
	- Respiratory frequency	Doxapram	Respiratory frequency
Neurophysiological	*Startle reflex during repeated exposure to sudden noise bursts*	Repeated exposure to sudden noise bursts (120-125 dB); multiple trial blocks	First choice: Habituation of startle reflex

Supplemental Table 4.

Domain	Outcome	Anxiety test	Preferred outcome measure
		Repeated exposure to sudden noise bursts (115-120 dB)	Second choice: Startle reflex
	Within-session fear extinction	Cued fear conditioning	Change in startle response from fear acquisition to test
	Extinction retention	Cued fear conditioning	Change in startle response from fear acquisition to test

Legend

ACC	anterior cingulate cortex
fMRI	functional magnetic resonance imaging
NA	not available
SPECT	single photon emission computed tomography

2.S.2.1 Included studies

Supplemental Table 5. Included studies

Author	Year	Title
Ahmad	2020	Kaempferol Facilitated Extinction Learning in Contextual Fear Conditioned Rats via Inhibition of Fatty-Acid Amide Hydrolase
Almeida	2013	Cannabidiol exhibits anxiolytic but not antipsychotic property evaluated in the social interaction test
Aso	2019	Adenosine A2A-Cannabinoid CB1 Receptor Heteromers in the Hippocampus: Cannabidiol Blunts Δ9-Tetrahydrocannabinol-Induced Cognitive Impairment
Assareh	2020	Cannabidiol disrupts conditioned fear expression and cannabidiolic acid reduces trauma-induced anxiety-related behaviour in mice
Bambico	2016	The fatty acid amide hydrolase inhibitor URB597 modulates serotonin-dependent emotional behaviour, and serotonin1A and serotonin2A/C activity in the hippocampus
Batista	2021	Intravenous doxapram administration as a potential model of panic attacks in rats
Bedse	2018	Therapeutic endocannabinoid augmentation for mood and anxiety disorders: comparative profiling of FAAH, MAGL and dual inhibitors
Bergamaschi	2011	Cannabidiol reduces the anxiety induced by simulated public speaking in treatment-naive social phobia patients
Bis-Humbert	2020	Decreased sensitivity in adolescent versus adult rats to the antidepressant-like effects of cannabidiol
Bortolato	2006	Anxiolytic-Like Properties of the Anandamide Transport Inhibitor AM404
Braida	2007	5-HT1A receptors are involved in the anxiolytic effect of Δ9-tetrahydrocannabinol and AM 404, the anandamide transport inhibitor, in Sprague-Dawley rats
Breuer	2016	Fluorinated Cannabidiol Derivatives: Enhancement of Activity in Mice Models Predictive of Anxiolytic, Antidepressant and Antipsychotic Effects
Busquets-Garcia	2011	Differential Role of Anandamide and 2-Arachidonoylglycerol in Memory and Anxiety-like Responses
Butler	2011	Fear-induced suppression of nociceptive behaviour and activation of Akt signalling in the rat periaqueductal grey: role of fatty acid amide hydrolase

Supplemental Table 5. Included studies

Author	Year	Title
Campos	2012	Cannabidiol blocks long-lasting behavioral consequences of predator threat stress: Possible involvement of 5HT1A receptors
Campos	2013	The anxiolytic effect of cannabidiol on chronically stressed mice depends on hippocampal neurogenesis: Involvement of the endocannabinoid system
Carnevali*	2015	Cardioprotective effects of fatty acid amide hydrolase inhibitor URB694, in a rodent model of trait anxiety
Casarotto	2010	Cannabidiol inhibitory effect on marble burying behaviour: involvement of CB1 receptors
Cheng	2014	Chronic cannabidiol treatment improves social and object recognition in double transgenic APPswe/PS1ΔE9 mice
Cheng	2014	Long-Term Cannabidiol Treatment Prevents the Development of Social Recognition Memory Deficits in Alzheimer's Disease Transgenic Mice
Chhatwal	2004	Enhancing Cannabinoid Neurotransmission Augments the Extinction of Conditioned Fear
Chicca*	2017	Chemical probes to potently and selectively inhibit endocannabinoid cellular reuptake
Coles	2020	Medium-Dose Chronic Cannabidiol Treatment Reverses Object Recognition Memory Deficits of APPSwe/PS1ΔE9 Transgenic Female Mice
Crippa	2011	Neural basis of anxiolytic effects of cannabidiol (CBD) in generalized social anxiety disorder: a preliminary report
Crippa	2004	Effects of Cannabidiol (CBD) on Regional Cerebral Blood Flow
Danandeh	2018	Effects of fatty acid amide hydrolase inhibitor URB597 in a rat model of trauma-induced long-term anxiety
Deiana	2011	Plasma and brain pharmacokinetic profile of cannabidiol (CBD), cannabidivarine (CBDV), Δ9-tetrahydrocannabivarin (THCV) and cannabigerol (CBG) in rats and mice following oral and intraperitoneal administration and CBD action on obsessive-compulsive behaviour
Duan	2017	Fatty acid amide hydrolase inhibitors produce rapid anti- anxiety responses through amygdala long-term depression in male rodents
ElBatsh	2012	Anxiogenic-like effects of chronic cannabidiol administration in rats
Ferizovic	2020	The fatty acid amide hydrolase inhibitor URB597 modulates splenic catecholamines in chronically stressed female and male rats

Supplemental Table 5. Included studies

Author	Year	Title
Fidelman	2018	Chronic treatment with URB597 ameliorates post-stress symptoms in a rat model of PTSD
Florensa-Zanuy	2021	Cannabidiol antidepressant-like effect in the lipopolysaccharide model in mice: Modulation of inflammatory pathways
Fogaça	2018	The anxiolytic effects of cannabidiol in chronically stressed mice are mediated by the endocannabinoid system: Role of neurogenesis and dendritic remodeling
Fusar-Poli	2009	Distinct Effects of Δ9-tetrahydrocannabinol and Cannabidiol on Neural Activation During Emotional Processing
Gáll	2020	Effects of Chronic Cannabidiol Treatment in the Rat Chronic Unpredictable Mild Stress Model of Depression
Gattinoni*	2010	Enol Carbamates as Inhibitors of Fatty Acid Amide Hydrolase (FAAH) Endowed with High Selectivity for FAAH over the Other Targets of the Endocannabinoid System
Gobira	2017	N-arachidonoyl-serotonin, a dual FAAH and TRPV1 blocker, inhibits the retrieval of contextual fear memory: Role of the cannabinoid CB1 receptor in the dorsal hippocampus
Gazarini	2014	PTSD-like memory generated through enhanced noradrenergic activity is mitigated by a dual step pharmacological intervention targeting its reconsolidation
Griebel*	2018	The selective reversible FAAH inhibitor, SSR411298, restores the development of maladaptive behaviors to acute and chronic stress in rodents
Guimarães	1990	Antianxiety effect of cannabidiol in the elevated plus-maze
Guimarães	1994	Anxiolytic effect of cannabidiol derivatives in the elevated plus-maze
Gunduz-Cinar*	2013	Convergent translational evidence of a role for anandamide in amygdala-mediated fear extinction, threat processing and stress-reactivity.
Gunduz-Cinar*	2016	Fluoxetine Facilitates Fear Extinction Through Amygdala Endocannabinoids.
Haller	2009	Interactions between environmental aversiveness and the anxiolytic effects of enhanced cannabinoid signaling by FAAH inhibition in rats
Heinz	2017	Enhanced anandamide signaling reduces flight behavior elicited by an approaching robo-beetle

Supplemental Table 5. Included studies

Author	Year	Title
Hermanson	2013	Substrate-selective COX-2 inhibition decreases anxiety via endocannabinoid activation
Hill*	2013	Disruption of fatty acid amide hydrolase activity prevents the effects of chronic stress on anxiety and amygdalar microstructure
Hill	2006	Endocannabinoids modulate stress-induced suppression of hippocampal cell proliferation and activation of defensive behaviours
Jankovic	2020	Inhibition of the fatty acid amide hydrolase changes behaviors and brain catecholamines in a sex-specific manner in rats exposed to chronic unpredictable stress
Jurkus	2016	Cannabidiol Regulation of Learned Fear: Implications for Treating Anxiety-Related Disorders
Kajero	2020	Investigation of the effects of cannabidiol on vacuous chewing movements, locomotion, oxidative stress and blood glucose in rats treated with oral haloperidol
Kasten	2019	Acute Cannabinoids Produce Robust Anxiety-Like and Locomotor Effects in Mice, but Long-Term Consequences Are Age- and Sex Dependent
Kathuria	2003	Modulation of anxiety through blockade of anandamide hydrolysis
Kinsey	2011	Inhibition of endocannabinoid catabolic enzymes elicits anxiolytic-like effects in the marble burying assay
Komaki	2015	Study the effect of endocannabinoid system on rat behavior in elevated plus-maze
Lemos	2010	Involvement of the prelimbic prefrontal cortex on cannabidiol-induced attenuation of contextual conditioned fear in rats
Lisboa	2015	Increased Contextual Fear Conditioning in Inos Knockout Mice: Additional Evidence for the Involvement of Nitric Oxide in Stress Related Disorders and Contribution of the Endocannabinoid System
Llorente-Berzal	2015	2-AG promotes the expression of conditioned fear via cannabinoid receptor type 1 on GABAergic neurons
Lomazzo	2017	Chronic stress leads to epigenetic dysregulation in the neuropeptide-Y and cannabinoid CB1 receptor genes in the mouse cingulate cortex
Long	2010	Behavioural comparison of acute and chronic Δ9-tetrahydrocannabinol and cannabidiol in C57BL/6JArc mice

Supplemental Table 5. Included studies

Author	Year	Title
Long	2012	Distinct Neurobehavioural Effects of Cannabidiol in Transmembrane Domain Neuregulin 1 Mutant Mice
Luján	2020	The pharmacological reduction of hippocampal neurogenesis attenuates the protective effects of cannabidiol on cocaine voluntary intake
Luján	2018	Repeated Cannabidiol treatment reduces cocaine intake and modulates neural proliferation and CB1R expression in the mouse hippocampus
Luque-Rojas	2013	Hyperactivity induced by the dopamine D2/D3 receptor agonist quinpirole is attenuated by inhibitors of endocannabinoid degradation in mice
Mahmud	2017	Effects of an acute cannabidiol treatment on cocaine self-administration and cue-induced cocaine seeking in male rats
Malone	2009	Cannabidiol reverses the reduction in social interaction produced by low dose Δ9-tetrahydrocannabinol in rats
Manna	2015	Paracetamol potentiates the antidepressant-like and anticompulsive-like effects of fluoxetine
Marco*	2015	Potential Therapeutic Value of a Novel FAAH Inhibitor for the Treatment of Anxiety
Markey	2020	Colonization with the commensal fungus Candida albicans perturbs the gut-brain axis through dysregulation of endocannabinoid signaling
Masataka*	2019	Anxiolytic Effects of Repeated Cannabidiol Treatment in Teenagers With Social Anxiety Disorders
Martín-Gonzáles	2018	Do psychoactive drugs have a therapeutic role in compulsivity? Studies on schedule-induced polydipsia
Matricon	2016	Distinct neuronal activation patterns are associated with PCP-induced social withdrawal and its reversal by the endocannabinoid enhancing drug URB597
Mayo*	2020	Elevated Anandamide, Enhanced Recall of Fear Extinction, and Attenuated Stress Responses Following Inhibition of Fatty Acid Amide Hydrolase: A Randomized, Controlled Experimental Medicine Trial
Micale	2009	Anxiolytic Effects in Mice of a Dual Blocker of Fatty Acid Amide Hydrolase and Transient Receptor Potential Vanilloid Type-1 Channels
Micale	2017	Extinction of avoidance behavior by safety learning depends on endocannabinoid signaling in the hippocampus

Supplemental Table 5. Included studies

Author	Year	Title
Moise	2008	An endocannabinoid signaling system modulates anxiety-like behavior in male Syrian hamsters
Moreira	2008	Reduced anxiety-like behaviour induced by genetic and pharmacological inhibition of the endocannabinoid-degrading enzyme fatty acid amide hydrolase (FAAH) is mediated by CB1 receptors
Morena	2018	Enhancing Endocannabinoid Neurotransmission Augments The Efficacy of Extinction Training and Ameliorates Traumatic Stress Induced Behavioral Alterations in Rats
Morena	2021	Sex-dependent effects of endocannabinoid modulation of conditioned fear extinction in rats
Morris*	2020	Alteration of the Canine Metabolome After a 3-Week Supplementation of Cannabidiol (CBD) Containing Treats: An Exploratory Study of Healthy Animals
Murkar	2019	Cannabidiol and the Remainder of the Plant Extract Modulate the Effects of Δ9-Tetrahydrocannabinol on Fear Memory Reconsolidation
Murphy	2017	Chronic Adolescent Δ9-Tetrahydrocannabinol Treatment of Male Mice Leads to Long-Term Cognitive and Behavioral Dysfunction, Which Are Prevented by Concurrent Cannabidiol Treatment
Myers	2019	Single and combined effects of plant-derived and synthetic cannabinoids on cognition and cannabinoid-associated withdrawal signs in mice
Naderi	2008	Interaction between cannabinoid compounds and diazepam on anxiety-like behaviour of mice
Naidu	2007	Evaluation of fatty acid amide hydrolase inhibition in murine models of emotionality
Nardo	2014	Cannabidiol reverses the mCPP-induced increase in marble-burying behavior
Natividad	2017	Constitutive Increases in Amygdalar Corticotropin-Releasing Factor and Fatty Acid Amide Hydrolase Drive an Anxious Phenotype
Navarrete	2018	Cannabidiol regulates behavioural alterations and gene expression changes induced by cannabinoid withdrawal
O'Brien	2013	Effect of chronic exposure to rimonabant and phytocannabinoids on anxiety-like behavior and saccharin palatability
Onaivi	1990	Pharmacological characterization of cannabinoids in the elevated plus maze

Supplemental Table 5. Included studies

Author	Year	Title
Pamplona	2008	Short- and long-term effects of cannabinoids on the extinction of contextual fear memory in rats
Patel	2006	Pharmacological Evaluation of Cannabinoid Receptor Ligands in a Mouse Model of Anxiety: Further Evidence for an Anxiolytic Role for Endogenous Cannabinoid Signaling
Paulus*	2021	The effects of FAAH inhibition on the neural basis of anxiety-related processing in healthy male subjects: a randomized clinical trial
Pavón	2021	Selective inhibition of monoacylglycerol lipase is associated with passive coping behavior and attenuation of stress-induced dopamine release in the medial prefrontal cortex
Qin	2015	Endocannabinoid-mediated improvement on a test of aversive memory in a mouse model of fragile X syndrome
Resstel	2006	Effects of cannabidiol and diazepam on behavioral and cardiovascular responses induced by contextual conditioned fear in rats
Rock	2017	Effect of prior footshock stress and Δ9-tetrahydrocannabinol, cannabidiolic acid, and cannabidiol on anxiety-like responding in the light-dark emergence test in rats
Rutkowska	2006	Effects of cannabinoids on the anxiety-like response in mice
Saletti*	2017	Cannabidiol Affects MK-801-Induced Changes in the PPI Learned Response of Capuchin Monkeys (Sapajus spp)
Scherma	2012	The anandamide transport inhibitor AM404 reduces the rewarding effects of nicotine and nicotine-induced dopamine elevations in the nucleus accumbens shell in rats
Scherma	2008	The endogenous cannabinoid anandamide has effects on motivation and anxiety that are revealed by fatty acid amide hydrolase (FAAH) inhibition
Schiavon	2016	Influence of single and repeated cannabidiol administration on emotional behavior and markers of cell proliferation and neurogenesis in non-stressed mice
Schleicher	2019	Prolonged Cannabidiol Treatment Lacks on Detrimental Effects on Memory, Motor Performance and Anxiety in C57BL/6J Mice
Schmidt*	2021	The effects of inhibition of fatty acid amide hydrolase (FAAH) by JNJ-42165279 in social anxiety disorder: a double-blind, randomized, placebo-controlled proof-of-concept study

Supplemental Table 5. Included studies

Author	Year	Title
Segev	2018	Role of endocannabinoids in the hippocampus and amygdala in emotional memory and plasticity
Seillier	2010	Inhibition of fatty-acid amide hydrolase and CB1 receptor antagonism differentially affect behavioural responses in normal and PCP treated rats
Seillier	2011	Inhibition of fatty acid amide hydrolase modulates anxiety-like behavior in PCP-treated rats
Seillier	2018	The cannabinoid transporter inhibitor OMDM-2 reduces social interaction: Further evidence for transporter-mediated endocannabinoid release
Seillier	2013	Phencyclidine-Induced Social Withdrawal Results from Deficient Stimulation of Cannabinoid CB1 Receptors: Implications for Schizophrenia
Servadio	2016	Targeting anandamide metabolism rescues core and associated autistic-like symptoms in rats prenatally exposed to valproicacid
Shallcross	2019	The Divergent Effects of CDPPB and Cannabidiol on Fear Extinction and Anxiety in a Predator Scent Stress Model of PTSD in Rats
Shoval	2016	Prohedonic Effect of Cannabidiol in a Rat Model of Depression
Silvestri	2020	Fish Oil, Cannabidiol and the Gut Microbiota: An Investigation in a Murine Model of Colitis
Simmons	2021	Effects of systemic endocannabinoid manipulation on social and exploratory behavior in prairie voles (Microtusochrogaster)
Song	2016	Bidirectional Effects of Cannabidiol on Contextual Fear Memory Extinction
Stern	2012	On Disruption of Fear Memory by Reconsolidation Blockade: Evidence from Cannabidiol Treatment
Stern	2015	Δ9-Tetrahydrocannabinol alone and combined with cannabidiol mitigate fear memory through reconsolidation disruption
Todd	2016	Neural correlates of interactions between cannabidiol and Δ9-tetrahydrocannabinol in mice: implications for medical cannabis
Todd	2017	Interactions between cannabidiol and Δ9-THC following acute and repeated dosing: Rebound hyperactivity, sensorimotor gating and epigenetic and neuroadaptive changes in the mesolimbic pathway
Twardowschy	2013	The role of 5-HT1A receptors in the anti-aversive effects of cannabidiol on panic attack-like behaviors evoked in the presence of the wild snake Epicrates cenchria crassus (Reptilia, Boidae)

Supplemental Table 5. Included studies

Author	Year	Title
Uribe-Mariño	2012	Anti-Aversive Effects of Cannabidiol on Innate Fear-Induced Behaviors Evoked by an Ethological Model of Panic Attacks Based on a Prey vs the Wild Snake Epicrates cenchria crassus Confrontation Paradigm
Vimalanathan	2020	Endocannabinoid modulating drugs improve anxiety but not the expression of conditioned fear in a rodent model of post- traumatic stress disorder
Watt	2020	Chronic cannabidiol (CBD) treatment did not exhibit beneficial effects in 4-month-old male TAU58/2 transgenic mice
Watt	2020	Chronic Treatment with 50‚Äämg/kg Cannabidiol Improves Cognition and Moderately Reduces Aβ40 Levels in 12-Month-Old Male AβPP swe/PS1ΔE9 Transgenic Mice
Zagzoog	2020	In vitro and in vivo pharmacological activity of minor cannabinoids isolated from Cannabis sativa
Zaitone	2012	Inhibition of fatty acid amide hydrolase by URB597 attenuates the anxiolytic-like effect of acetaminophen in the mouse elevated plus-maze test
Zieba	2019	Cannabidiol (CBD) reduces anxiety-related behavior in mice via an FMRP-independent mechanism
Zuardi	1993	Effects of ipsapirone and cannabidiol on human experimental anxiety
Zuardi	2017	Inverted U-Shaped Dose-Response Curve of the Anxiolytic Effect of Cannabidiol during Public Speaking in Real Life

* Excluded from meta-analyses.

Supplemental Table 6. Ongoing and incomplete studies, Fear learning, fear expression, anxiety symptoms

Study ID	Status	Title and brief description
NCT04286594	Recruiting	A Clinical Trial of a Hemp-Derived Cannabidiol Product for Anxiety "0.5ml of sublingual CBD solution (30mg/ml) administered twice daily for six weeks in subjects with moderate or" severe anxiety in addition to their normal treatment regimen"
NCT04269252	Recruiting	CHI-907 CBD Extract and Experiences of Test Anxiety "randomized, placebo-controlled study examining the effects of CHI-907 (CBD extract) on test anxiety specifically, and state anxiety more broadly"
NCT04577612	Recruiting	A Randomized Controlled Test of the Effects of CHI-554 on Fear "to reduce fear elicited via a safe, well-established, controlled, laboratory-based carbon dioxide (CO2)-enriched air biological challenge that causes abrupt increases in bodily arousal with 150-600 mg CBD in healthy volunteers"
NCT04726475	Recruiting	Use of CBD Oil in the Treatment of Panic Attack-Related Fear "whether 300 mg cannabidiol (CBD) (vs placebo) can interfere with the reconsolidation of naturally acquired pathological interoceptive fear memory in humans with DSM-5 panic disorder or subthreshold elevated concerns about having additional panic attacks"
NCT03549819	Not yet recruiting	Cannabidiol for the Treatment of Anxiety Disorders: An 8-Week Pilot Study "In DSM-5 anxiety disorders (GAD, SAD, PD or agoraphobia), 200 mg CBD- titrated as tolerated up to a maximum 2 capsules twice daily (200 mg- 800 mg total dose), or placebo"

Supplemental Table 6. Ongoing and incomplete studies, Fear learning, fear expression, anxiety symptoms

2014-004094-17	Ongoing	Cannabidiol enhancement of exposure therapy in treatment refractory patients with phobias "To test the hypothesis that administration of cannabidiol as an augmentation step in combination with exposure therapy can strengthen treatment outcome in patients with phobic disorders (generalized social anxiety and panic disorder with agoraphobia) who do not respond satisfactorily to treatment as usual."
NCT01665573	Active, not recruiting	Cannabinoid Augmentation of Fear Response in Humans The effects of cannabinoid receptor augmentation on the facilitation of fear conditioning by PF-04457845 (vs placebo) in healthy individuals
NCT04086342	Withdrawn	CHI-902 for Treatment of Social Anxiety Disorder "This randomized doubleblind, placebo-controlled trial of CBD in adults with SAD will evaluate the efficacy, tolerability and safety of CBD oil (CHI-902) in SAD."

2.S.2.2 Outcomes across tests of conditioned anxiety

Supplemental Table 7. Type of anxiety tests used and number of effects per test included in the data-analysis.

Animal studies							
538 (100%)							
(Fear) Conditioned	Cued	Contextual					
113 (21%)	38 (7%)	75 (14%)					
Unconditioned	Approach avoidance	Acoustic startle	Defense	Repetitive compulsive-like behavior	Social interaction	Novelty suppressed feeding	Doxapram induced panic
425 (79%)	300 (56%)	25 (5%)	9 (2%)	40 (7%)	31 (6%)	17 (3%)	3 (1%)

Human studies			
Experimentally induced anxiety	Anxiety induction by SPECT	Simulated public speaking	Viewing fearful faces
19 (100%)	2 (11%)	16 (84%)	1(5%)

Note: SPECT: single-photon emission computerized tomography.

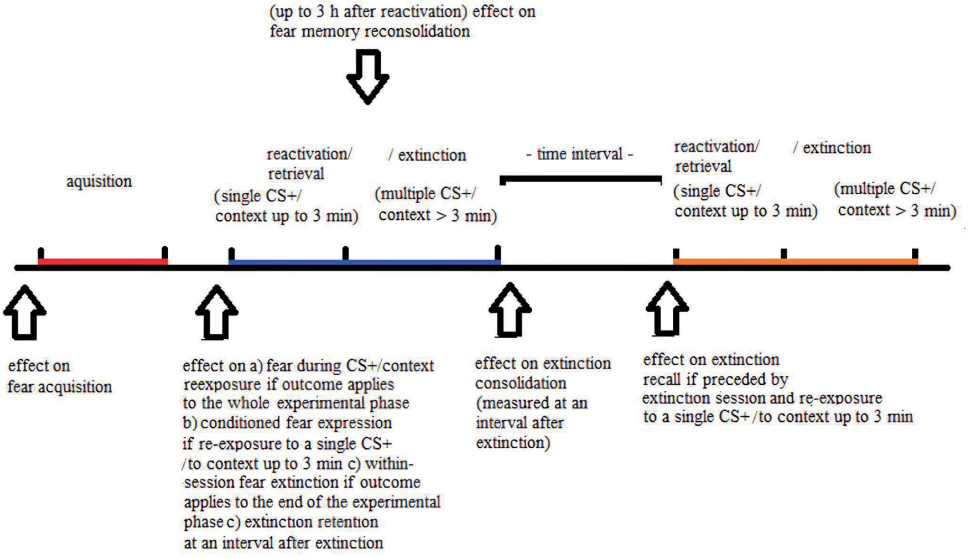
[1] Assuming a vigilant state

[2] During direct confrontation with an innate fear eliciting stimulus

Supplemental Table 8. Distribution of outcomes across tests of conditioned anxiety

Outcome	n (%)
Extinction retention	32 (28)
Fear during CS reexposure	18 (16)
Fear memory reconsolidation	20 (18)
Retention of safety learning	3 (3)
Within-session fear extinction	22 (19)
Conditioned fear expression	9 (8)
Extinction consolidation	6 (5)
Extinction recall	3 (3)
	113 (100)

Note: number of effects and percentages are given. See Supplemental Figure 1 for more detailed definition of the selected outcomes. Note that the terminology used by some study authors deviated from this categorization.



Supplemental Figure 1. Types of outcome measured in tests of conditioned anxiety along the experimental timeline. Arrows indicate time drug administration (single or multiple dose).

Note: CS+: conditioned stimulus; CS-: unconditioned stimulus. Studies into effects on fear acquisition were not included in the study selection procedure. A duration of 3 min context re-exposure for fear memory retrieval and drug administration up to 3 h thereafter for measuring effects on fear memory reconsolidation were adapted from studies into CBD effects on fear memory reconsolidation.[3,4]

2.S.2.3 Planned moderator analyses

Supplemental Table 9. Tested moderators per combination of drug type and conditioned and unconditioned anxiety in non-human mammals, and experimentally induced anxiety in humans.

	Pub year	Dose	Dose2	HED	HED2	Frequency of drug	Timing of drug	Anxiety test	Anxiety condition	Type of outcome	Sex	Species
CBD												
Unconditioned	✓			✓	✓	✓	✓	✓	✓		✓	✓
Conditioned	✓			✓	✓	✓	✓	✓		✓		✓
Experimental	✓	✓	✓					✓a	✓		✓a	
URB597												
Unconditioned	✓			✓	✓	✓	✓	✓	✓		✓	✓
Conditioned	✓			✓	✓	✓	✓	✓	✓b	✓b	✓	✓
AM404												
Unconditioned	✓			✓	✓			✓	✓		✓	✓
Conditioned	✓			✓	✓	✓		✓c		✓c		✓
PF-3845												
Unconditioned	✓			✓	✓	✓	✓	✓	✓		✓	✓

Note: Studies of conditioned and unconditioned anxiety were conducted in animals, studies of experimentally induced anxiety were conducted in humans. CBD: cannabidiol, Pub year: publication year; HED: human equivalent dose (dose corrected by allometric scaling factors for animal studies). Predictors were omitted when studies within a meta-analysis did not differ with respect to the characteristic.[a,b,c] Dummy variables were redundant because studies had identical values on multiple dummy variables. Only one of these redundant dummy variables was retained, and its name updated to represent both variables.

Below are presented, in tabular form (Table 10-24), the results of the planned moderator analyses with Bayesian regularized meta-regression (BRMA) and meta-regression analyses with the maximum likelihood approach. In meta-regression analyses with the maximum likelihood approach, similar to the BRMA analyses, publication year (larger effects for more recent publications), presence of a pre-existing anxiety condition (larger effects for pre-existing anxiety condition present vs absent) and anxiety test (larger effects for tests of repetitive compulsive-like behavior compared to approach avoidance tests) moderated CBD effects on unconditioned anxiety. In addition to the significant moderators with the BRMA analyses, meta-regression with the maximum likelihood approach suggested moderation of CBD effects on unconditioned anxiety by HED^2. This suggests the existence of a non-linear dose-response association.

Results of meta-regression analyses with the maximum likelihood approach differed from BRMA results in two instances: Type of outcome moderated the effect of AM404 on conditioned anxiety, such that a larger effect of AM404 was observed for within-session extinction compared to extinction retention. Further, frequency of drug administration moderated the effect of PF-3845 on unconditioned anxiety, such that (sub)chronic dosing regimens were associated with a smaller effect compared to single dosing regimens. In the BRMA analyses, these

moderators were not significant.

2.S.2.3.1 Planned moderator analyses for anxiolytic effects of CBD

Supplemental Table 10. Most important moderators of CBD effects on unconditioned anxiety in animals selected with Bayesian regularized meta-regression.

Predictor	Effect [95% CI]
Intercept	57.64 [3.66, 110.61]*
Pubyear	-0.03 [-0.05, <-0.01]*
HED	0.03 [-0.02, 0.10]
HED^2	-0.01 [-0.03, <0.01]
Frequency_MD	-0.66 [-2.02, 0.11]
Timing_delay	0.14 [-0.09, 0.45]
Female	-0.24 [-0.88, 0.19]
Male + female	-0.10 [-0.77, 0.42]
Disease_Yes	0.67 [0.12, 1.18]*
Acoustic startle	-10 [-0.38, 0.11]
Defense	0.31 [-0.21, 1.19]
Novelty suppressed feeding	0.03 [-0.43, 0.52]
Repetitive compulsive-like behavior	0.69 [0.24, 1.11]*
Social interaction test	-0.19 [-0.62, 0.10]
Rats	-0.09 [-0.45, 0.17]
$\tau^2_{within} = 0.20^*$, $\tau^2_{between} = 0.21^*$	

Note: * 95% credible interval excludes zero. HED: human equivalent dose. Single dose was used as a reference category for Frequency_MD: studies with (sub)chronic dosing regimens; Acute effects were used as a reference for Timing_delay: delayed drug effects; Male was used as a reference category for female and male + female. Approach avoidance tests was used as a reference category for the other anxiety tests. Mice was used as a reference category for rats.

Supplemental Table 11. Results of preplanned meta-regression analyses with the maximum likelihood approach for CBD effects on unconditioned anxiety in animals.

Predictor	Effect [95% CI]
Intercept	67.87 [19.10, 116.63]*
Pubyear	-0.03 [-0.06, -0.01]*
HED	0.06 [-0.01, 0.13]
HED^2	-0.02 [-0.03, -0.00]*
Female	-0.47 [-1.13, 0.20]
Male and female	-0.31 [-1.28, 0.66]
Timingdelay	0.24 [-0.07, 0.55]
Disease_Yes	0.83 [0.33, 1.32]*
Acoustic startle	-0.17 [-0.47, 0.13]
Defense	0.77 [-0.15, 1.70]
Novelty suppressed feeding	-0.03 [-0.72, 0.67]
Repetitive compulsive-like behavior	0.78 [0.36, 1.21]*
Social interaction	-0.29 [-0.70, 0.11]
Rats	-0.19 [-0.58, 0.20]
FrequencyMD	-0.09 [-0.36, 0.18]
$\tau^2_{within} = 0.18^*$, $\tau^2_{between} = 0.19^*$	

Note: * 95% confidence interval excludes zero. HED: human equivalent dose. Single dose was used as a reference category for Frequency_MD: studies with (sub)chronic dosing regimens; Acute effects were used as a reference for Timing_delay: delayed drug effects; Male was used as a reference category for female and male + female. Approach avoidance tests was used as a reference category for the other anxiety tests. Mice was used as a reference category for rats.

Supplemental Table 12. Most important moderators of CBD effects on conditioned anxiety in animals selected with Bayesian regularized meta-regression.

Predictor	Effect [95% CI]
Intercept	80.24 [-57.34, 364.45]
Pubyear	-0.04 [-0.18, 0.03]
HED	0.01 [-0.06, 0.11]
HED^2	<-0.01 [-0.03, 0.02]
Frequency_MD	-0.45 [-1.71, 0.13]
Timing_delay	-0.02 [-0.70, 0.65]
Conditioned fear expression	-0.02[-0.43, 0.24]
Extinction retention	0.02 [-0.32, 0.38]
Fear during CS reexposure	0.01 [-0.35, 0.38]
Within session fear extinction	-0.15 [-0.64, 0.08]
Cued fear conditioning	0.01 [-0.21, 0.26]
Mice	-0.04 [-0.70, 0.47]
$\tau^2_{within} = 0.02^*$, $\tau^2_{between} = 1.06^*$	

Note: * 95% credible interval excludes zero. HED: human equivalent dose. Single dose was used as a reference category for Frequency_MD: studies with (sub)chronic dosing regimens; Acute effects were used as a reference for Timing_delay: delayed drug effects; Fear memory reconsolidation was used as a reference category for the other outcomes types for conditioned anxiety; Rats was used as a reference category for mice. Contextual fear conditioning was used as a reference category for cued fear conditioning.

Supplemental Table 13. Results of preplanned meta-regression analyses with the maximum likelihood approach for CBD effects on conditioned anxiety in animals.

Predictor	Effect [95% CI]
Intercept	159.39 [-121.68, 440.46]
Pubyear	-0.08 [-0.22, 0.06]
HED	0.05 [-0.09, 0.20]
HED^2	<-0.01 [-0.05, 0.02]
Timingdelay	1.21 [-1.28, 3.70]
Cued fear conditioning	0.13 [-0.25, 0.50]
Mice	-0.14 [-2.19, 1.90]
Conditioned fear expression	-0.91 [-2.12, 0.30]
Extinction retention	-0.76 [-1.94, 0.42]
Fear during CS reexposure	-0.58 [-1.81, 0.64]
Within-session fear extinction	-1.12 [-2.30, 0.06]
FrequencyMD	-1.69 [-3.22, -0.16]*
$\tau^2_{within} = 0.00, \tau^2_{between} = 0.50$*	

Note: * 95% confidence interval excludes zero. HED: human equivalent dose. Single dose was used as a reference category for Frequency_MD: studies with (sub)chronic dosing regimens; Acute effects were used as a reference for Timing_delay: delayed drug effects; Fear memory reconsolidation was used as a reference category for the other outcomes types for conditioned anxiety; Rats was used as a reference category for mice. Contextual fear conditioning was used as a reference category for cued fear conditioning.

Supplemental Table 14. Most important moderators of CBD effects on experimental anxiety in humans selected with Bayesian regularized meta-regression.

Predictor	Effect [95% CI]
Intercept	10.22 [-82.00, 117.80]
Pub year	<-0.01 [-0.06, 0.04]
Dose	<0.01 [<-0.01, <0.01]
$Dose^2$	<-0.01 [<-0.01, <0.01]
Disease_yes	<-0.01 [-0.68, 0.62]
Sex_male	0.13 [-0.47, 1.00]
SPECT (at resting state)	0.15 [-0.48, 1.14]
Viewing fearful faces	0.01 [-0.77, 0.80]
$\tau^2_{within} = 0.05$*, $\tau^2_{between} = 0.52$*	

Note: * 95% credible interval excludes zero. SPECT = single photon emission computed tomography. No anxiety disorder was used as a reference category for Disease_yes: participants with an anxiety disorder. Male and female was used as a reference category for Sex_male: male participants. The simulated public speaking test was used as a reference category for SPECT at resting state and viewing fearful faces.

2.S.2.3.2 Planned moderator analyses for anxiolytic effects of URB597

Supplemental Table 15. Most important moderators of URB597 effects on unconditioned anxiety in animals selected with Bayesian regularized meta-regression.

Predictor	Effect [95% CI]
Intercept	43.55 [-62.27, 214.40]
Pubyear	-0.02 [-0.11, 0.03]
HED	-0.04 [-0.74, 0.71]
HED^2	-0.04 [-0.30, 0.18]
Frequency_MD	-0.10 [-0.61, 0.25]
Timing_delay	-0.14 [-0.62, 1.47]
Disease_yes	0.53 [-0.09, 1.56]
Defense	0.13 [-0.12, 0.72]
Doxapram	0.05 [-0.16, 0.38]
Novelty suppressed feeding	0.01 [-0.45, 0.54]
Social interaction test	-0.03 [-0.66, 0.45]*
Female	-0.19 [1.26, 0.50]
Sex_NI	0.14 [-0.53, 1.28]
Mice	<0.01 [-0.56, 0.56]
Cricetidae	<-0.01 [-0.87, 0.88]
$\tau^2_{within} = 0.11^*$, $\tau^2_{between} = 1.66^*$	

Note: * 95% credible interval excludes zero. HED: human equivalent dose. Frequency_MD (studies with (sub)chronic dosing regimens) was used as reference category for single dose studies. Timing_delay was used as a reference category for acute drug effects. No pre-existing anxiety was used as a reference category for Disease_yes: subjects with pre-existing anxiety. Approach avoidance tests was used as a reference category the other anxiety tests. Male was used as a reference category for female and Sex_NI: no information. Rats was used as a reference category for mice and Cricetidae.

Supplemental Table 16. Results of preplanned meta-regression analyses with the maximum likelihood approach for URB597 effects on unconditioned anxiety in animals.

Predictor	**Effect [95% CI]**
Intercept	47.58 [-178.47, 273.64]
Pubyear	-0.02 [-0.14, 0.09]
HED	0.04 [-1.53, 1.62]
HED^2	-0.08 [-0.59, 0.44]
Female	-0.91 [-2.43, 0.61]
sexNI	0.46 [-1.30, 2.22]
Timingdelay	1.35 [-0.88, 3.58]
DiseaseYes	0.98 [0.08, 1.88]*
Defense	0.27 [-1.91, 2.46]
Doxapram	1.35 [-4.28, 1.58]
Novelty suppressed feeding	-0.82 [-2.28, 0.64]
Social interaction	-4.60 [-6.09, -3.12]*
Cricetidae	-0.45 [-2.54, 1.63]
Mice	-0.39 [-1.43, 0.65]
FrequencyMD	-0.37 [-1.04, 0.30]
$\tau^2_{within} = 0.11, \tau^2_{between} = 1.73$*	

Note: * 95% confidence interval excludes zero. HED: human equivalent dose. Frequency_MD (studies with (sub)chronic dosing regimens) was used as the reference category for single-dose studies. Timing_delay was used as a reference category for acute drug effects. No pre-existing anxiety was used as a reference category for Disease_yes: subjects with pre-existing anxiety. Approach avoidance tests were used as a reference category for the other anxiety tests. Male was used as a reference category for female and Sex_NI: no information. Rats were used as a reference category for mice and Cricetidae.

Supplemental Table 17. Most important moderators of URB597 effects on conditioned anxiety in animals selected with Bayesian regularized meta-regression.

Predictor	Effect [95% CI]
Intercept	-37.78 [-305.97, 138.52]
Pubyear	0.02 [-0.07, 0.15]
HED	0.70 [-2.51, 6.04]
HED^2	-0.34 [-28.49, 23.35]
Frequency_SD	-0.07 [-0.60, 0.21]
Timing_delay	-0.11 [-0.72, 0.20]
Cued fear conditioning	-0.17 [-0.90, 0.15]
Disease_yes_Extinction recall	-0.07 [-0.77, 0.33]
Extinction consolidation	0.14 [-0.14, 0.74]
Outcome_Extinction retention	0.06 [-0.13, 0.41]
Outcome_Fear during CS reexposure	-0.03 [-0.63, 0.42]
Outcome_Fear memory reconsolidation	-0.04 [-0.68, 0.43]
Sex_female	-0.16 [-1.09, 0.27]
Species_mice	-0.08 [-0.66, 0.24]
$\tau^2_{within} = 0.06^*$, $\tau^2_{between} = 0.30^*$	

Note: * 95% credible interval excludes zero. HED: human equivalent dose. Multiple doses were used as a reference category for Frequency_SD: studies with single dosing regimens; Acute effects were used as a reference for Timing_delay: delayed drug effects; Contextual fear conditioning was used as a reference for cued fear conditioning; No pre-existing anxiety was used as a reference for Disease_yes_outcome extinction recall: test of extinction recall in animals with pre-existing anxiety. Within-session fear extinction was used as a reference category for the other outcome types for conditioned anxiety; Male was used as a reference category for Sex_male: male participants; Rats were used as a reference category for Species_mice.

Supplemental Table 18. Results of preplanned meta-regression analyses with the maximum likelihood approach for URB597 effects on conditioned anxiety in animals.

Predictor	Effect [95% CI]
Intercept	-384.60 [-931.44, 162.25]
Pubyear	0.19 [-0.08, 0.46]
HED	4.64 [-3.40, 12.68]
HED^2	-21.54 [-73.16, 30.09]
Female	-1.10 [-3.01, 0.82]
Timingdelay	-0.66 [-1.42, 0.11]
DiseaseYes;Extinction.recall	-0.50 [-2.06, 1.06]
Cued fear conditioning	-0.97 [-2.39, 0.46]
Mice	-0.11 [-1.61, 1.40]
Extinction consolidation	0.38 [-0.25, 1.01]
Extinction retention	0.11 [-0.30, 0.52]
Fear during CS reexposure	-0.54 [-2.60, 1.52]
Fear memory reconsolidation	-0.78 [-2.27, 0.72]
FrequencySD	-0.37 [-1.43, 0.69]
$\tau^2_{within} = 0.00, \tau^2_{between} = 0.45$*	

Note: * 95% confidence interval excludes zero. HED: human equivalent dose. Multiple dose was used as a reference category for Frequency_SD: studies with single dosing regimens; Acute effects were used as a reference for Timing_delay: delayed drug effects; Contextual fear conditioning was used as a reference for cued fear conditioning; No pre-existing anxiety was used as a reference for Disease_yes_outcome extinction recall: test of extinction recall in animals with pre-existing anxiety. Within-session fear extinction was used as a reference category for the other outcomes types for conditioned anxiety; Male was used as a reference category for Sex_male: male participants; Rats was used as a reference category for Species_mice.

2.S.2.3.3 Planned moderator analyses for anxiolytic effects of AM404

Supplemental Table 19. Most important moderators of AM404 effects on unconditioned anxiety in animals selected with Bayesian regularized meta-regression.

Predictor	Effect [95% CI]
Intercept	-21.06 [-283.11, 215.38]
Pubyear	0.01 [-0.11, 0.14]
HED	0.26 [-0.51, 1.41]
HED^2	-0.33 [-0.55, 1.66]
Disease_yes	-0.15 [-1.49, 0.69]
Defense	-0.01 [-1.06, 0.91]
Acoustic startle	-0.13 [-1.07, 0.44]
Repetitive compulsive-like behavior	0.11 [-0.58, 1.16]
Rats	-0.03 [-0.68, 0.57]
$\tau^2_{within} = 0.76$*, $\tau^2_{between} = 0.74$*	

Note: * 95% credible interval excludes zero. HED: human equivalent dose. No pre-existing anxiety was used as a reference category for Disease_yes: subjects with pre-existing anxiety. Approach avoidance tests were used as a reference category for the other anxiety tests. Mice were used as a reference category for rats.

Supplemental Table 20. Results of preplanned meta-regression analyses with the maximum likelihood approach for AM404 effects on unconditioned anxiety in animals.

Predictor	Effect [95% CI]
Intercept	-36.46 [-606.54, 533.62]
Pubyear	0.02 [-0.27, 0.30]
HED	0.29 [-1.40, 1.98]
HED^2	0.77 [-1.09, 2.63]
DiseaseYes	-2.18 [-5.40, 1.04]
Acoustic startle	-0.72 [-2.19, 0.76]
Defense	-0.20 [-3.05, 2.66]
Repetitive compulsive-like behavior	1.29 [-1.43, 4.01]
Rats	0.20 [-1.16, 1.56]
$\tau^2_{within} = 0.82^*$, $\tau^2_{between} = 0.55$	

Note: * 95% confidence interval excludes zero. HED: human equivalent dose. No pre-existing anxiety was used as a reference category for Disease_yes: subjects with pre-existing anxiety. Approach avoidance tests were used as a reference category for the other anxiety tests. Mice were used as a reference category for rats.

Supplemental Table 21. Most important moderators of AM404 effects on conditioned anxiety in animals selected with Bayesian regularized meta-regression.

Predictor	Effect [95% CI]
Intercept	-7.96 [-221.75, 181.30]
Pubyear	<0.01 [-0.09, 0.11]
HED^2	0.30 [-0.84, 1.94]
Frequency_MD	-0.09 [-1.02, 0.61]
Contextual fear conditioning	0.14 [-0.57, 1.10]
Conditioned fear expression	-0.15 [-1.21, 0.55]
Inhibitory avoidance_Retention of safety learning	0.09 [-0.59, 1.09]
Within-session fear extinction	0.31 [-0.19, 1.18]
Mice	-0.02 [-0.99, 0.87]
$\tau^2_{within} = 0.17^*$, $\tau^2_{between} = 0.28^*$	

Note: * 95% credible interval excludes zero. HED: human equivalent dose. Single dose was used as a reference category for Frequency_MD: studies with (sub)chronic dosing regimens; Cued fear conditioning was used as a reference category for contextual fear conditioning and inhibitory avoidance training, Extinction retention was used as a reference category for the other outcomes types for conditioned anxiety; Rats was used as a reference category for Species_mice.

Supplemental Table 22. Results of preplanned meta-regression analyses with the maximum likelihood approach for AM404 effects on conditioned anxiety in animals.

Predictor	Effect [95% CI]
Intercept	121.01 [-158.89, 400.92]
Pubyear	-0.06 [-0.20, 0.08]
HED^2	0.63 [-0.76, 2.01]
Contextual fear conditioning	0.43 [-0.95, 1.81]
Inhibitory avoidance task;Retention.of.safety.learning	1.01 [-0.43, 2.44]
Conditioned fear expression	-0.73 [-2.34, 0.87]
Within-session fear extinction	0.88 [0.14, 1.62]*
$\tau^2_{within} = 0.00, \tau^2_{between} = 0.18$	

Note: * 95% confidence interval excludes zero. HED: human equivalent dose. Single dose was used as a reference category for Frequency_MD: studies with (sub)chronic dosing regimens; Cued fear conditioning was used as a reference category for contextual fear conditioning and inhibitory avoidance training, Extinction retention was used as a reference category for the other outcomes types for conditioned anxiety; Rats was used as a reference category for Species_mice.

2.S.2.3.4 Planned moderator analyses for anxiolytic effects of PF-3845

Supplemental Table 23. Most important moderators of PF-3845 effects on unconditioned anxiety in animals selected with Bayesian regularized meta-regression.

Predictor	Effect [95%CI]
Intercept	16.11 [-222.45, 269.42]
Pubyear	-0.01 [-0.13, 0.11]
HED	0.09 [-0.45, 0.83]
HED^2	-0.34 [-1.53, 0.34]
Disease_no	-0.03 [-0.56, 0.78]
Timing_delay	0.02 [-0.43, 0.52]
Frequency_MD	-0.46 [-1.22, 0.05]
Novelty suppressed feeding	<-0.01 [-0.44, 0.43]
Repetitive compulsive-like behavior	0.03 [-0.67, 0.81]
Rats	-0.23 [-1.40, 0.45]
$\tau^2_{within} = 0.14^*, \tau^2_{between} = 0.64^*$	

Note: * 95% credible interval excludes zero. HED: human equivalent dose. Pre-existing anxiety was used as a reference category for Disease_no, subjects without pre-existing anxiety; Acute drug effects were used as a reference category for Timing_delay. Participants with an anxiety disorder; Single dose studies were used as a reference category for Frequency_MD (studies with (sub)chronic dosing regimens); Approach avoidance tests were used as a reference category for the other anxiety tests; Mice were used as a reference category for rats.

Supplemental Table 24. Results of preplanned meta-regression analyses with the maximum likelihood approach for PF-3845 effects on unconditioned anxiety in animals.

Predictor	Effect [95% CI]
Intercept	71.03 [-463.22, 605.28]
Pubyear	-0.03 [-0.30, 0.23]
HED	0.45 [-0.43, 1.33]
HED^2	-0.69 [-1.91, 0.52]
Timingdelay	0.17 [-0.52, 0.85]
DiseaseNo	0.27 [-1.36, 1.90]
Novelty suppressed feeding	0.12 [-0.50, 0.74]
Repetitive compulsive-like behavior	-0.32 [-3.08, 2.44]
Rats	-1.29 [-3.34, 0.75]
FrequencyMD	-1.03 [-1.71, -0.35]*
$\tau^2_{within} = 0.09$, $\tau^2_{between} = 0.61$*	

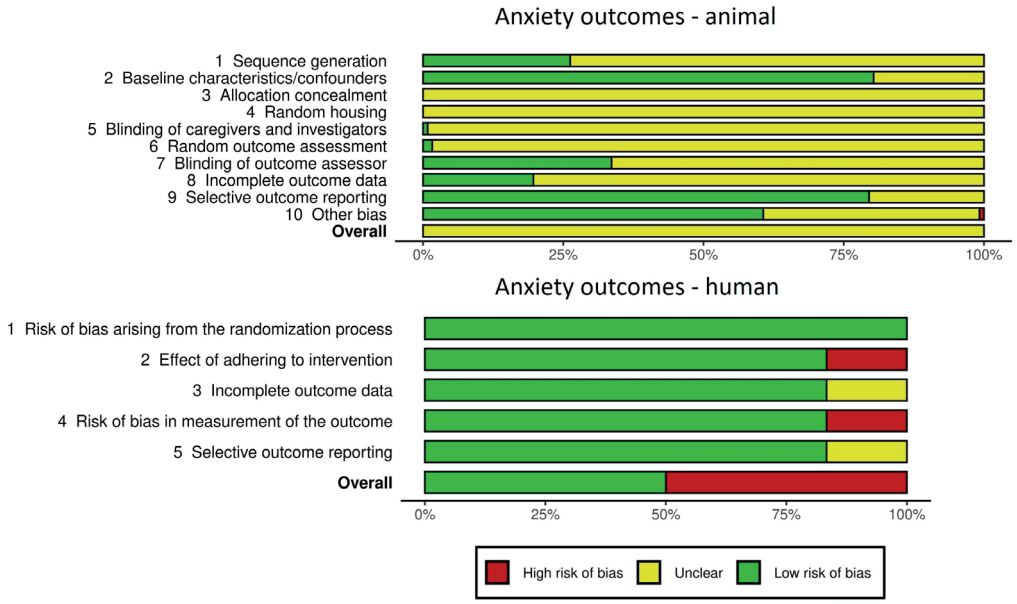
Note: * 95% confidence interval excludes zero. HED: human equivalent dose. Pre-existing anxiety was used as a reference category for Disease_no, subjects without pre-existing anxiety; Acute drug effects were used as a reference category for Timing_delay. Participants with an anxiety disorder; Single dose studies were used as a reference category for Frequency_MD (studies with (sub)chronic dosing regimens); Approach avoidance tests were used as a reference category for the other anxiety tests; Mice were used as a reference category for rats.

2.S.2.4 Quality of evidence

Supplemental Table 25. GRADE assessment of the quality of evidence.

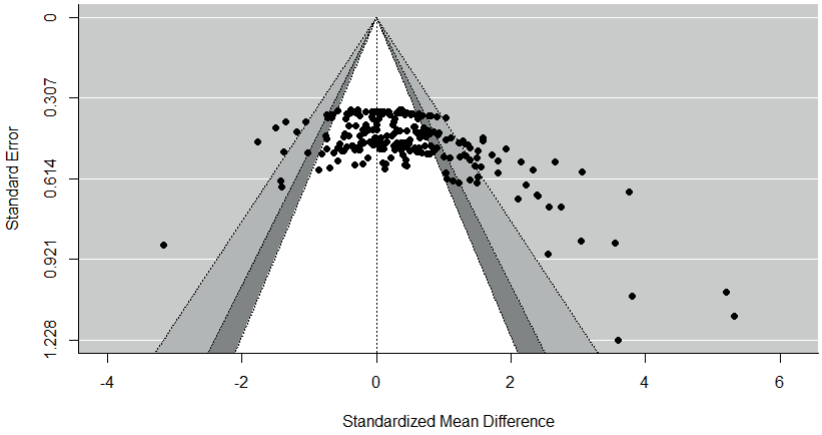
Intervention	Risk of Bias	Inconsistency	Indirectness	Imprecision	Publication Bias	Other Factors	Quality of Evidence
CBD (Experimental)	Serious	Serious	Serious	Not Serious	Strongly Suspected	Upgrade	Low
CBD (Conditioned)	Serious	Serious	Serious	Not Serious	Very Strongly Suspected	Upgrade	Low
CBD (Unconditioned)	Serious	Serious	Very Serious	Not Serious	Very Strongly Suspected	Upgrade	Low
URB597 (Conditioned)	Serious	Serious	Serious	Not Serious	Strongly Suspected	Upgrade	Low
URB597 (Unconditioned)	Serious	Serious	Very Serious	Serious	Strongly Suspected	Upgrade	Low
AM404 (Conditioned)	Serious	Serious	Serious	Not Serious	Strongly Suspected	Upgrade	Low
AM404 (Unconditioned)	Serious	Serious	Very Serious	Not Serious	Strongly Suspected	Upgrade	Low
PF-3845 (Unconditioned)	Serious	Serious	Serious	Not Serious	Strongly Suspected	Upgrade	Low

Note: CBD: cannabidiol; GRADE: Grading of Recommendations, Assessment, Development and Evaluation.



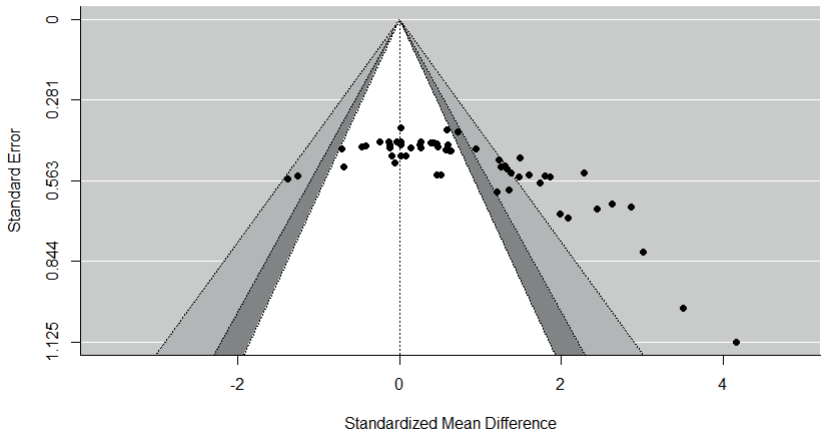
Supplemental Figure 10. Summary risk of bias plots for anxiety outcomes.

Note: Only studies that were included in the meta-analysis are included in the plots.



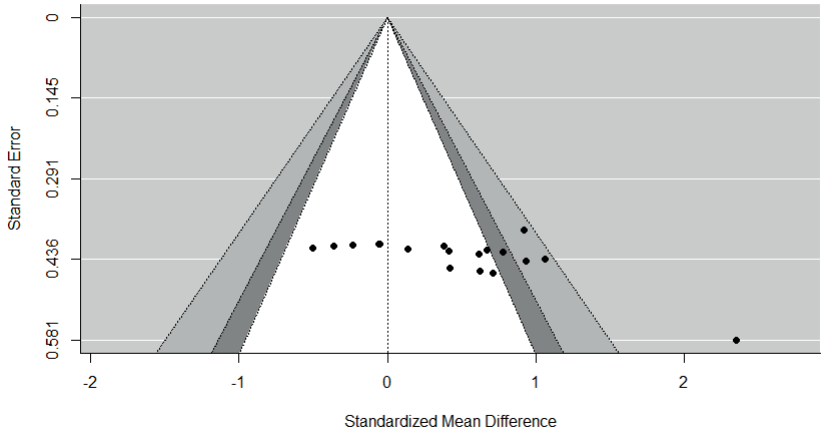
Supplemental Figure 11. Funnel plot for CBD effects on unconditioned anxiety in animals, suggesting non-significant results in small studies remained unpublished.

Note: Triangle covers the 95% pseudoconfidence interval specified around a (hypothetical) pooled effect of zero. Dark grey: p<.05; medium gray: p<.01; light gray: p <.001.



Supplemental Figure 12. Funnel plot for CBD effects on conditioned anxiety in animals, suggesting non-significant results in small studies remained unpublished.

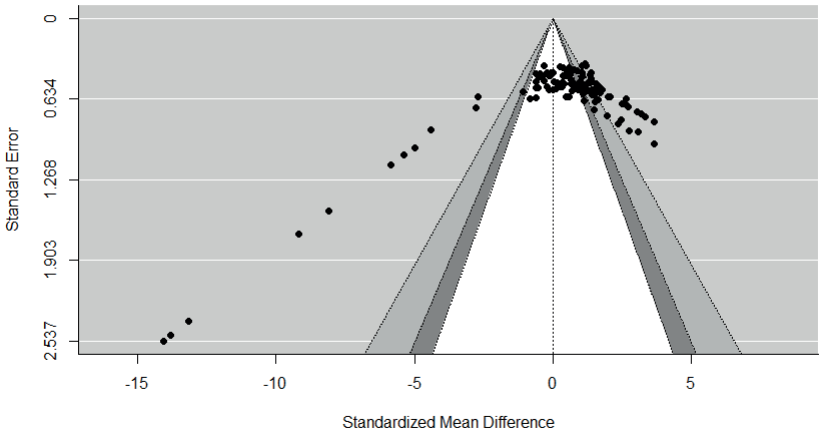
Note: Triangle covers the 95% pseudoconfidence interval specified around a (hypothetical) pooled effect of zero. Dark grey: p<.05; medium gray: p<.01; light gray: p <.001.



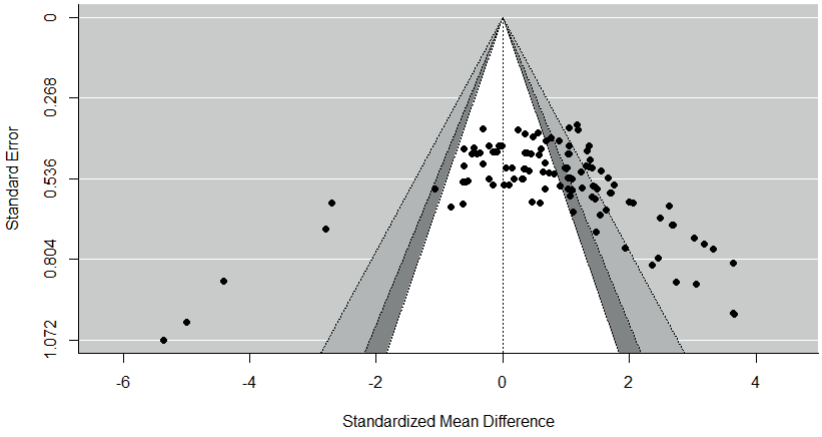
Supplemental Figure 13. Funnel plot for CBD effects on experimental anxiety in humans.

Note: Triangle covers the 95% pseudoconfidence interval specified around a (hypothetical) pooled effect of zero. Dark grey: p<.05; medium gray: p<.01; light gray: p <.001.

a)

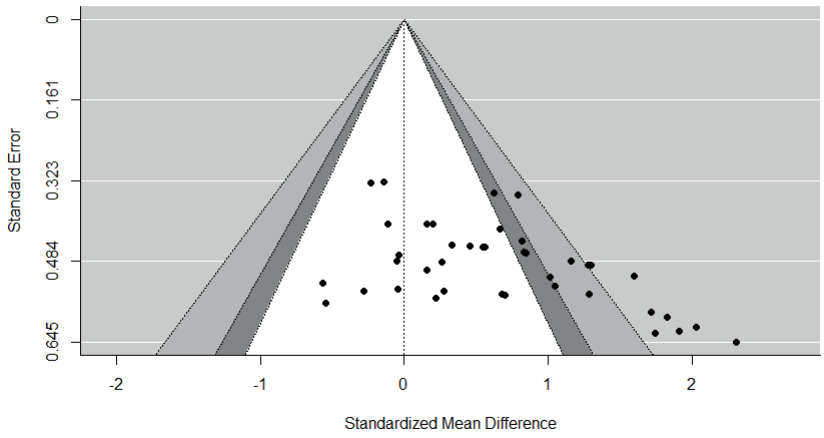


b)



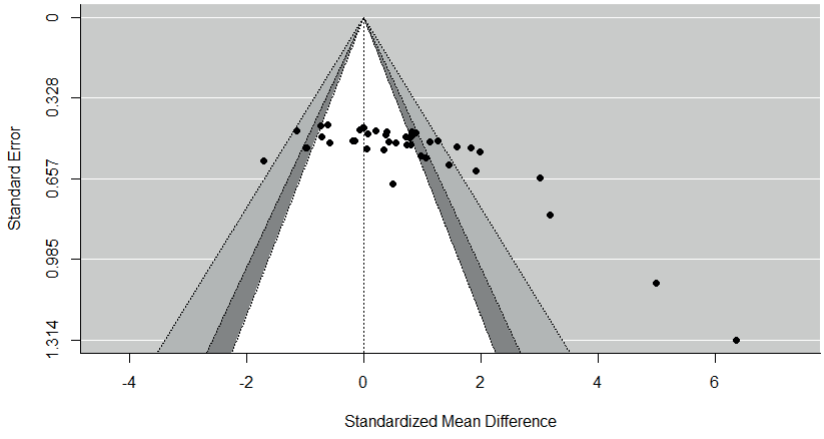
Supplemental Figure 14. Funnel plot for URB597 effects on unconditioned anxiety in animals (a), excluding statistical outliers (b), suggesting non-significant results in small studies remained unpublished.

Note: Triangle covers the 95% pseudoconfidence interval specified around a (hypothetical) pooled effect of zero. Dark grey: p<.05; medium gray: p<.01; light gray: p <.001.



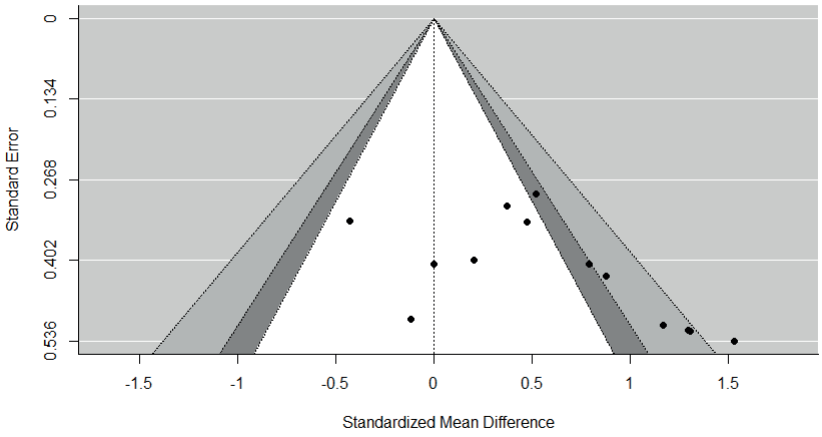
Supplemental Figure 15. Funnel plot for URB597 effects on conditioned anxiety in animals, suggesting non-significant results in small studies remained unpublished.

Note: Triangle covers the 95% pseudoconfidence interval specified around a (hypothetical) pooled effect of zero. Dark grey: p<.05; medium gray: p<.01; light gray: p <.001.



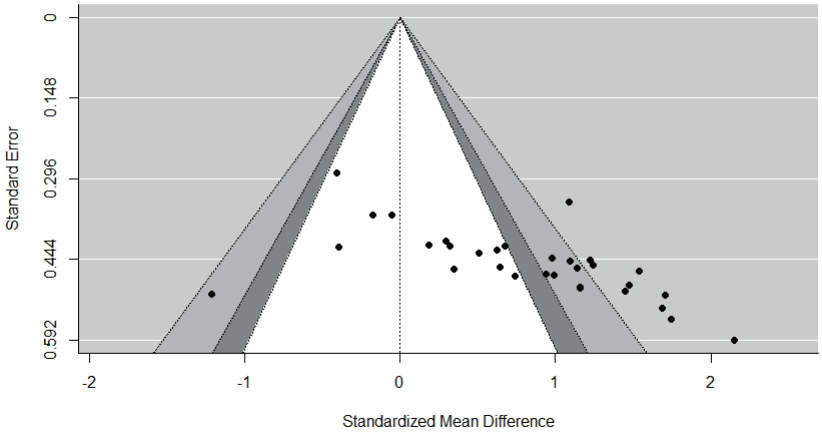
Supplemental Figure 16. Funnel plot for AM404 effects on unconditioned anxiety in animals, suggesting non-significant results in small studies remained unpublished.

Note: Triangle covers the 95% pseudoconfidence interval specified around a (hypothetical) pooled effect of zero. Dark grey: p<.05; medium gray: p<.01; light gray: p <.001.



Supplemental Figure 17. Funnel plot for AM404 effects on conditioned anxiety in animals.

Note: Triangle covers the 95% pseudoconfidence interval specified around a (hypothetical) pooled effect of zero. Dark grey: p<.05; medium gray: p<.01; light gray: p <.001.



Supplemental Figure 18. Funnel plot for PF-3845 effects on unconditioned anxiety in animals, suggesting non-significant results in small studies remained unpublished.

Note: Dark grey: p<.05; medium gray: p<.01; light gray: p <.001.

2.S.2.5 Sensitivity analyses

Supplemental Table 26. Excluded effects in sensitivity analyses

Reason	Explanation	No. of effects, type of study (studies)
Assessed as high risk of bias with SYRCLE (Hooijmans et al., 2014) and Cochrane RoB 2.0 (Sterne et al., 2019) tools	- Increased mental sedation in the CBD condition and, as a potential consequence, unsuccessful blinding	1 effect of CBD in an experimental study in humans (Crippa et al., 2004)
	- Highly variable CBD plasma concentrations (4.7 (7) and 17 (29) ng/mL 1 and 2 hours after administration, that led to concerns about failures in implementing the intervention	1 effect of CBD in an experimental study in humans (Fusar-Poli et al., 2009)
	- Unclear bias due to missing outcome data and concerns about selective outcome reporting	2 effects of CBD in an experimental study in humans (Zuardi et al., 1993)
	- Competing financial interests	3 effects of URB597 on unconditioned anxiety in animals (Kathuria et al., 2003)
Oral administration	Bioavailability may be different than with intraperitoneal administration	22 effects of CBD on unconditioned anxiety in animals (Cheng, Spiro et al., 2014; Deiana et al., 2011; Kajero et al., 2020) and on conditioned anxiety in animals (Cheng, Spiro et al., 2014; Murkar et al., 2019; Shoval et al., 2016; Silvestri et al., 2020)
Statistical outliers (effect >3 SD smaller than mean effect)	Great care was taken to prevent stress and anxiety in animals (personal correspondence with the study author), which raises the possibility that no anxiety-reducing drug effects could be measured	8 effects of URB597 on unconditioned anxiety in animals (Seillier et al., 2013; 2018)

Supplemental Table 26. Excluded effects in sensitivity analyses

Less reliable estimates of human equivalent dose (HED)	For the Cricetidae family of rodents no FDA conversion factors to calculate HED are available. Therefore, we used conversion factors for hamsters as a best estimate, which may not be accurate (CDER, 2015)	9 effects of URB597 on unconditioned anxiety in animals (Moise et al., 2008; Simmons et al., 2021)

Note: SYRCLE: Systematic Review Centre for Laboratory animal Experimentation; CBD: Cannabidiol; RoB: Risk of Bias; SD: standard deviation; HED: human equivalent dose; FDA: Food and Drug Administration.

2.S.2.6 Exploratory moderator analyses

Below are presented, in Tables 27-42, the results of the exploratory moderator analyses with Bayesian regularized meta-regression (BRMA) and meta-regression analyses with the maximum likelihood approach. In the exploratory BRMA analyses, similar to the planned BRMA analyses, the social interaction test was associated with smaller URB597 anxiolytic effects compared to approach avoidance tests. Further, only effects of AM404 in tests of repetitive compulsive-like behavior depended on dose. Within the range of tested doses (HED 0.0081-1.62), higher HED was associated with larger drug effects (Supplemental Figure 19). Results of the exploratory meta-regression analyses with the maximum likelihood approach and exploratory moderator analyses with Bayesian regularized meta-regression (BRMA) differed for CBD effects on unconditioned anxiety (Tables 27-28), CBD effects on conditioned anxiety (Tables 29-30), and CBD effects on experimentally induced anxiety in humans (Tables 31-32).

2.S.2.6.1 Exploratory moderator analyses for anxiolytic effects of CBD

Supplemental Table 27. Exploratory moderator analyses with BRMA for CBD effects on unconditioned anxiety in animals.

Predictor	Effect [95% CI]
Intercept	0.36[0.15, 0.60]*
HED	0.01[-0.03, 0.07]
HED^2	<-0.01[-0.02, 0.01]
Acoustic startle.HED	-0.03[-0.15, 0.06]
Defense.HED	-0.09[-0.80, 0.64]
Novelty suppressed feeding.HED	0.52[-3.29, 5.39]
Repetitive compulsive-like behavior.HED	0.22[-0.02, 0.59]
Social interaction.HED	0.04[-0.06, 0.21]
Acoustic startle.HED^2	-0.01[-0.04, 0.01]
defense.HED^2	0.06[-0.24, 0.50]
Novelty suppressed feeding.HED^2	1.09[-13.43, 15.84]
Repetitive compulsive-like behavior.HED^2	-0.04[-0.11, 0.00]
Social interaction.HED^2	<-0.01[-0.03, 0.01]
Acoustic startle	-0.02[-0.25, 0.15]
Defense	0.07[-0.30, 0.86]
Novelty suppressed feeding	0.03[-0.53, 0.69]
Repetitive compulsive-like behavior	1.03[0.01, 1.73]
Social interaction	-0.11[-0.55, 0.10]
$\tau^2_{within} = 0.19^*$, $\tau^2_{between} = 0.32^*$	

Note: * 95% credible interval excludes zero. HED: human equivalent dose. Approach avoidance tests were used as a reference category for the other anxiety tests.

Supplemental Table 28. Exploratory meta-regression analyses with the maximum likelihood approach for CBD effects on unconditioned anxiety in animals.

Predictor	Effect [95% CI]
Intercept	0.32[0.10, 0.55]*
Acoustic startle	-0.08[-0.50, 0.34]
Defense	0.93[-1.11, 2.98]
Novelty suppressed feeding	0.41[-0.26, 1.08]
Repetitive compulsive-like behavior	1.58[1.03, 2.13]*
Social interaction	-0.33[-0.80, 0.13]
HED	0.02[-0.07, 0.11]
HED^2	-0.01[-0.03, 0.02]
Acoustic startle:HED	-0.05[-0.24, 0.14]
Defense:HED	-1.09[-6.51, 4.33]
Repetitive compulsive-like behavior:HED	0.53[0.26, 0.80]*
Social interaction:HED	0.12[-0.10, 0.34]
Acoustic startle:HED^2	-0.01[-0.05, 0.04]
defense:HED^2	-0.56[-3.69, 2.57]
Repetitive compulsive-like behavior:HED^2	-0.1[-0.15, -0.05]*
Social interaction:HED^2	-0.01[-0.06, 0.03]
τ^2 within= 0.19*, $\tau^2_{between}$= 0.26*	

Note: * 95% confidence interval excludes zero. HED: human equivalent dose. Approach avoidance tests were used as a reference category for the other anxiety tests.

Supplemental Table 29. Exploratory moderator analyses with BRMA for CBD effects on conditioned anxiety in animals.

Predictor	Effect [95% CI]
Intercept	0.71[0.09, 1.34]*
HED	<0.01[-0.04, 0.05]
HED^2	<-0.01[-0.01, 0.01]
Conditioned fear expression.HED	0.02[-0.07, 0.23]
Extinction retention.HED	<-0.01[-0.17, 0.13]
Fear during CS reexposure.HED	<-0.01[-0.07, 0.05]
Within session fear extinction.HED	0.01[-0.09, 0.15]
Conditioned fear expression.HED^2	-0.02[-0.25, 0.05]
Extinction retention.HED^2	0.01[-0.11, 0.17]
Fear during CS reexposure.HED^2	<-0.01[-0.01, 0.01]
Within session fear extinction.HED^2	-0.04[-0.33, 0.04]
HED.Cued fear conditioning	0.01[-0.03, 0.13]
HED^2.Cued fear conditioning	<-0.01[-0.02, 0.01]
Conditioned fear expression	<0.01[-0.10, 0.13]
Extinction retention	<0.01[-0.13, 0.15]
Fear during CS reexposure	0.01[-0.10, 0.20]
Within-session fear extinction	-0.03[-0.40, 0.05]
Cued fear conditioning	<0.01[-0.09, 0.10]
τ^2_{within}= 0.03*, $\tau^2_{between}$= 1.44*	

Note: *95% credible interval excludes zero. HED: human equivalent dose. Contextual fear conditioning was used as a reference category for the cued fear conditioning; Fear memory reconsolidation was used as a reference category for the other outcome types.

Supplemental Table 30. Exploratory meta-regression analyses with the maximum likelihood approach for CBD effects on conditioned anxiety in animals.

Predictor	Effect [95% CI]
Intercept	1.82[0.65, 2.99]*
Conditioned fear expression	-1.25[-2.79, 0.30]
Extinction retention	-1.64[-3.14, -0.14]*
Fear during CS reexposure	-1.32[-2.68, 0.05]
Within-session fear extinction	-1.88[-3.42, -0.33]*
HED	0.02[-0.33, 0.37]
HED^2	-0.03[-0.13, 0.08]
Cued fear conditioning	0.24[-0.21, 0.70]
Conditioned fear expression:HED	0.05[-0.52, 0.63]
Extinction retention:HED	-0.11[-0.68, 0.47]
Fear during CS reexposure:HED	-0.16[-0.60, 0.28]
Within-session fear extinction:HED	-0.14[-0.69, 0.41]
Conditioned fear expression:HED^2	-0.35[-0.78, 0.08]
Extinction retention:HED^2	0.07[-0.47, 0.62]
Fear during CS reexposure:HED^2	0.05[-0.07, 0.17]
Within-session fear extinction:HED^2	-0.1[-0.52, 0.32]
HED:Cued fear conditioning	0.21[-0.06, 0.48]
HED^2:Cued fear conditioning	-0.03[-0.10, 0.03]
τ^2within <0.01, $\tau^2_{between}$=0.78*	

Note: * 95% confidence interval excludes zero. HED: human equivalent dose. Contextual fear conditioning was used as a reference category for the cued fear conditioning; Fear memory reconsolidation was used as a reference category for the other outcome types.

Supplemental Table 31. Exploratory moderator analyses with BRMA for CBD effects on experimental anxiety in humans.

Predictor	Effect [95% CI]
Intercept	0.73[0.01, 1.57]*
Dose	<0.01[-0.00, 0.00]
$Dose^2$	<-0.01[-0.00, 0.00]
SPECT (resting state).dose	-0.01[-0.05, 0.03]
Viewing fearful faces.dose	<0.01[-0.01, 0.02]
SPECT (resting state).$dose^2$	<0.01[<-0.01, <0.01]
Viewing fearful faces.$dose^2$	<0.01[<-0.01, <0.01]
SPECT (resting state)	0.06[-0.83, 1.13]
Viewing fearful faces	0.02[-0.87, 1.02]
τ^2within <0.05, $\tau^2_{between}$=0.53*	

Note: * 95% credible interval excludes zero. SPECT = single photon emission computed tomography. The simulated public speaking test was used as a reference category for SPECT at resting state and viewing fearful faces.

Supplemental Table 32. Exploratory meta-regression analyses with the maximum likelihood approach for CBD effects on experimental anxiety in humans.

Predictor	Effect [95% CI]
Intercept	0.78[0.41, 1.16]*
SPECT (resting state)	0.60[-0.20, 1.39]
Viewing fearful faces	0.18[-0.66, 1.03]
Dose	<0.01[<0.01, <0.01]
$Dose^2$	<0.01[<0.01, <0.01]*
τ^2within<0.01, τ^2between<0.01	

Note: * 95% confidence interval excludes zero. SPECT = single photon emission computed tomography. The simulated public speaking test was used as a reference category for SPECT at resting state and viewing fearful faces.

2.S.2.6.2 Exploratory moderator analyses for anxiolytic effects of URB597

Supplemental Table 33. Exploratory moderator analyses with BRMA for URB597 effects on unconditioned anxiety in animals.

Predictor	Effect [95% CI]
Intercept	0.83[0.39, 1.27]*
HED	-0.04[-0.54, 0.44]
HED^2	-0.03[-0.22, 0.11]
Defense.HED	-3.62[-26.59, 12.60]
Doxapram.HED	-1.36[-14.55, 10.41]
Novelty suppressed feeding.HED	0.31[-12.32, 15.39]
Social interaction.HED	2.87[-8.69, 27.96]
Defense.HED^2	43.5[-84.58, 226.50]
Doxapram.HED^2	23.04[-108.26, 201.42]
Novelty suppressed feeding.HED^2	16.64[-87.68, 201.99]
Social interaction.HED^2	-9.81[-177.05, 104.04]
Defense	0.01[-0.63, 0.74]
Doxapram	-0.10[-1.38, 0.45]
Novelty suppressed feeding	-0.04[-0.84, 0.51]
Social interaction	-3.19[-5.15, -0.09]*
τ^2_{within} = 0.12*, $\tau^2_{between}$ = 1.71*	

Note: * 95% credible interval excludes zero. HED: human equivalent dose. Approach avoidance tests were used as a reference category for the other anxiety tests.

Supplemental Table 34. Exploratory meta-regression analyses with the maximum likelihood approach for URB597 effects on unconditioned anxiety in animals.

Predictor	Effect [95% CI]
Intercept	1.00[0.55, 1.44]*
Defense	-0.78[-3.28, 1.71]
Doxapram	-2.36[-5.41, 0.69]
Novelty suppressed feeding	0.13[-7.19, 7.45]
Social interaction	-5.25[-7.57, -2.92]*
HED	0.16[-1.40, 1.71]
HED^2	-0.12[-0.64, 0.40]
Defense:HED	-18.12[-41.54, 5.29]
Doxapram:HED	7.66[-28.37, 43.70]
Novelty suppressed feeding:HED	52.02[-171.89, 275.93]
Social interaction:HED	-5.91[-47.25, 35.43]
Doxapram:HED^2	220.83[-276.35, 718.02]
Novelty suppressed feeding:HED^2	498.02[-1021.30, 2017.35]
Social interaction:HED^2	67.12[-308.64, 442.88]
τ^2 within= 0.14*, τ^2 between= 1.46*	

Note: * 95% confidence interval excludes zero. HED: human equivalent dose. Approach avoidance tests were used as a reference category for the other anxiety tests.

Supplemental Table 35. Exploratory moderator analyses with BRMA for URB597 effects on conditioned anxiety in animals.

Predictor	Effect [95% CI]
Intercept	0.54[0.13, 0.95]*
HED	0.17[-1.48, 2.76]
HED^2	0.03[-12.13, 12.13]
Extinctionconsolidation.HED	-1.74[-18.14, 6.03]
Extinction.recall.HED	-0.61[-18.02, 13.43]
Extinction.retention.HED	-1.91[-16.02, 3.20]
Fear.during.CS.reexposure.HED	3.68[-3.24, 23.58]
Fear.memory.reconsolidation.HED	22.44[-255.30, 418.15]
Extinction.consolidation.HED^2	49.44[-169.49, 517.36]
Extinction.recall.HED^2	-8.2[-401.69, 308.90]
Extinction.retention.HED^2	7.87[-220.13, 254.93]
Fear.during.CS.reexposure.HED^2	-62.32[-649.51, 164.78]
Fear.memory.reconsolidation.HED^2	-7985.54[-190233.63, 121540.17]
HED.Cued.fear.conditioning	0.41[-6.35, 8.96]
HED^2.Cued.fear.conditioning	-18.94[-348.28, 207.39]
Extinction.consolidation	0.03[-0.10, 0.47]
Extinction.recall	-0.01[-0.30, 0.15]
Extinction.retention	0.02[-0.08, 0.24]
Fear.during.CS.reexposure	<-0.01[-0.20, 0.18]
Fear.memory.reconsolidation	-0.01[-0.26, 0.19]
Cued.fear.conditioning	-0.05[-0.54, 0.10]
τ^2_{within}= 0.06*, $\tau^2_{between}$= 0.33*	

Note: * 95% credible interval excludes zero. HED: human equivalent dose. Contextual fear conditioning was used as a reference category for cued fear conditioning. Within-session fear extinction was used as a reference category for the other outcome types for conditioned anxiety.

Supplemental Table 36. Exploratory meta-regression analyses with the maximum likelihood approach for URB597 effects on conditioned anxiety in animals.

Predictor	Effect [95% CI]
Intercept	0.80[0.12, 1.48]*
Extinction consolidation	0.71[-0.45, 1.87]
Extinction recall	-0.05[-1.51, 1.41]
Extinction retention	0.26[-0.24, 0.77]
Fear during CS reexposure	0.40[-1.51, 2.30]
Fear memory reconsolidation	-0.44[-1.93, 1.05]
HED	3.01[-13.09, 19.11]
HED^2	-16.25[-106.01, 73.52]
Cued fear conditioning	-0.67[-1.86, 0.51]
Extinction consolidation:HED	-0.48[-40.04, 39.09]
Extinction recall:HED	-16.02[-115.04, 82.99]
Extinction retention:HED	-21.58[-44.06, 0.90]
Fear during CS reexposure:HED	11.43[-14.56, 37.43]
Extinction retention:HED^2	-365.05[-1177.57, 447.47]
Fear during CS reexposure:HED^2	-619.25[-1825.78, 587.28]
HED:Cued fear conditioning	9.71[-15.32, 34.73]
HED^2:Cued fear conditioning	155.07[-1970.22, 2280.36]
τ^2 within= 0.01, τ^2 between= 0.42*	

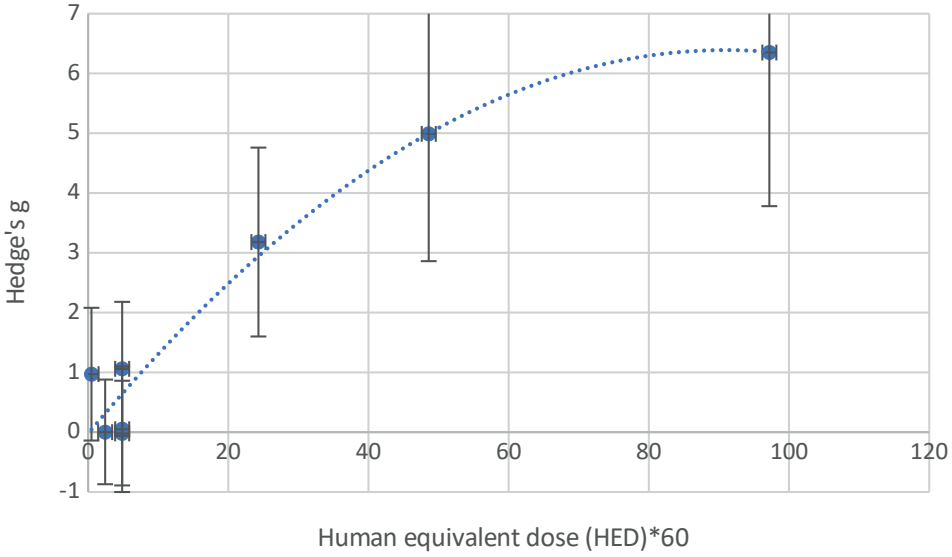
Note: * 95% confidence interval excludes zero. HED: human equivalent dose. Contextual fear conditioning was used as a reference category for cued fear conditioning. Within-session fear extinction was used as a reference category for the other outcome types for conditioned anxiety.

2.S.2.6.3 Exploratory moderator analyses for anxiolytic effects of AM404

Supplemental Table 37. Exploratory moderator analyses with BRMA for AM404 effects on unconditioned anxiety in animals.

Predictor	Effect [95% CI]
Intercept	0.74[0.18, 1.37]*
HED	-0.15[-1.24, 0.71]
HED^2	-0.25[-2.44, 1.15]
Acoustic startle.HED	0.16[-2.14, 2.79]
Defense.HED	-12.52[-767.67, 775.02]
Repetitive compulsive-like behavior.HED	3.73[0.55, 6.65]*
Acoustic startle.HED^2	0.27[-1.45, 2.72]
Defense.HED^2	-3730.13[-155625.37, 132453.63]
Repetitive compulsive-like behavior.HED^2	0.25[-1.94, 2.91]
Acoustic startle	-0.07[-0.96, 0.63]
Defense	<-0.01[-1.09, 1.08]
Repetitive compulsive-like behavior	0.28[-0.36, 1.42]
τ^2_{within}= 0.49*, $\tau^2_{between}$= 0.61*	

Note: * 95% credible interval excludes zero. HED: human equivalent dose. Cued fear conditioning was used as a reference category for contextual fear conditioning. Extinction retention was used as a reference category for the other outcome types for conditioned anxiety.



Supplemental Figure 19. Hedge's g plotted against human equivalent dose for AM404 effects on repetitive compulsive-like behavior. Error bars display 95% confidence intervals. A second order polynomial trendline is drawn.

Supplemental Table 38. Exploratory meta-regression analyses with the maximum likelihood approach for AM404 effects on unconditioned anxiety in animals.

Predictor	Effect [95% CI]
Intercept	0.91[0.02, 1.81]*
Acoustic startle	-0.94[-3.31, 1.42]
Defense	-0.42[-2.75, 1.90]
Repetitive compulsive-like behavior	1.31[-0.32, 2.94]
HED	-0.05[-2.08, 1.98]
HED^2	-3.36[-10.35, 3.63]
Acoustic startle:HED	0.92[-8.41, 10.24]
Repetitive compulsive-like behavior:HED	6.41[2.26, 10.56]*
Acoustic startle:HED^2	3.01[-6.31, 12.33]
Repetitive compulsive-like behavior:HED^2	0.83[-7.13, 8.79]
τ^2 within= 0.47*, τ^2 between= 0.26	

Note: * 95% confidence interval excludes zero. HED: human equivalent dose. Cued fear conditioning was used as a reference category for contextual fear conditioning. Extinction retention was used as a reference category for the other outcome types for conditioned anxiety.

Supplemental Table 39. Exploratory moderator analyses with BRMA for AM404 effects on conditioned anxiety in animals.

Predictor	Effect [95% CI]
Intercept	0.33[-0.63, 1.09]
HED2	0.22[-0.68, 1.74]
Conditioned fear expression.HED	-0.11[-2.12, 1.73]
Retention of safety learning.HED.HED.Inhibitory avoidance task	-0.11[-2.16, 1.60]
Within-session fear extinction.HED	-0.21[-1.45, 0.45]
Conditioned fear expression.HED2	-0.19[-2.65, 1.86]
Retention of safety learning.HED2.HED2.Inhibitory avoidance task	0.23[-3.34, 4.57]
Within-session fear extinction.HED2	0.33[-0.88, 2.51]
HED.Contextual fear conditioning	0.22[-0.91, 2.05]
HED2.Contextual fear conditioning	0.22[-1.18, 2.32]
Conditioned fear expression	-0.04[-0.86, 0.59]
Retention of safety learning.Inhibitory avoidance task	0.02[-0.61, 0.77]
Within-session fear extinction	0.11[-0.36, 0.97]
Contextual fear conditioning	0.05[-0.57, 0.90]
$\tau^2_{within} = 0.16^*$, $\tau^2_{between} = 0.33^*$	

Note: * 95% credible interval excludes zero. HED: human equivalent dose. Cued fear conditioning was used as a reference category for contextual fear conditioning. Extinction retention was used as a reference category for the other outcome types for conditioned anxiety.

Supplemental Table 40. Exploratory meta-regression analyses with the maximum likelihood approach for AM404 effects on conditioned anxiety in animals.

Predictor	Effect [95% CI]
Intercept	-0.21[-1.11, 0.69]
Conditioned fear expression	-0.43[-2.00, 1.14]
Retention of safety learning; Inhibitory avoidance task	0.63[-0.40, 1.66]
Within-session fear extinction	0.63[-0.12, 1.38]
HED2	0.74[-0.62, 2.11]
Contextual fear conditioning	0.99[-0.60, 2.58]
Within-session fear extinction:HED	-0.77[-1.97, 0.43]
$\tau^2_{within} < 0.01$, τ^2 between= 0.21	

Note: * 95% confidence interval excludes zero. HED: human equivalent dose. Cued fear conditioning was used as a reference category for contextual fear conditioning. Extinction retention was used as a reference category for the other outcome types for conditioned anxiety.

2.S.2.6.4 Exploratory moderator analyses for anxiolytic effects of PF-3845

Supplemental Table 41. Exploratory moderator analyses with BRMA for PF-3845 effects on unconditioned anxiety in animals.

Predictor	Effect [95% CI]
Intercept	0.67[0.01, 1.21]*
HED	-0.06[-0.65, 0.42]
HED^2	-0.14[-1.16, 0.53]
Novelty suppressed feeding.HED	-0.16[-1.55, 1.13]
Repetitive compulsive-like behavior.HED	0.69[-0.47, 2.70]
Novelty suppressed feeding.HED^2	-0.44[-2.20, 0.46]
Repetitive compulsive-like behavior.HED^2	-0.06[-6.12, 5.50]
Novelty suppressed feeding	-0.01[-0.37, 0.30]
Repetitive compulsive-like behavior	0.03[-0.42, 0.65]
τ^2_{within}= 0.18*, $\tau^2_{between}$= 0.42*	

Note: * 95% credible interval excludes zero. HED: human equivalent dose. Approach avoidance tests were used as a reference category for the other anxiety tests.

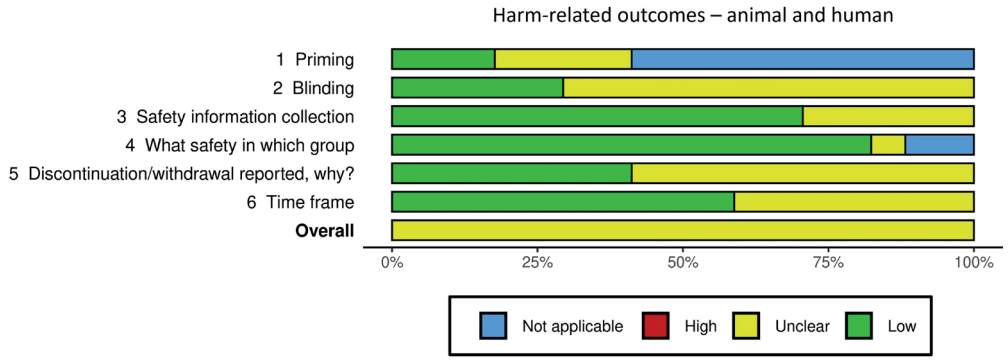
Supplemental Table 42. Exploratory meta-regression analyses with the maximum likelihood approach for PF-3845 effects on unconditioned anxiety in animals.

Predictor	Effect [95% CI]
Intercept	0.61[0.05, 1.17]*
Novelty suppressed feeding	0.08[-0.69, 0.84]
Repetitive compulsive-like behavior	0.52[-1.65, 2.69]
HED	-0.37[-1.48, 0.74]
HED^2	0.10[-1.42, 1.61]
Novelty suppressed feeding:HED	1.31[-2.49, 5.12]
Repetitive compulsive-like behavior:HED	1.65[-0.97, 4.26]
Novelty suppressed feeding:HED^2	-2.23[-6.14, 1.68]
Repetitive compulsive-like behavior:HED^2	1.59[-18.30, 15.11]
τ^2_{within}= 0.20*, $\tau^2_{between}$= 0.22	

Note: * 95% confidence interval excludes zero. HED: human equivalent dose. Approach avoidance tests were used as a reference category for the other anxiety tests.

2.S.2.7 Risk of bias for harm-related outcomes

The majority of studies with harm-related objectives clearly reported the methods to assess harm-related outcomes; which effect, or lack thereof, was observed in which treatment condition; and included assessments that were taken during an extended period of time (during and after drug treatment). However, poor reporting of information on blinding 1); on whether study discontinuation/withdrawal was attributed to adverse effects 2) and on instructions to participants about drug safety and potential side effects 3).



Supplemental Figure 20. Summary risk of bias plot for harm-related outcomes.

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Chapter 3

Cannabidiol in clinical and preclinical anxiety research. A systematic review into concentration-effect relations using the IB-de-risk tool

Caroline M.B. Kwee
Joop M.A. van Gerven
Fleur L.P. Bongaerts
Danielle C. Cath
Gabriël Jacobs
Johanna M.P. Baas
Lucianne Groenink

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JB and DC obtained funding for this study from the Helmholtz Institute and GGZ Drenthe. LG, DC, JB, GJ, JvG and CK contributed to the study design. FB and CK screened and selected studies, assessed risk of bias and extracted data. JvG, GJ, LG, JB and CK contributed to data synthesis. LG, DC, JB, GJ, JvG and CK interpreted the data. CK drafted the manuscript. All authors critically reviewed the manuscript and provided the final approval.

Abstract

Background: Preclinical research suggests that cannabidiol (CBD) may have therapeutic potential in pathological anxiety. Dosing guidelines to inform future human studies are however lacking.

Aim: We aimed to predict the therapeutic window for anxiety-reducing effects of CBD in humans based on preclinical models.

Methods: We conducted two systematic searches in Pubmed and Embase up to August 2021, into pharmacokinetic (PK) and pharmacodynamic (PD) data of systemic CBD exposure in humans and animals, which includes anxiety-reducing and potential side effects. Risk of bias was assessed with SYRCLE's RoB tool and Cochrane RoB 2.0. A control group was an inclusion criterion in outcome studies. In human outcome studies, randomization was required. We excluded studies that co-administered other substances. We used the IB-de-risk tool for a translational integration of outcomes.

Results: We synthesized data from 87 studies. For most observations (70.3%) CBD had no effect on anxiety outcomes. There was no identifiable relation between anxiety outcomes and drug levels across species. In all species (humans, mice, rats), anxiety-reducing effects seemed to be clustered in certain concentration ranges, which differed between species.

Discussion: A straightforward dosing recommendation was not possible, given variable concentration-effect relations across species, and no consistent linear effect of CBD on anxiety reduction. Currently, these results raise questions about the broad use as a drug for anxiety. Meta-analytic studies are needed to quantitatively investigate drug efficacy, including aspects of anxiety symptomatology. Acute and (sub)chronic dosing studies with integrated PK and PD outcomes are required for substantiated dose recommendations.

3.1 Introduction

The endocannabinoid (eCB) system is a modulator of multiple neurotransmitter systems (Kogan and Mechoulam, 2006). One of its receptors, the cannabinoid type 1 receptor (CB1R), is densely expressed throughout the brain (Herkenham et al., 1990). Consequently, cannabinoids induce a wide range of central nervous system (CNS)-mediated effects (Breivogel and Childers, 1998). Following isolation of the CB1R-binding eCBs anandamide (AEA) and 2-arachidonoyl-glycerol (2-AG) in the 1990s (Hanuš, 2007), preclinical anxiety research has increasingly focused on the eCB system (Griebel and Holmes, 2013). Marsicano et al. (2002) showed that in mice, genetic deletion or pharmacological blockade of CB1R impaired fear extinction. Inactivation of CB1R by genetic deletion or by administration of a CB1R antagonist has also been studied with respect to its effect on unconditioned anxiety, with diverging outcomes seemingly dependent on dose, animal strain and testing conditions (Lafenêtre et al., 2007). No additional studies were performed to explain this variability in outcomes.

In humans, increased subjective anxiety has been associated with disrupted AEA signaling. For example, moderate to large negative correlations between baseline serum AEA content and anxiety levels were demonstrated in healthy volunteers (n=71) (Dlugos et al., 2012) and in females with a depressive episode (n=28) (Hill et al., 2008). Conversely, in a small study in unaccompanied refugee minors (n=93), no significant correlations were found between hair AEA content and psychopathological symptoms (Croissant et al., 2020), and very recently negative correlations were found with plasma eCB levels and self-report anxiety scales in post-traumatic stress disorder (Leen et al., 2022).

Despite these somewhat conflicting findings, it has been argued that pharmacological inhibition of hydrolysis or reuptake of AEA, an endogenous ligand of the CB1R (Hanuš, 2007), could attenuate pathological anxiety. Bisogno et al. (2001) demonstrated that cannabidiol (CBD) inhibits AEA hydrolysis and cellular uptake of AEA. This mechanism, as well as other pharmacological activities like 5HT1A-activation (Campos and Guimarães, 2008), has been related to the frequently discussed anxiety-reducing properties of this important cannabis sativa constituent (e.g. Crippa et al., 2018). In addition, CBD and AEA are agonists of the transient receptor potential vanilloid subtype 1 (TRPV1) at higher concentrations (Bisogno et al., 2001; Ross, 2003), which could be involved in the anxiogenic effects that were also reported with this compound (Campos and Guimarães, 2009). Interestingly, after initial activation of TRPV1, CBD desensitizes the channel (Bisogno et al., 2001). This could, in theory, abrogate the effects of AEA at TRPV1 (Ross, 2003). However, this has yet to be experimentally confirmed.

The effects on anxiety outcomes reported in CB1R inactivation studies (Lafenêtre et al.,

2007; Marsicano et al., 2002), the possible association between subjective anxiety and disrupted AEA signaling (Dlugos et al., 2012; Hill et al., 2008), and the potential of CBD to enhance levels of this endogenous ligand of the CB1R (Bisogno et al., 2001) suggest that CBD may be suitable for therapeutic use.

This is further supported by data suggesting that the compound has a favorable safety and tolerability profile. Literature reviews on human studies suggest that CBD is well tolerated up to chronic oral doses between 1500 mg (Bergamaschi et al., 2011a) and 3000 mg CBD per day (Chesney et al., 2020). The only adverse event (AE) reported to occur more frequently with CBD compared to placebo was diarrhea (Chesney et al., 2020). In childhood epilepsy, abnormal liver function tests, pneumonia, decreased appetite, diarrhea and somnolence occurred more frequently in CBD compared to placebo conditions (Chesney et al., 2020). These AEs could be attributed to CBD inhibiting the hepatic metabolism of other medications including anti-epileptics (Bergamaschi et al., 2011a; Chesney et al., 2020). The authors concluded that the controlled use of CBD in humans is safe, although careful monitoring for interactions with other medications is necessary (Bergamaschi et al., 2011a; Chesney et al., 2020).

Uncertainty about the effective dose range of CBD may explain the somewhat conflicting results regarding the anxiety-reducing properties of this compound. A previous narrative review by Melas et al. (2021) described anxiety reduction by CBD in anxiety tests in rodents with certain doses. Some evidence for an inverted U-shaped dose-response curve was seen in the CBD condition with the elevated plus maze (EPM) test (Melas et al., 2021). However, the dose range in which anxiety-reducing effects in the EPM test were reported varied considerably, and there were also negative results. One study in rats reported anxiety-reducing effects at 2.5, 5 and 10 mg/kg intraperitoneally (i.p.), but not at 20 mg/kg (Guimarães et al., 1990), whereas in a second study in rats, beneficial effects occurred with doses ranging from 0.5 mg/kg up to 50 mg/kg i.p. (Onaivi et al., 1990). In humans, too, an inverted U-shaped dose-response curve has been found for CBD in small samples of healthy subjects who performed a public speaking task. A dose of 300 mg orally ingested CBD elicited anxiety-reducing effects, lower and higher doses did not (Linares et al., 2019; Zuardi et al., 2017).

This limited availability of results on the relationship between dose and effect provides only an initial guideline for CBD administration in humans. The range between minimum dose for anxiety-reducing effects and maximum tolerated dose of CBD in humans is still unclear (Skelley et al., 2020). Since dosing guidelines or maximum doses for CBD are lacking (MacCallum and Russo, 2018), there is the risk of dosing too low for a therapeutic effect, which may ultimately lead to confusion about unexpected null findings. Furthermore, subjects may be exposed to undesirable drug effects that could have been avoided when knowledge about maximum tol-

erable exposure was available. Despite the importance of integrated assessment of preclinical and clinical dose-effect relationships of a new compound before it is administered in humans (Van Gerven and Cohen, 2018), pharmacology-based dose-selection has not been performed for CBD. This omission may at least partly be responsible for the 'slow dawn of the long-predicted era of cannabinoid medicines' (Young and Nutt, 2021).

The primary aim of this study was to predict the CBD plasma concentration range in which anxiety-reducing effects of CBD can be expected to occur in humans. To achieve this objective, we used the IB-de-risk tool, developed by Van Gerven and Cohen (2018). This tool summarizes pharmacokinetic (PK), pharmacodynamic (PD) and safety data from an Investigator's Brochures of novel drugs in development, but it can also be used to obtain an overview of published preclinical and clinical literature (see, e.g., Cohen et al., 2022). For the current work data on CBD doses, CBD plasma exposure levels, effects on anxiety outcomes and undesirable effects, were obtained with systematic review of the literature, and were entered in the tool. The IB-de-risk approach yields a structured, tabular and color-coded overview from which patterns become apparent that would otherwise be very hard—if not impossible—to derive from a narrative synthesis alone. The obtained semiquantitative color-coded overview of all the preclinical and clinical data maximizes understanding of what would otherwise be separate chunks of data (Van Gerven and Cohen, 2018), and hence can aid in predicting the therapeutic window for anxiety-reducing effects of CBD in humans.

3.2 Methods

This review was preregistered on PROSPERO (CRD42021251490 and CRD42021236572).

Protocol CRD42021236572 had already been registered with the aim of meta-analytically summarizing the evidence of PD effects of anandamide breakdown and/or cellular reuptake inhibitors, including CBD. For the current review, we included only the studies in which CBD was used as a pharmaceutical, as was described in protocol CRD42021251490: 'In order to address our overall research aim of establishing the therapeutic window of CBD in which anxiolytic effects in humans are to be expected, PK data extracted in the present review will be combined with data from a second review. This review on fear expression, fear learning and anxiety symptoms has been registered with PROSPERO (CRD42021236572)'. Hence, for the current paper, we analyzed all results that concerned CBD from this broader review, as per protocol.

3.2.1 Search strategy

The two systematic literature searches were conducted in line with Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines. PRISMA checklists are included as Supplemental Tables 1 and 2. Studies were searched in the electronic databases PubMed and Embase using both free text and underlying terms (MeSH and Emtree, respectively) up to 19 May 2021. Only peer-reviewed studies were included. No restrictions were placed on publication year or language.

The full search strategies are found in Supplemental Table 3.

Preregistered but as of yet unpublished studies were searched as well in ClinicalTrials.gov, the EU Clinical Trials Register, the Australian and New Zealand Clinical Trials Registry, Animal Study Registry (German Centre for the Protection of Laboratory Animals) and Preclinicaltrials.eu, to get an indication of potential positive results bias.

3.2.2 Inclusion and exclusion criteria

3.2.3 Studies.

For human studies with anxiety outcomes, only randomized designs, in which a CBD condition was compared to a non-active placebo/vehicle condition, were eligible. The use of randomization is usually not reported in animal research (Muhlhausler et al., 2013). Due to underreporting of this important aspect of study design, it has of yet not been empirically demonstrated whether the use of randomization would influence outcomes. Therefore, in animals we considered vehicle-controlled experiments without information about randomization and explicitly non-randomized but controlled studies to be eligible as well. For studies with PK outcomes, both studies with and without a control condition were considered eligible.

3.2.4 Participants

Included were studies with healthy, adult non-human mammals with a common naturally occurring phenotype, or bred or engineered for having an anxious phenotype, and with healthy adult humans or subjects diagnosed with an anxiety disorder according to the DSM criteria applied in included studies. This includes DSM-IV and DSM-5 specific phobia, social anxiety disorder (social phobia), panic disorder, agoraphobia, and generalized anxiety disorder, and DSM-IV hypochondriasis, post-traumatic stress disorder and obsessive-compulsive disorder. Experimental procedures in animals primarily aimed at inducing stress (e.g. restraint stress), rather than an anxiety(-like) response, fell beyond the scope of this paper. With regard to hu-

man studies, we excluded studies that tested chronic users of cannabis compounds; occasional use of cannabis compounds in the past was allowed, provided that subjects were in a drug-free state while participating in the experiment. Studies that allowed stable concomitant medication for anxiety and/or depression were included. Because of pregnancy-associated changes in PKs (Verstegen and Ito, 2019), studies in pregnant or lactating subjects were excluded.

3.2.5 Intervention

Studies that employed single or repeated administration of CBD were included. For within-subject designs, a washout period of at least 24 h was required to reduce carryover effects. Excluded were:

a Experimental arms with intracerebral/ intracerebroventricular/intravenous administration;

b Experimental arms in which other substances (e.g. other cannabinoids) were co-administered as part of the investigation;

c Experimental arms with products containing more than 0.3 % Δ9-tetrahydrocannabinol on a dry weight basis;

d For single-dose studies with anxiety outcomes, time between drug administration and anxiety assay of $\geq$ 24 h. (For (sub)chronic dosing studies, which frequently employ $\geq$ 24 h to distinguish delayed from acute CBD effects, time between last drug administration and anxiety assay of $\geq$ 24 h were allowed.)

3.2.6 Outcomes

For search 1, studies were eligible for inclusion when they reported on the outcome of fear expression, fear learning (within Pavlovian fear conditioning paradigms) and/or anxiety disorder symptoms. Eligible outcome domains were subjective (humans only), neurophysiological, neuroendocrine, autonomic, behavioral and neuronal activity or connectivity during an anxiety test in brain regions involved in emotion processing and regulation. For an outcome to be eligible, outcome type had to be continuous.

For search 2, to be eligible for inclusion studies had to report on the PK outcome of Cmax and/or area under the curve (AUC). In humans, absorption of CBD typically does not continue for more than 10 h after administration (even in powder form, which is associated with delayed

Tmax) (Millar et al., 2018; Izgelov, Davidson et al., 2020a). After CBD administration via various routes in humans, Tmax occurs between 0 and 5 h (Millar et al., 2018). Rats and mice treated orally with various commercially available drugs also show average Tmax between 0 and 5 h (Yoshimatsu et al., 2020). To have a broad enough search window, highest reported plasma CBD levels measured within 10 h of drug administration were included as Cmax. Our second alternative outcome measure was the reported area under the plasma concentration curve (AUC).

3.2.7 Study screening and selection

Titles and abstracts of studies retrieved using the search strategy were independently screened by the first reviewer (CK) and second reviewer (FB or one of the collaborators on the PROSPERO CRD42021236572 project) to identify studies that appeared to meet the inclusion criteria. They then independently screened the full text of these studies for eligibility. Disagreements about inclusion or exclusion were resolved through discussion, if no consensus was reached a third (LG) or fourth reviewer (JB) was consulted.

3.2.8 Data extraction

All relevant data were extracted by one author (CK), 10% was extracted by a second reviewer (FB or one of the collaborators on the PROSPERO CRD42021236572 project). The results were compared, discrepancies identified and resolved through discussion (with a third reviewer (LG) and fourth reviewer (JB) when necessary). According to the population, intervention, comparison, outcome framework (Schardt et al., 2007), we recorded the details of the populations, interventions (including concomitant medication in human studies) and outcomes. The comparison group, if there was any, always received placebo/vehicle.

If PK parameters of interest (Cmax, AUC) were not fully reported in numbers, we requested the corresponding author to provide this information. In case no answer was received within 2 months and data were presented graphically, Cmax was estimated using Plot Digitizer software (`http://plotdigitizer.sourceforge.net/`).

If AUC to infinity (AUC0-inf) was reported, we chose this outcome rather than AUC until the last measurable concentration (AUC0-t), provided that the dose-corrected difference between these two parameters was < 20 % of AUC0-t. This was used as a criterion to gauge whether the sampling interval was sufficient to adequately estimate total exposure (PhUSE CSS, 2014). If the difference was > 20 %, AUC0-inf was deemed inadequate and was not extracted. If AUC0-inf was not reported, we used AUC0-t, provided that the PK profile showed that plasma levels approached zero at the last measurable concentration. If this was not the

case, AUC0-t was deemed inadequate and was not extracted, unless a reported elimination rate constant or elimination half-life allowed for extrapolation of AUC0-t to AUC0-inf (PhUSE CSS, 2014).

3.2.9 Primary and secondary outcomes

In general, if available, we always selected results for the primary endpoint as predefined by the authors. In case of comparisons at multiple timepoints, the anxiety assessment during the anxiety test was selected as primary endpoint. If applicable (in humans), we also collected results for the assessment most closely preceding the anxiety test to assess anticipatory anxiety. Often, for the outcome of conditioned freezing (but not for other outcomes), multiple comparisons over time were reported. We then opted for the last comparison made.

We used decision rules when multiple results were available in studies. The preferred outcome measures are listed per outcome domain in Supplemental Table 4. To decide which result to collect, we established a priori, how well an outcome measure represented the outcome that it was aimed to operationalize. If the preferred outcome measures were not reported, we selected the most frequently reported outcome measure across studies employing the same anxiety test to avoid unnecessary heterogeneity. If this outcome measure was not reported either, we selected the second most frequently reported outcome measure across studies employing the same anxiety test, etc.

For harm-related information, we searched in the included studies with the terms 'harm', 'adverse', 'side', 'unwanted', 'undesirable', 'safe*', 'toler*'. We also included assessments of body temperature, locomotor activity and catalepsy as harm-related outcomes.

3.2.10 Assessment of risk of bias

Included studies were assessed independently by two authors (CK and FB or one of the collaborators on the PROSPERO CRD42021236572 project) using the Systematic Review Centre for Laboratory animal Experimentation's risk of bias tool for animal studies (SYRCLE's RoB tool) (Hooijmans et al., 2014) with a vehicle control group. We used version 2 of the Cochrane risk of bias tool (RoB 2.0) (Sterne et al., 2019) for human outcome studies.

The following types of bias were assessed for the review of anxiety outcomes (terms corresponding to the SYRCLE's RoB tool and Cochrane RoB 2 tool, respectively):

For animal studies:

1. Sequence generation; 2. Baseline characteristics; 3. Allocation concealment; 4. Random housing; 5. Blinding of caregivers and investigators; 6. Random outcome assessment; 7. Blinding of outcome assessor; 8. Incomplete outcome data; 9. Selective outcome reporting and 10. Other conflicting interests.

For human studies:

1. Bias arising from the randomization process; 2. Bias due to deviations from intended interventions; 3. Bias due to missing outcome data; 4. Bias in measurement of the outcome 5. and Bias in selection of the reported result.

Since existing tools are aimed at effect studies, and are not applicable to studies with PK outcomes, we assessed bias for the PK review by considering the following:

1. Bias due to confounders
   Was food intake prior to dosing reported? Was, in human studies, concomitant medication reported? Were, in human studies, drugs-of-abuse tests conducted? Was dissolving vehicle reported when drugs were administered orally?

2. Bias due to missing data
   Were outcome data available for all, or nearly all, participants? Were reasons for missing data reported and if yes, is it likely that results were biased because of missing data? Is there evidence that results were robust to the presence of missing data?

3. Bias in measurement of the outcome
   Were analytical quality control and method validation procedures reported?

To assess bias in studies with harm-related objectives, we considered the following (based on the CONSORT extension on reporting of HARMS) (Ioannidis et al., 2004):

1. To what extent were study subjects aware of the potential AEs associated with the substance they were taking?

2. Was collection and assessment of safety information blinded?

3. Was the manner in which safety information was collected described clearly and thoroughly?

4. Was it clearly reported which AEs/safety outcomes occurred in which treatment arm?

5. Were discontinuations/withdrawals due to safety-related events clearly reported?

6. Was it clear up until when participants safety information was collected?

The Rob 2.0 tool has the option for judging each type of bias as 'high'. In addition, the development group for Cochrane RoB 2.0 recommends that a result should be judged as high risk of bias when some concerns exist for multiple types of bias at the same time (Higgins et al., 2021). For the other risk assessments, we opted for the term 'unclear' rather than 'some concerns' or 'high concerns', because these assessments do not have a strong empirical basis (Ioaniddis et al., 2004; Vollert et al., 2020).

3.2.11 Data synthesis

3.2.11.1 Outcome categorization.

Comparisons between CBD and placebo/vehicle arm(s) as mentioned by the authors in the studies were used to decide on the presence/absence and direction of CBD treatment effects on fear learning, fear expression and anxiety symptoms. If authors did not explicitly report statistical significance for a CBD versus vehicle/placebo comparison (Todd and Arnold, 2016), we interpreted this as a non-significant difference.

If multiple outcomes belonging to more than one outcome domain (see Supplemental Table 4) were used within an experiment, we considered these as one observation. If the authors reported a significant result on at least one of these outcomes, the observation was categorized as representing a significant CBD effect.

Harm-related hypotheses and hypothesis tests are uncommon (Ioannidis et al., 2004). Nevertheless, for CBD we expected CNS inhibition (Rosenkrantz et al., 1981), which could lead, for instance, to decreased motor activity, sedation or somnolence. Next to type of AE, we gauged relatedness of AE to CBD by comparing AE occurrence between CBD and placebo/vehicle conditions. A higher frequency in the CBD condition would argue for relatedness to this compound. Also, a dose-response relationship in the form of increasing occurrence of an AE with increasing doses of CBD increases the probability of CBD relatedness.

We categorized information on harms based on clinical severity. We based our categorization on The Veterinary Cooperative Oncology Group – Common Terminology Criteria for Adverse Events (LeBlanc et al., 2016). Our categories were 'AE very mild/infrequent and/or uncertain relationship to CBD', 'undesirable effects' (mild or moderate clinical signs, self-limiting,

not requiring intervention, or non-invasive intervention indicated, relatedness to CBD probable), 'more severe AEs'(medically significant but not immediately life threatening, relatedness to CBD probable), 'serious irreversible toxicity and/or death'.

A color-coded overview of the outcomes was construed using the IB-de-risk tool (Van Gerven and Cohen, 2018), which contains all the studies included in the data synthesis. Each row contains a separate observation. First, experiments within studies were considered as separate observations. Second, studies in which different doses were used within an experiment were considered as separate observations for each administration. Third, measurements of anxiety outcomes and of potential side effects were considered as separate observations. Rows were sorted by Cmax and AUC, measured or otherwise imputed, to obtain an impression of a concentration-effect association. The color coding scheme, which was based on outcome categorization, is shown in Table 1.

Table 1. Color coding scheme used for the overview of the outcomes.

White No anxiety outcomes measured
Light green Anxiety outcomes measured, no effect observed
Green Anxiolytic effects
Light yellow AE very mild/infrequent and/or uncertain relationship to CBD
Yellow Undesirable effects
Orange More severe adverse effects
Red Serious irreversible toxicity and/or death
Pink Imputed PK parameter
Grey No PK estimation could be made

Note: AE: adverse event; CBD: cannabidiol; PK: pharmacokinetic.

3.2.11.2 Imputation of PK parameters

To estimate the relation between systemic exposure and therapeutic or undesirable effects across different species and studies, we inferred maximum plasma concentration (Cmax) and AUC for studies that measured anxiety outcomes, but did not include this PK information. We did so by using papers that reported CBD's PK parameters of systemic exposure in the same species.

Results from an earlier review suggest that the use of lipid formulations and subjects being in a fed state increases Cmax and AUC (Millar et al., 2018). We therefore matched PK studies and experiments that focused on anxiety outcomes on these parameters, before estimating missing PK parameters. We used linear inter- or extrapolation (per administration mode, per

species) for our estimations. Non-linear trendlines were fit when visual inspection of plots suggested a non-linear association. We subsequently selected the method with the largest explained variance. Rows were then sorted by Cmax or AUC(measured or otherwise imputed).

With multiple dosing, accumulation of CBD in human adipose tissues leads to prolonged elimination half-life (Lucas et al., 2018) up to around 68 h with multiple dosing (Hosseini et al., 2020; Taylor et al., 2018). Therefore, even with administration once daily, CBD is eliminated incompletely from the body at the time a new dose is given. Dose-dependent moderate drug accumulation was reported at steady state (1.8- to 2.6-fold for 750 and 1500 mg bidaily doses) (Hosseini et al., 2020; Taylor et al., 2018). This indicates that PK estimates for multiple dose studies would require complex PK modelling. Since we consider this to be beyond the scope of this paper, we limited ourselves to estimating missing plasma exposure levels only for single-dose studies.

Details about PK estimates for single-dose human and animal studies are described in the Supplemental Material.

3.2.12 Interpretation

We provided a narrative synthesis of the findings discussing between-species translatability, anticipated effective human dose and safety margin using the color-coded overview. More specifically, we inspected our color-coded overview for the presence/absence of different levels of severity of AEs, and the drug concentrations with which these AEs occurred. The lowest drug concentrations with predominantly 'desirable effects' constitute the lower level of the therapeutic range.

3.2.12.1 Risk of bias due to missing results in the synthesis

To assess selective reporting bias, we compared the tests and outcomes planned by the original investigators with those reported in the published study. When published protocols were not available, we compared the methods and the results sections.

3.3 Results

3.3.1 Results of searches

With our PD search that was focused on anxiety outcomes, we found 7248 records. After duplicates removal, we screened 5887 records, from which we reviewed 244 full-text articles and included 69 studies. Of these studies, 53 were included in the data synthesis. With our PK search

that focused on PK outcomes, we found 2404 records. After duplicates removal, we screened 1843 records, from which we reviewed 176 full-text articles and included 43 studies. Of these studies, 34 were included in the data synthesis. The selection processes for both searches are displayed in Figure 1. Ongoing and incomplete studies are displayed in Supplemental Table 5.

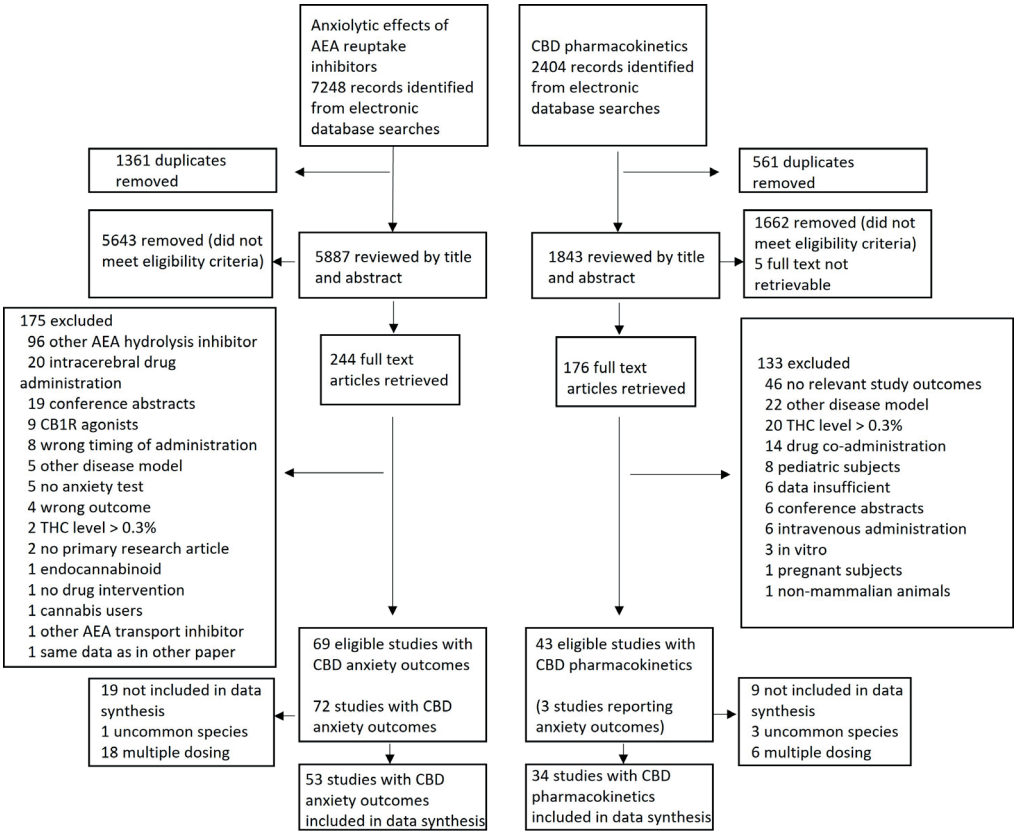

Figure 1. Flowchart displaying the study selection process.

Note: AEA: anandamide; CBD: cannabidiol; CB1R: cannabinoid type 1 receptor; THC: Δ9-tetrahydrocannabinol.

3.3.2 Description of studies included in the data synthesis

The included studies and their characteristics are presented in Supplemental Table 6 (studies with anxiety outcomes) and Supplemental Table 7 (studies with PK data).

In all human studies, the administration route was oral (p.o.). In mice and rats, drugs were predominantly administered via the i.p. route. Across studies, the most frequently assessed outcome domain was behavioral and the most frequently used anxiety test the EPM.

In humans, nine studies reported anxiety outcomes (Bergamaschi et al., 2011b; Crippa et al., 2004, 2011, 2021; Fusar-Poli et al., 2009, 2010; Linares et al., 2019; Zuardi et al., 1993, 2017) and 14 studies reported PK outcomes but no anxiety outcomes (Atsmon et al., 2018; Crockett et al. 2020; Grimm et al., 2018; Hosseini et al., 2020; Knaub et al., 2019; Martín-Santos et al., 2012; Patrician et al., 2019; Perkins et al., 2020; Schoedel et al., 2018; Taylor et al., 2018, 2019; Tayo et al., 2020; Verrico et al., 2020; Williams et al., 2021). In three studies, both types of outcomes were reported (Crippa et al., 2021; Fusar-Poli et al., 2009, 2010).

In mice, 20 studies reported anxiety outcomes (Aso et al., 2019; Assareh et al., 2020; Breuer et al., 2016; Casarotto et al., 2010; Deiana et al., 2012; Florensa-Zanuy et al., 2021; Kasten et al., 2019 ; Long et al., 2010, 2012; Myers et al., 2019; Nardo et al., 2014; Navarrete et al., 2018; Onaivi et al., 1990; Schiavon et al., 2016; Todd and Arnold, 2016; Todd et al., 2017; Twardowschy et al., 2013; Uribe-Mariño et al., 2012; Zagzoog et al., 2020; Zieba et al., 2019) and four reported PK outcomes but no anxiety outcomes, or no CBD-vehicle comparison was reported (Anderson et al., 2021; Brzozowska et al., 2016; Majimbi et al., 2021; Pang et al., 2021). In two studies, both types of outcomes were reported (Deiana et al., 2012; Zieba et al., 2019).

In rats, 24 studies reported anxiety outcomes (Almeida et al., 2013; Espejo-Porras et al., 2013; Gáll et al., 2020; Gazarini et al., 2014; Guimarães et al., 1994; Hložek et al., 2017; Javadi-Paydar et al., 2019; Jurkus et al., 2016; Kajero et al., 2020; Karniol et al., 1974; Lemos et al., 2010; Mahmud et al., 2017; Malone et al., 2009; Martín-González et al., 2018; Moreira et al., 2006; Murkar et al., 2019; O'Brien et al., 2013; Resstel et al., 2006; Rock et al., 2017; Shallcross et al., 2019; Shoval et al., 2016 ; Song et al., 2016; Stern et al., 2012, 2015) and 10 studies reported only PK outcomes (Cherniakov et al., 2017; Deiana et al., 2012; Feng et al., 2021; Izgelov et al., 2020b, 2020c, 2020d; Nagao et al., 2020; Paudel et al., 2010; Xu et al., 2019; Zgair et al., 2016). In one study, both types of outcomes were reported (Hložek et al., 2017).

As shown in Figure 1, 28 studies (n=19 with anxiety outcome, n=9 with CBD PKs) that were initially eligible for this review, were not included in the data synthesis.

First, multiple dose regimens were not included because of the complex PK modelling that would be required which would not be conceivable with the available data. For this reason, one study with anxiety outcomes (Masataka, 2019) and one with PK outcomes in humans (Taylor et al., 2020) were not included in the synthesis. Two studies with multiple dosing and PK objectives (Bartner et al., 2018; Vaughn et al., 2020) and one with anxiety outcomes in dogs (Morris et al., 2020) were not included. For the same reason, the following studies with PD outcomes in mice and rats were not included: Bis-Humbert et al. (2020), Campos et al. (2012, 2013), Cheng et al. (2014a, 2014b), Coles et al. (2020), Elbatsh et al. (2012), Fogaça et al. (2018), Luján et al. (2018, 2020), Murphy et al. (2017), Pang et al. (2021), Schleicher et al. (2019), Silvestri et al.

(2020), Watt et al. (2020a, 2020b).

Second, the following studies that contained PK data for cats (Kulpa et al., 2021), horses (Ryan et al., 2021), minipigs (Wray et al., 2017), guinea pigs (Paudel et al., 2010) and rabbits (Mannila et al., 2007), and PD data from one study which assessed CBD effects on startle in capuchin monkeys (Saletti et al., 2017) were excluded. We could either not use the PK data to estimate plasma exposure levels in similar species (Manilla et al., 2007; Paudel et al., 2010; Wray et al., 2017) or PK data were not available to estimate plasma exposure levels (Saletti et al., 2017). Third, PK and PD results for cats (Kulpa et al., 2021) and horses (Ryan et al., 2021) were not included in the synthesis. These species are uncommonly used as a model to predict human kinetics and toxicity; the translational value may be limited (The National Institute of Public Health and the Environment and 3Rs-Centre Utrecht Life Sciences, 2015).

3.3.3 Risk of bias of studies included in the data synthesis

We analyzed risk of bias per study, given the overlap of aspects that could lead to bias between experiments in the same studies. A summary is provided in Supplemental Figure 5.

Overall risk of bias was unclear for anxiety outcomes in all animal studies due to lack of information about blinding, dropout and/or handling of missing data, and randomization. A high degree of similarity between CBD and control condition could often be assumed, since animals were housed under controlled conditions, were almost invariably of the same sex (male), and often, animals were habituated to the testing environment before submission to the anxiety test.

Our overall risk of bias judgements for human studies with respect to anxiety outcomes ranged from low to high. All human studies were randomized and used identical appearing capsules to conceal the allocation to CBD and placebo treatments. In general, risk of bias due to missing outcome data was considered low, as in most studies, numbers of patients after randomization were equal to the number of patients for whom results were available (Bergamaschi et al., 2011b; Crippa et al., 2004, 2011; Linares et al., 2019). In contrast, highly variable CBD plasma concentrations (M=17; standard deviation=29 ng/ml) (Fusar-Poli et al., 2009, 2010) may have led to biased estimates of per-protocol effects. Furthermore, increased mental sedation in the CBD condition may have affected subjective anxiety ratings (Crippa et al., 2004).

Most human studies used healthy volunteers and described restrictions concerning the use of recreational drugs. However, concomitant medication use and drugs-of-abuse tests were not reported in several papers (Bergamaschi et al., 2011b; Crippa et al., 2004, 2011, 2021; Crockett et al., 2020; Hosseini et al., 2020; Izgelov et al., 2020a; Knaub et al., 2019; Linares et al., 2019; Patrician et al., 2019; Schoedel et al., 2018; Taylor et al., 2018, 2019; Tayo et al., 2020; Williams et al., 2021; Zuardi et al., 1993). Information about bioanalytical methods validation in studies

with PK data varied from no description (e.g. Izgelov et al., 2020a) to a detailed one (e.g. Perkins et al., 2020). For the majority of PK studies, overall risk of bias was unclear.

For animal studies with harm-related outcomes but without explicit harm-related study objectives, overall risk of bias was unclear due to underreporting of information needed to assess bias. For all human studies, it was unclear whether participants knew beforehand whether information on harms was collected, and whether assessment of safety information was blinded. Method of assessing AEs was usually described, although sometimes concise. Period of assessing safety was usually specified, but relatively short, with some exceptions (e.g. a follow-up period of 8-14 days (Schoedel et al., 2018) and 2 weeks (Taylor et al., 2019).

3.3.4 PD effects across species

PD results are summarized using the color coding scheme in Table 1. As shown in Supplemental Table 8, no clear pattern of associations between Cmax, AUC and frequency of anxiety-reducing or adverse effects were discernible, when looking at all species and anxiety tests together. Across species, for the majority of observations, CBD had no effect on anxiety outcomes (121 out of 172; 70.3%). Importantly, 138 of 172 rows (80.2%) were observations from studies that investigated multiple doses of CBD without necessarily expecting an anxiety-reducing effect with each dose. Anxiety-reducing effects were reported across the entire range of systemic exposure (300 $\sim$ 53,000 ng/mLxh). Regardless of effect on anxiety outcome, sample sizes per experimental condition were rather small (between n=5 and n=22). There were in total 19 rows with very mild AEs, infrequent AEs and/or AEs with an uncertain relationship to CBD. A comparable number of rows (n=22) contained observations of mild or moderate clinical signs that were probably related to CBD. The absence of severe AEs (which would be colored orange or red) with plasma levels that are adequate to measure anxiety-reducing effects (indicated by the color green) is in line with the advantageous safety profile of CBD in humans reported in earlier work (Bergamaschi et al., 2011a; Chesney et al., 2020).

3.3.5 PD effects within species

In the paragraphs below, we describe the most active AUC range per species, that is, the range in which anxiety-reducing effects of CBD occurred relatively frequently, when compared to other AUC levels. Sorting the data on Cmax did not change the pattern of results that was obtained by sorting on AUC.

In humans, we identified the AUC range (Table 2) with the most frequent observations of anxiety-reducingc effects, to be between $\sim$2000-2800 ng/mLxh. In this most active AUC range,

there were four of seven rows with anxiety-reducing effects with 300-600 mg CBD doses (Bergamaschi et al., 2011b; Crippa et al., 2004, 2011; Linares et al., 2019). Within the most active range, 2 of 12 effects were consistent with CNS inhibition that could be related to CBD: Increased sedation in the CBD condition compared to placebo (Bergamaschi et al., 2011b; Crippa et al., 2004). Only one study was conducted that measured anxiety outcomes with higher total systemic exposure (~3700 ng/mLxh). This study reported no anxiety-reducing effect of CBD (Zuardi et al., 2017).

Table 2. Color-coded overview of the most active AUC range in humans.

Study ID	Type of anxiety test	Dose (mg)	Effects	AUC (ng/mLxh)	Classification (see legend)
Linares 2019	SPS	300	Anxiety-reducing effect on VAMS during speech	2003	
Zuardi 1993	SPS	300	No effect	2003	
Zuardi 2017	SPS	300	No effect	2003	
Crippa 2004	fnct	400	Lower VAMS anxiety at 75 min, modulated ECD uptake	2277	
Crippa 2004	fnct	400	Higher mental sedation at 75 min	2277	
Crippa 2011	fnct	400	Lower VAMS anxiety at 75 min, modulated ECD uptake	2277	
Bergamaschi 2011	SPS	600	Lower VAMS anxiety during speech	2827	
Bergamaschi 2011	SPS	600	Less decrease in sedation anticipating speech	2827	
Linares 2019	SPS	600	No effect	2827	

Low AUC ↓ High AUC

Note: Light green: anxiety outcomes measured, no effect observed; green: anxiety-reducing effects; yellow: undesirable effects; pink: imputed PK parameter; SPS: Simulated Public Speaking; fnct: functional neuronal activation. Effects are displayed in brief, an overview of all rows and more elaborate results can be found in Supplemental Table 8.

In rats, sorting rows on AUC yielded the most active range between ~ 1500 and 2900 ng/mLxh (Table 3), with 16 of 26 anxiety-reducing effects (Gazarini et al., 2014; Guimarães et al., 1990, 1994; Lemos et al., 2010; Mahmud et al., 2017; Martín-Gonzáles et al., 2018; Moreira et al., 2006; Resstel et al., 2006; Rock et al., 2017; Shallcross et al., 2019; Song et al., 2016; Stern et al., 2012) and two effects that may be interpreted as anxiety increasing (Gáll et al., 2020; Song et al., 2016). As shown in Supplemental Table 8, null effects became more frequent with lower AUC; between ~300 and 1100 ng/mLxh there were only 3 of 15 anxiety-reducing effects. One study reported increased motor activity after CBD administration, with AUC of ~500 ng/mLxh (Hložek et al., 2017). Similarly, with larger AUC; between ~ 4400 and 17,700 ng/mLxh, there were only 4 of 22 anxiety-reducing effects (Murkar et al., 2019; Shoval et al., 2016; Stern et al., 2012). There were three cases of CBD effects on vertical and horizontal activities (Espejo-Porras et al., 2013). Drowsiness and piloerection in rats, which were categorized as severe AEs, occurred after a single dose at the high end of the AUC range (>40,000 ng/mLxh; Deiana et al., 2012).

Table 3. Color-coded overview of the most active AUC range in rats.

Study ID	Type of anxiety test	Dose (mg/kg)	HED (mg) (*60kg)	Effects	AUC (ng/mLxh)	Classification (see legend)
Almeida 2013	SI	5	1440	No effect	1473	
Guimarães 1990	AA	5	1440	Higher % open arm entries	1473	
Guimarães 1994	AA	5	1440	Higher % open arm entries	1473	
Jurkus 2016	FC d	5	1440	No effect	1473	
Malone 2009	SI	5	1440	No effect	1473	
Rock 2017	AA	5	1440	No effect	1473	
Rock 2017	S_AA	5	1440	Higher mean time in light box	1473	
Shallcross 2019	AA	5	1440	Decreased latency to enter the light compartment	1473	
Moreira 2006	FC d	5	1440	No effect	1473	
Moreira 2006	FC d	5	1440	No effect	1473	
Gáll 2020	AA	10	2880	Less rearing compared to vehicle	2945	
Lemos 2010	fnct	10	2880	Attenuation of c-Fos expression in BNST after conditioning	2945	
Mahmud 2010	AA	10	2880	More time in open arms than vehicle	2945	
Moreira 2006	FC d	10	2880	Higher no. of punished licks	2945	
Moreira 2006	FC d	10	2880	Higher no. of punished licks	2945	
Resstel 2006	FC	10	2880	Lower % time freezing, less increase in heart rate	2945	
Song 2016	FC	10	2880	Lower % freezing time at test	2945	
Song 2016	FC	10	2880	Increased % freezing time at test	2945	
Stern 2012	FC	10	2880	Lower % freezing time during context reexposure	2945	
Stern 2012	FC	10	2880	Lower % freezing time during context reexposure	2945	
Stern 2012	FC	10	2880	Lower % freezing time during context reexposure	2945	
Karniol 1974	AA	10	2880	No effect	2945	
Lemos 2010	FC	10	2880	Lower % freezing during context reexposure	2945	
Gáll (2020)	AA	10	2880	No effect	2945	
Gáll (2020)	AA	10	2880	No effect	2945	
Gazarini 2014	FC	10	2880	Lower % freezing during context test	2945	
Guimarães 1990	AA	10	2880	Higher % open arm entries	2945	
Jurkus 2016	FC d	10	2880	No effect	2945	

Low AUC ↓ High AUC

Note: Light green: anxiety outcomes measured, no effect observed; green: anxiety-reducing effects; yellow: undesirable effects; pink: imputed PK parameter; SI: Social Interaction; AA: Approach Avoidance; FC d: fear conditioning to discrete cue; S: exposed to stressor(s); fnct: functional neuronal activation; FC: fear conditioning to context. Effects are displayed in brief, an overview of all rows and more elaborate results can be found in Supplemental Table 8.

In mice, the most active range (Table 4) seemed to be between ~10,500 and 13,300 ng/mLxh, with 11 of 17 anxiety-reducing effects (Assareh et al., 2020; Breuer et al., 2016; Casarotto et al., 2010; Nardo et al., 2014; Uribe- Mariño et al., 2012). With lower AUC, between ~4400 and 8800 ng/mLxh, there were 4 of 34 rows with an anxiety-reducing effect

(Deiana et al., 2012; Todd and Arnold, 2016; Todd et al., 2017; Zieba et al., 2019) and 1 of 34 rows with an anxiety increasing effect (Kasten et al., 2019). With higher AUC, between ~22,100 and 53,000 ng/mLxh, (1/9) of results were anxiety reducing (Deiana et al., 2012) and 1 of 9 anxiety-increasing (Long et al., 2012). Mice seemed less sensitive to CBD compared to humans and rats. Within the most active AUC range in humans and rats, between ~1700 and 2200 ng/mLxh, only 2 of 13 effects in mice were anxiety reducing (Kasten et al., 2019; Schiavon et al., 2016; Twardowschy et al., 2013; Uribe-Mariño et al., 2012). There were no publications of undesirable effects in mice other than the above-mentioned anxiety-increasing effects (Kasten et al., 2019; Long et al., 2012).

Table 4. Color-coded overview of the most active AUC range in mice.

Study ID	Type of anxiety test	Dose (mg/kg)	HED (mg) (*60kg)	Effects		AUC (ng/mLxh)	Classification (see legend)
Breuer 2016	RCLB	15	72	No effect		10458	
Casarotto 2010	RCLB	15	72	Reduced marble burying		10458	
Assareh 2020	FC d	30	144	Lower % freezing	Low AUC	13254	
Assareh 2020	FC	30	144	No effect		13254	
Assareh 2020	S_AA	30	144	No effect		13254	
Breuer 2016	RCLB	30	144	Reduced marble burying		13254	
Breuer 2016	RCLB	30	144	Reduced marble burying		13254	
Breuer 2016	RCLB	30	144	Reduced marble burying		13254	
Florensa-Zanuy 2021	AA	30	144	No effect		13254	
Nardo 2014	RCLB	30	144	Reduced marble burying		13254	
Nardo 2014	AA	30	144	No effect		13254	
Schiavon 2016	AA	30	144	No effect		13254	
Uribe-Mariño 2012	Defense	30	144	Lower behavioural index for defensive immobility outside		13254	
Casarotto 2010	RCLB	30	144	Reduced marble burying		13254	
Casarotto 2010	RCLB	30	144	Reduced marble burying	High AUC	13254	
Casarotto 2010	RCLB	30	144	Reduced marble burying		13254	
Casarotto 2010	RCLB	30	144	Reduced marble burying		13254	

Note: Light green: anxiety outcomes measured, no effect observed; green: anxiety-reducing effects; yellow: undesirable effects; pink: imputed PK parameter. RCLB: Repetitive Compulsive-Like Behavior; FC d: Fc to discrete cue; FC: fear conditioning to context; S: exposed to stressor(s); AA: Approach Avoidance. Effects are displayed in brief, an overview of all rows and more elaborate results can be found in Supplemental Table 8.

In summary, our synthesis revealed a predominance of null effects on anxiety outcomes in all investigated species. Yet, for all species, the color-coded patterns may suggest an AUC range with a relatively high number of anxiety-reducing effects. For mice, the anxiety-reducing effects were predominantly observed in the marble burying test of repetitive, compulsive-like behavior. Therefore, it is not possible to differentiate between a contribution of type of anxiety

test and level of CBD exposure to these anxiety-reducing effects.

3.3.6 Risk of bias due to missing results in the synthesis

Overall, there were some concerns about risk of bias due to missing results in the synthesis.

Four preregistered studies that were completed have not yet published their results (NCT03164512, NCT04577612, NCT04790136, ACTRN12620000891921), which may be indicative of positive results bias. Within published studies, anxiety tests and outcomes described in the methods sections generally matched those reported in the results sections. However, in EPM tests, authors sometimes reported one, but not both of the conventional indices of anxiety (% open arm entries and open arm time) (Rodgers et al., 1997). This may be indicative of outcome reporting bias. In addition, more extensive reporting of an animals behavioral repertoire was rare. Safety assessments were often described in a concise way in methods and results sections, which led to an unclear risk of bias due to selective reporting.

Our synthesis was limited to single-dose regimens; multiple dose regimens were not included because the required complex PK modeling, combined with the sparsity of PK data after multiple dosing. While it is unknown how the plasma levels with multiple dose regimens relate to those with single dose regimens, the majority of effects with multiple dose regimens (93 out of 114; 81.6%) constituted no differences between CBD and placebo on anxiety outcomes.

3.4 Discussion

Preclinical research suggests that CBD may have beneficial effects in the treatment of pathological anxiety. To inform future human studies, the purpose of this study was a translational prediction of the exposure range for anxiety-reducing effects of CBD, based on its minimum exposure for anxiety-reducing effects and maximum tolerated exposure. We used the IB-de-risk tool (Van Gerven and Cohen, 2018) to synthesize PK and PD data of systemic CBD exposure in humans and animals.

Our data synthesis did not show straightforward dose-response relationships, between systemic exposure and anxiety-reducing effects, which would be expected for typical pharmacologically active drugs (Van Gerven and Cohen, 2018). None of the species showed a dose-related transition from a no-effect range, through a therapeutic anxiety-reducing range, to increasingly frequent and severe adverse effects. Across species, anxiety-reducing effects were reported within an exorbitant range of CBD exposures ($\sim$300 to 53,000 ng/mLxh). Within this range of systemic exposure, a majority of studies in our review reported no anxiety-reducing effects of CBD. Furthermore, mild to moderate AEs were observed with the same levels of

drug exposure that produced anxiety-reducing effects, and the intensity of adverse effects did not increase clearly with dose. Within species, concentration ranges were discernible in which anxiety-reducing effects of CBD occurred relatively frequent. Importantly, even in these ranges, anxiety-reducing effects were interspersed with null effects.

These findings seem to be in contrast with the therapeutic potential in treating anxiety symptoms which has been described by other authors (e.g. Crippa et al., 2018) and might be an explanation for the 'slow dawn of the long-predicted era of cannabinoid medicines' (Young and Nutt, 2021). Although this review showed exposure-response relationships that are poorly translational and far from conventional, it would be premature to conclude that CBD does not have anxiety-reducing properties. Several alternative explanations for the lack of a clear cross-species concentration-effect relation are conceivable. Up until the beginning of the 21th century, the scientific literature contained fewer null findings than nowadays, because these findings were less likely to be to published (Shrout and Rodgers, 2018). Our comprehensive systematic literature searches may have yielded a more or less balanced representation of the literature. This includes studies with null findings, which may be attributed to individual study characteristics.

First, anxiety tests typically tap into only certain aspects of anxiety symptomatology (Sams-Dodd, 2006). It is conceivable that potentially beneficial effects of CBD are limited to some symptom dimensions of anxiety. Moreover, some anxiety tests are poor models of an anxiety disorder or anxiety symptoms, and suitability to measure anxiety-reducing drug effects may differ greatly between these tests (Bach, 2022). However, the strength of the IB-de-risk tool is to summarize all effects, without cherry picking, to allow an overall perspective.

Beneficial effects of CBD might also be specific for anxious sub-populations with specific biological features. For example, sex- and brain region-specific differences in CB1R density in mice were induced by early life stress (Dow-Edwards, 2020) and sub-chronic stress during adult life (Zoppi et al., 2011). Furthermore, in healthy humans, changes in eCB plasma levels in response to acute stress were larger in men than in women (Dlugos et al., 2012). Behavioral effects of exogenous cannabinoids may be more or less pronounced dependent on such differences in the eCB system (Martín-Sanchez et al., 2021).

A third explanation for null effects may be the less than optimal timing between drug administration and anxiety tests to measure therapeutic effects in some studies. Levels of CBD in the brain may continue to rise after peak concentration in plasma (Deiana et al., 2012). While the former is of primary interest considering expected CNS-related effects, estimations of the latter, are commonly being used as benchmark for test commencement. In addition, after oral administration, the time at which plasma levels are highest may differ substantially between in-

dividual subjects. This has been demonstrated for rats (Cherniakov et al., 2017: 27) and humans (Taylor et al., 2018: 1061), but to a lesser extent for mice; Pang et al. (2021: 2044) reported no differences in Tmax between six mice. In addition, Tmax strongly depends on the formulation used in rats (Cherniakov et al., 2017: 27), mice (Majimbi et al., 2021: 8) and humans (Izgelov, Davidson et al., 2020a: 3). Thus, in some cases, the anxiety test may have already been terminated at the time plasma levels of CBD have reached their peak.

Lastly, included studies may have been underpowered to detect modest CBD effects, because of the generally small samples sizes used. A future meta-analysis may be helpful to qualitatively summarize the findings across studies while taking imprecision of reported effects into account. Moreover, such an endeavor may help to elucidate whether the effect of CBD on anxiety outcomes is dependent on certain study characteristics, such as the specific anxiety features that are under investigation, and the corresponding anxiety-related tests.

As stated above, there was no evidence of a clear exposure- or concentration-effect association across species. The lack thereof could at least partly be attributed to differences in active ranges between species. That is, rats and humans seemed more sensitive to CBD effects than mice. In rats and humans, beneficial CBD effects on anxiety outcomes were clustered in a range of concentrations around ∼2000 ng/mLxh. In humans, this corresponds to oral dosages between 300 and 600 mg. Studies using higher dosages are largely lacking in humans, but in rats null effects became again more frequent with higher concentrations. In mice, the same pattern as in rats was observed in the order of fivefold increased concentrations. Anxiety-reducing effects clustered at moderate plasma concentrations (∼ 11000 ng/mLxh) and more numerous null effects occurred at higher plasma concentrations. It has been suggested that CBD exhibits a complex inverted U-shaped exposure-response relationship (Zuardi et al., 2017).

At present, there is no agreed upon explanation for why anxiety-reducing effects would disappear with higher concentrations. There are various explanations for such patterns (Calabrese & Baldwin, 2001). One possibility is that therapeutic activity is overcome by adverse effects at higher doses or concentrations. This review does not provide arguments for this explanation, because none of the species showed a clear increase of adverse effects with higher CBD levels. It has also been suggested that biphasic effects of CBD could be attributed to its multiple, partly antagonistic receptor targets that may be activated at different concentrations. This could, for example, involve the activation of the TRPV1 by CBD at higher concentrations (Campos and Guimarães, 2009). Data from Campos and Guimarães (2009) lend support to this notion. That is, the anxiety-reducing effect of CBD in the EPM test in lower doses disappeared with increasing doses, but was rescued by coadministration of a TRPV1 antagonist.

This is the first study to synthesize PK and PD data from the large and diverse body of lit-

erature on systemic CBD exposure in humans and animals. It comes with several strengths and limitations. The strength of the employed IB-de-risk approach is integration of all this data to make predictions of expected drug effects in humans. At the other side of the coin, effects of CBD were assessed on highly variable outcomes, including subjective, neurophysiological, autonomic and behavioral outcomes and changes in neuronal activity or connectivity were measured during various anxiety tests. Many of these tests elicit behavior that belongs to an animal's standard repertoire, and may not be controlled by the same neurobiological mechanisms as maladaptive avoidance behaviour in patients (Bach, 2022). Moreover, many anxiety tests are sensitive to specific classes of medication for anxiety, but less so to other drug classes (Griebel and Holmes, 2013). This may explain the inconsistent effects of CBD on anxiety outcomes and the absence of clear dose-effect patterns in the current work. A meta-analytic approach is needed to elucidate potential moderators of CBD effects, including type of anxiety test.

Some limitations are worth mentioning that are related to the imputations of missing PK data. First, the synthesis was limited to acute CBD effects. No PK estimates were made for multiple dose regimens, because this would require complex PK modelling and there was not sufficient data to reliably perform such calculations. PD drug effects may accumulate over time, or have a delayed onset (Agid et al., 2003). Depending on CBD's mechanism of action for anxiety-reducing effects (the interested reader can refer to Crippa et al., 2018, for an overview), either an acute or (sub)chronic treatment regimen may be needed for the drug to reliably exert these effects. Second, we accounted for type of formulation and diet in our imputations of missing PK data with oral administration of CBD in humans, because Cmax (like Tmax) depends on the formulation used (Cherniakov et al., 2017: 27; Izgelov et al., 2020a: 3; Majimbi et al., 2021: 8). Furthermore, evidence exists that PK parameters in humans are affected by food intake (Taylor et al., 2018: 1064). For rats and mice, however, there was too little PK data looking into effects of different formulations and diets available to take these parameters into account. That being said, in rats and mice CBD was mostly administered via the i.p., instead of the oral route.

Unfortunately, however, only three studies reported PK data for i.p. administration (Deiana et al., 2012; Javadi-Paydar et al., 2019; Zieba et al., 2019), which may have introduced another source of bias. That is, by basing our estimations of missing PK parameters on such sparse data we may have influenced the sorting on Cmax and AUC across species, from which, indeed, no interpretable pattern could be identified.

3.4.1 Conclusion

This systematic analysis of the literature regarding anxiety-reducing properties of CBD is a first attempt to estimate its active and safe dose range in humans, from a translational cross-species

perspective. The majority of effects were null effects, and anxiety-reducing effects were not concentrated in a particular range of blood levels across species, although some evidence for an inverted U-shaped dose-response curve was perhaps suggested when looking within species. So far, human studies that use oral doses in the 300-600 mg range tend to report anxiety-reducing effects. More data are needed to decide whether this range indeed provides a reliable anxiety-reducing effect, and what underlies the loss of a possible effect with higher concentrations seen in mice and rat studies.

3.4.2 Recommendations for future work

The current systematic review yielded a mixture of beneficial and null effects of CBD on anxiety outcomes, which raises questions about the broad therapeutic use as a drug for anxiety. Meta-analyses may provide summary effects and investigate for which aspects of anxiety symptomatology CBD could be efficacious. A meta-analysis with this objective (PROSPERO CRD42021236572) is currently ongoing.

Furthermore, little is known about the pharmacological validity of preclinical anxiety tests for measuring the effects of CBD, which should include corresponding effects in preclinical anxiety tests and in humans who suffer from anxiety disorders (Ferreira et al., 2020). These knowledge gaps suggest fruitful avenues for future research.

In the current review, there was evidence of underreporting of aspects that could lead to bias in preclinical research, which included animal research and studies with PK and harm-related objectives. By reporting aspects of design, conduct, and analysis, confusion about underreporting or a study not possessing a certain quality (e.g. blinding) can be eliminated. Recommendations to optimizing design, conduct and analysis of animal research are widely available (Vollert et al., 2020). The CONSORT extension on reporting of HARMS (Ioannidis et al., 2004) could be a useful guideline for studies with safety outcomes.

Lastly, there is an urgent need for integrated acute and (sub)chronic dosing PK/PD studies that measure both types of outcomes, especially in humans. This integration is needed to account for the influence of PKs on anxiety-reducing effects and to overcome the limitations inherent in synthesizing these different types of data across publications and species. Together, these efforts will greatly advance the translation of preclinical research to clinical applications of CBD in humans.

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3.4.5 Conflict of interest

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3.S Supplemental Material

Contents

Supplemental Table 1. PRISMA 2020 for Abstracts Checklist

Section and Topic	Item #	Checklist item	Reported (Yes/No)
TITLE			
Title	1	Identify the report as a systematic review.	Yes
BACKGROUND			
Objectives	2	Provide an explicit statement of the main objective(s) or question(s) the review addresses.	Yes
METHODS			
Eligibility criteria	3	Specify the inclusion and exclusion criteria for the review.	Yes
Information sources	4	Specify the information sources (e.g. databases, registers) used to identify studies and the date when each was last searched.	Yes
Risk of bias	5	Specify the methods used to assess risk of bias in the included studies.	Yes
Synthesis of results	6	Specify the methods used to present and synthesise results.	Yes
RESULTS			
Included studies	7	Give the total number of included studies and participants and summarise relevant characteristics of studies.	Yes
Synthesis of results	8	Present results for main outcomes, preferably indicating the number of included studies and participants for each. If meta-analysis was done, report the summary estimate and confidence/credible interval. If comparing groups, indicate the direction of the effect (i.e. which group is favoured).	Yes
DISCUSSION			
Limitations of evidence	9	Provide a brief summary of the limitations of the evidence included in the review (e.g. study risk of bias, inconsistency and imprecision).	Yes

Section and Topic	Item #	Checklist item	Reported (Yes/No)
Interpretation	10	Provide a general interpretation of the results and important implications.	Yes
OTHER			
Funding	11	Specify the primary source of funding for the review.	Yes
Registration	12	Provide the register name and registration number.	Yes

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

Supplemental Table 2. PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Reported (Yes/No)
TITLE			
Title	1	Identify the report as a systematic review.	p. 139
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	p. 140
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	p. 141
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	p. 143
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	p. 144
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	p. 144
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Supplemental Table 3
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	p. 146

Section and Topic	Item #	Checklist item	Reported (Yes/No)
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	p.146
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	p. 145, Supplemental Table 4
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	p. 145
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	p. 147
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	p. 149
	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	Supplemental Table 6-7
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	p. 149 Supplemental Material

Section and Topic	Item #	Checklist item	Reported (Yes/No)
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	p. 150, Table 1
Synthesis methods	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	p. 149
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	NA
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	NA
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	p. 151
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	p. 151
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	p. 151 , Figure 1
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	p. 151 , Figure 1
Study characteristics	17	Cite each included study and present its characteristics.	p. 152, Supplemental Table 6-7
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	p. 154 Supplemental Figure 5

Section and Topic	Item #	Checklist item	Reported (Yes/No)
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Supplemental Table 8
	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	p. 154 Supplemental Figure 5
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	NA
Results of syntheses	20c	Present results of all investigations of possible causes of heterogeneity among study results.	p. 155 , Table 2-4
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	NA
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	p. 159
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	p. 159
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	p. 159
	23b	Discuss any limitations of the evidence included in the review.	p. 162
	23c	Discuss any limitations of the review processes used.	p.161
	23d	Discuss implications of the results for practice, policy, and future research.	p. 162

Section and Topic	Item #	Checklist item	Reported (Yes/No)
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	p. 143
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	p. 143
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	Supplemental Material
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	p. 164
Competing interests	26	Declare any competing interests of review authors.	p. 164
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Supplemental Tables 6-8

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

Supplemental Table 3.a Search strategies Search CBD pharmacodynamic effects

Pubmed

Search number	Search term
1	"cbd" [All Fields]
2	"cannabidiol"[MeSH Terms] OR "cannabidiol"[All Fields] OR "cannabidiolic" [All Fields]
3	"cannabinoids"[MeSH Terms] OR "cannabinoids"[All Fields] OR "cannabinoid"[All Fields]
4	"FAAH"[All Fields]
5	"Fatty Acid Amide Hydrolase"[All Fields]
6	"anandamide"[Supplementary Concept] OR "anandamide"[All Fields] OR "anandamide s"[All Fields] OR "anandamides"[All Fields]
7	#1 OR #2 OR #3 OR #4 OR #5 OR #6
8	"fear"[MeSH Terms] OR "fear"[All Fields]
9	"phob*" [All Fields]
10	"anxi*" [All Fields]
11	"defens*"
12	#8 OR #9 OR #10 OR #11
13 (final search query)	#7 AND #12

Embase

Search number	Search term
1	cbd
2	cannabidiol'/exp OR 'cannabidiol'
3	cannabinoid'/exp OR 'cannabinoid'
4	'epidiolex'/exp OR epidiolex
5	faah
6	fatty acid amide hydrolase'/exp OR 'fatty acid amide hydrolase'
7	anandamide'/exp OR 'anandamide'
8	#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7
9	fear'/exp OR 'fear'
10	phob*

Supplemental Table 3.a Search strategies Search CBD pharmacodynamic effects

11	anxi*
12	defens*
13	#9 OR #10 OR #11 OR #12
14	#8 AND #13
15	case report'/de OR 'conference abstract'/de OR 'review'/de OR 'cross sectional study'/de
16 (final search query)	#14 NOT #15

Supplemental Table 3.b Search strategies Search CBD pharmacokinetics

PubMed	
Search number	Search term
1	"CBD"[All Fields]
2	"cannabidiol"[MeSH Terms] OR "cannabidiol"[All Fields] OR "cannabidiolic"[All Fields]
3	"cannabidiol"[MeSH Terms] OR "cannabidiol"[All Fields] OR "epidiolex"[All Fields] OR "cannabidiolic"[All Fields]
4	#1 OR #2 OR #3
5	"apparent"[All Fields] AND ("volum"[All Fields] OR "volume"[All Fields] OR "volumes"[All Fields] OR "voluming"[All Fields]) AND ("distribute"[All Fields] OR "distributed"[All Fields] OR "distributer"[All Fields] OR "distributers"[All Fields] OR "distribut
6	"cmin"[All Fields]
7	"tmax"[All Fields]
8	"AUC"[All Fields]
9	"bioavailable"[All Fields] OR "bioavailibility"[All Fields] OR "biological availability"[MeSH Terms] OR ("biological"[All Fields] AND "availability"[All Fields]) OR "biological availability"[All Fields] OR "bioavailabilities"[All Fields] OR "bioavailabili
10	"absorptance"[All Fields] OR "absorptances"[All Fields] OR "absorption"[MeSH Terms] OR "absorption"[All Fields] OR "absorptions"[All Fields] OR "absorptive"[All Fields] OR "absorptivities"[All Fields] OR "absorptivity"[All Fields]
11	"peak"[All Fields] AND ("concentrate"[All Fields] OR "concentrated"[All Fields] OR "concentrates"[All Fields] OR "concentrating"[All Fields] OR "concentration"[All Fields] OR "concentrations"[All Fields])
12	"half life"[MeSH Terms] OR "half life"[All Fields] OR ("half"[All Fields] AND "life"[All Fields]) OR "half life"[All Fields]
13	("plasma"[MeSH Terms] OR "plasma"[All Fields] OR "plasmas"[All Fields] OR "plasma s"[All Fields]) AND ("level"[All Fields] OR "levels"[All Fields])
14	("plasma"[MeSH Terms] OR "plasma"[All Fields] OR "plasmas"[All Fields] OR "plasma s"[All Fields]) AND ("concentrate"[All Fields] OR "concentrated"[All Fields] OR "concentrates"[All Fields] OR "concentrating"[All Fields] OR "concentration"[All Fields] OR "

Supplemental Table 3.b Search strategies Search CBD pharmacokinetics

15	"cmax"[All Fields]
16	"pharmacokinetic"[All Fields] OR "pharmacokinetical"[All Fields] OR "pharmacokinetically"[All Fields] OR "pharmacokinetics"[MeSH Subheading] OR "pharmacokinetics"[All Fields] OR "pharmacokinetics"[MeSH Terms]
17	#5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16
18 (final search query)	#4 AND #17

Embase

Search number	Search term
1	cbd
2	cannabidiol
3	epidiolex
4	#1 OR #2 OR #3
5	pharmacokinetics
6	cmax
7	plasma AND concentrations
8	plasma AND levels
9	half AND life
10	peak AND concentrations
11	absorption
12	bioavailability
13	auc
14	tmax
15	cmin
16	apparent AND volume AND of AND distribution
17	#5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16
18 (final search query)	#4 AND #17

Supplemental Table 4. Preferred outcome measures per outcome domain

Domain	Outcome	Anxiety test	Preferred outcome measure
Subjective experience	*State anxiety*		
		Simulated public speaking	Visual Analogue Mood Scale - Anxiety factor (items calm–excited, relaxed–tense, and tranquil–troubled)
		SPECT scanning	
	Social-evaluative anxiety		
		NA	Fear of Negative Evaluation questionnaire
Behaviour	*Repetitive, compulsive-like behaviours (Angoa-Pérez, 2013; Moreno & Flores, 2012; Thomas, 2009)*		
	- Defensive burying	Marble burying test	Number of buried marbles
	- Polydipsia	Schedule-induced polydipsia	Consumed water volume
	- Nestlet shredding	Nestlet shredding test	Percentage of Nestlet shredded
	Fear memory expression/within-session fear extinction	Contextual and/or cued fear conditioning	(percentage of) time freezing
	Extinction retention	Contextual/cued fear conditioning	(percentage of) time freezing
	Fear memory reconsolidation	Contextual fear conditioning	(percentage of) time freezing

Supplemental Table 4. Preferred outcome measures per outcome domain

Domain	Outcome	Anxiety test	Preferred outcome measure
	Approach behaviour during approach/ avoidance conflict (unconditioned approach behaviour and elicited aversion; Dulawa & Hen, 2005; Carobrez & Bertoglio, 2005; Prut & Belzung, 2003)		
	- Exploration of environment	Elevated plus maze	First choice: proportion/percentage of open arm entries Second choice: proportion/percentage of open arm time or distance traveled in open arms
		Light dark test	First choice: proportion/percentage of entries in light compartment Second choice: proportion/percentage of open arm time or distance traveled in light compartment
		Open field test, actitrack	(Percentage) of time in centre of arena, Percentage of distance traveled in centre of arena
		Novel object exploration test	Time spent near a novel object
	- Food consumption	Novelty suppressed feeding test	Latency to feed
	Suppression of punished responses		
	- Water consumption	Vogel conflict test	Number of punished licks

Supplemental Table 4. Preferred outcome measures per outcome domain

Domain	Outcome	Anxiety test	Preferred outcome measure
	Panic-like behaviour - Defensive immobility (immobility followed by autonomic reactions, such as defecation, exophthalmia and/or micturition)	Prey versus predator paradigm	Frequency of defensive immobility outside the burrow proportional to time spent outside the burrow
	Social interaction	Social interaction test	First choice: Time in active social interaction Second choice: Total time in social interaction
	Tail posture	Exposure to noise (3 min 90 dB)	Time tail stiff
	Defecations	Open field test	Number of fecal boluses
Autonomic	*Sympathetic nervous system activation (Joyner et al., 2008; Kim et al., 2018; Rosebrock et al., 2017)*		
	- Heart rate	Exposure to noise (3 min 90 dB)	Heart rate
		Fear memory expression	
		Simulated public speaking	
	- Blood pressure	Simulated public speaking	Arterial systolic and/or diastolic pressure
	- Electrodermal activity	Simulated public speaking	Skin conductance level, fluctuations
		fMRI paradigm: Viewing fearful faces with different intensity of fearfulness	Skin conductance level, fluctuations, fluctuation latency

Supplemental Table 4. Preferred outcome measures per outcome domain

Domain	Outcome	Anxiety test	Preferred outcome measure
Neurophysiological	*Startle reflex during repeated exposure to sudden noise bursts*	Repeated exposure to sudden noise bursts (120-125 dB) ; multiple trial blocks	First choice: Habituation of startle reflex
		Repeated exposure to sudden noise bursts (115-120 dB)	Second choice: Startle reflex
(Functional) neuronal activity			
	c-Fos expression	Fear memory expression, within-session fear extinction	Prespecified hypothesis by authors
		Open field test	Prespecified hypothesis by authors
	Resting cerebral regional blood flow	SPECT scanning	Prespecified hypothesis by authors
		SPECT scanning	Prespecified hypothesis by authors
	Blood oxygenation level–dependent signal	fMRI paradigm: Viewing fearful faces with different intensity of fearfulness	Prespecified hypothesis by authors
	Effective connectivity between left amygdala and ACC	fMRI paradigm: Viewing fearful faces with different intensity of fearfulness	Prespecified hypothesis by authors

Legend

ACC	anterior cingulate cortex
fMRI	functional magnetic resonance imaging
NA	not available
SPECT	single photon emission computed tomography

3.S.1 Estimations of Cmax and AUC

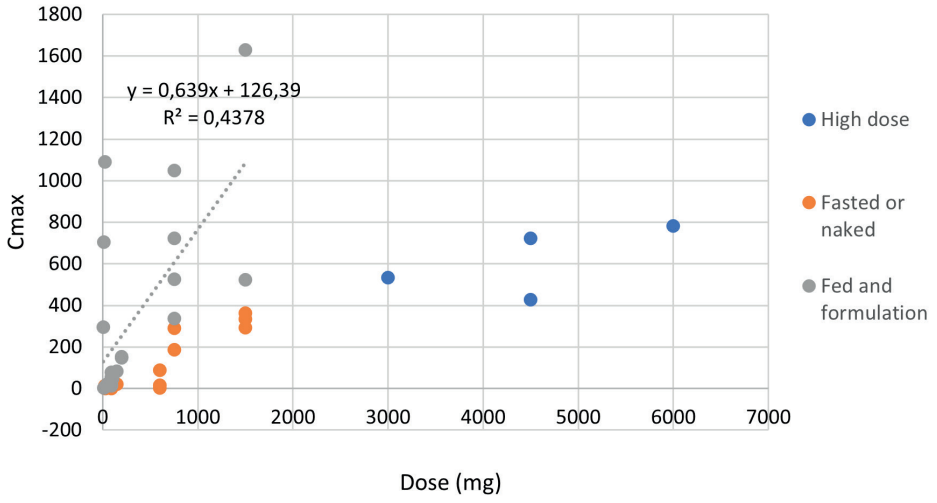
3.S.1.1 Humans

Given the study designs from the included studies with missing maximum plasma concentration (Cmax), intra-/extrapolations were based on studies that used a formulation to improve cannabidiol (CBD)'s bioavailability and tested subjects who were in a fed state. These studies are indicated by the grey data points in Supplemental Figure 1a, in which measured peak plasma concentrations (a) and area under the curve (b) are plotted against doses for oral CBD administration in human subjects.

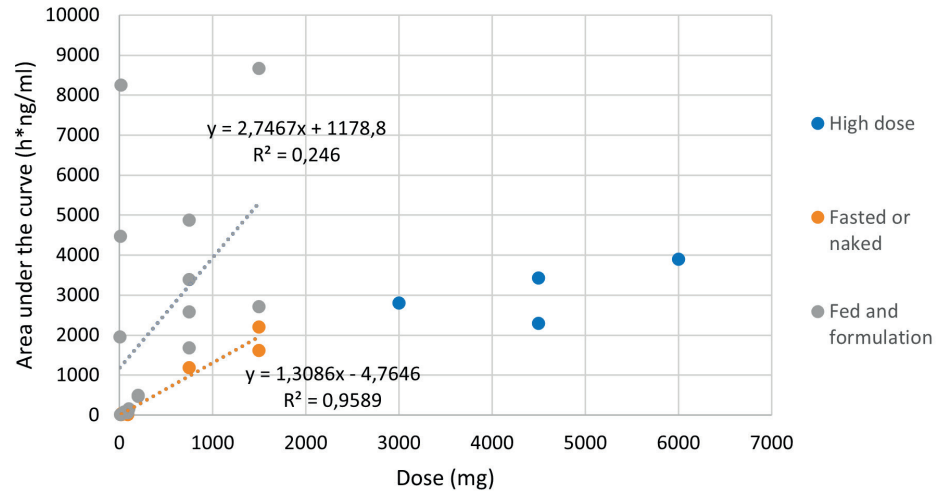
For studies with missing area under the curve (AUC), intra-/extrapolations were based either on studies that used a formulation to improve CBD's bioavailability and tested subjects who were in a fed state, or on studies with fasted individuals / studies that did not report a dissolving vehicle. This depended on the study designs of the studies with missing pharmacokinetic (PK) data.

Saturation of drug absorption appeared to occur at doses between 3000 and 6000 mg. Further, articles that focused on anxiety outcomes did not test CBD at such high dosages. Hence, we based our estimations of missing Cmax and AUC on PK data with CBD doses up to 1500 mg.

a)



b)

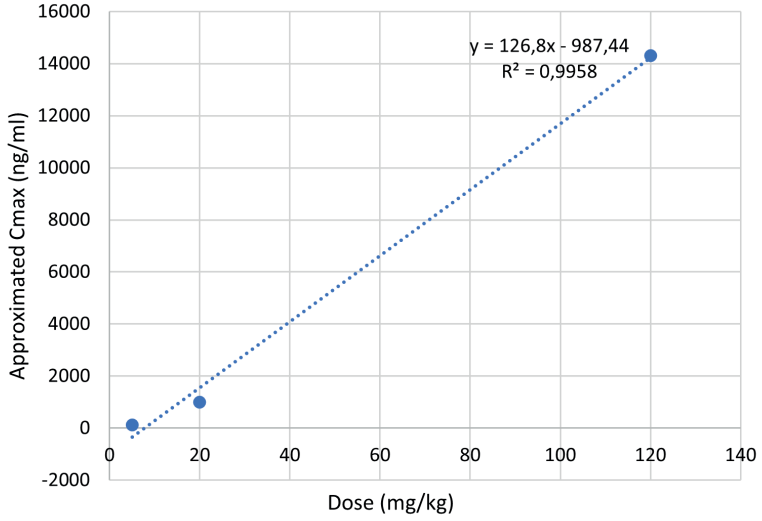


Supplemental Figure 1. Maximum plasma concentrations (a) and area under the curve (b) plotted against orally administered single CBD dose in humans. Two data points in the 'fasted or naked' condition are an approximation of true Cmax; in these studies too little measurements were taken to identify the peak concentration (Fusar-Poli et al., 2009, 2010; Verrico et al., 2020).

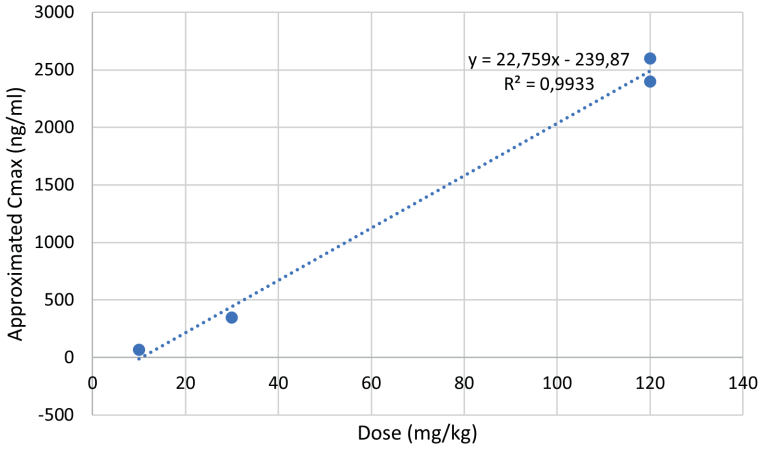
3.S.1.2 Mice and rats

With an eye on compatibility with studies with missing plasma concentrations, which were restricted to male animals, we only used data obtained from male animals for our predictions in mice (Deiana et al., 2012; Zieba et al., 2019) (Figure 3a) and in rats (Deiana et al., 2012; Javadi-Paydar et al., 2019) (Figure 3b). Of note, Javadi-Paydar et al. (2019) showed that in rats, bioavailability of CBD after i.p. administration was higher in females than in males. Estimations of Cmax after doses of 0.01-5 mg/kg i.p. in mice and 1-10 mg/kg in rats were negative. We used (fractions of) the lowest measured Cmax (Javadi-Paydar et al., 2019; Zieba et al., 2019) instead of intra-or extrapolation based on multiple data points to estimate these missing values. In Zieba et al. (2019) plasma levels were measured in the same mice who were subjected to behavioral tests, so in this case we used these PK data from the same article.

a)



b)

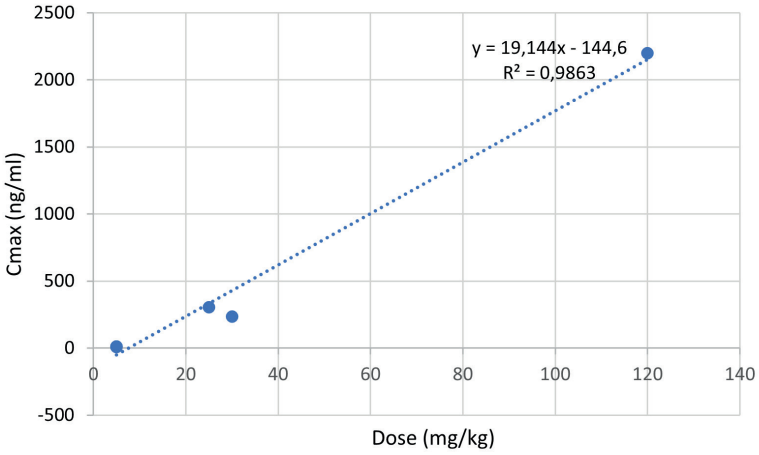


Supplemental Figure 2. Approximated maximum plasma concentrations plotted against single CBD doses administered intraperitoneally in male mice (a) and rats (b).Measured plasma concentrations with 15 and 30 mg/kg doses (Javadi-Paydar et al., 2019; Zieba et al., 2019) are approximations of true Cmax, too little measurements were taken to identify the peak concentration.

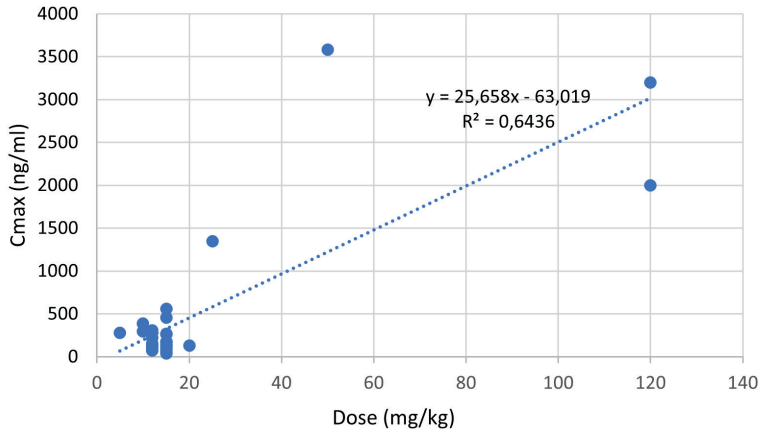
Two rodent articles had pharmacodynamic–pharmacokinetic objectives: One article with p.o. administration in mice (Deiana et al., 2012); and one with p.o. and subcutaneous admin-

istration in rats (Hložek et al., 2017). In the rat study, animals from the behavioral tests were reused for plasma level measurements more than 1 h after drug administration (Hložek et al., 2017). We used linear intra- or extrapolation for other studies with p.o. administration in mice and rats (see Supplemental Figure 3a and 4b). As in mice, the dose vs. approximated maximum plasma concentration relationship in rats was best described with a linear function (R^2 = .64); an exponential function did not increase explained variance (R^2 = .49).

a)



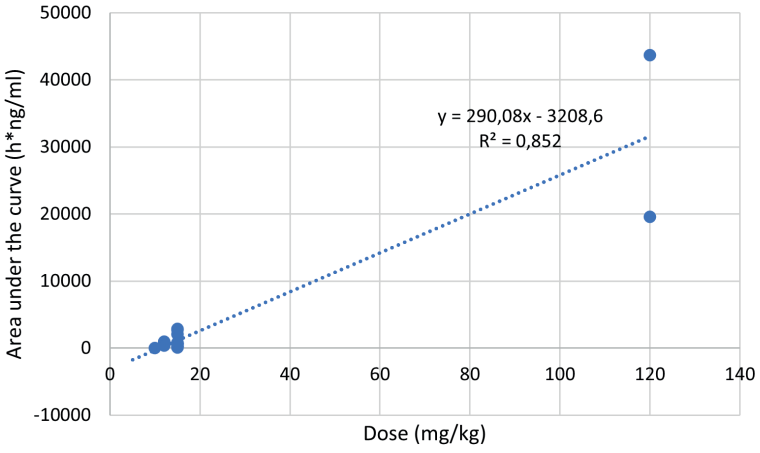
b)



Supplemental Figure 3. Maximum plasma concentrations plotted against single oral CBD dose in mice (**a**) and rats (**b**)

.

Estimations of AUC after i.p. and oral administration in mice, and after i.p. administration in rats were based on PK data of Deiana et al. (2012), by taking fractions of their reported AUC values. Data on total systemic exposure from more than one article was available for oral administration in rats. We used linear interpolation to estimate missing AUC for this administration route and species. Extrapolation of AUC for a 10 mg/kg oral dose in rats (Hložek et al., 2017) yielded a negative value. We therefore used a fraction of the averaged lowest measured AUCs (Feng et al., 2021; Zgair et al., 2016) instead of linear interpolation to estimate this missing value.



Supplemental Figure 4. Area under the curve plotted against orally administered single CBD dose in rats.

Supplemental Table 5. Ongoing and incomplete studies
Fear learning, fear expression, anxiety symptoms

Study ID	Status	Title and brief description
NCT04286594	Recruiting	A Clinical Trial of a Hemp-Derived Cannabidiol Product for Anxiety "0.5ml of sublingual CBD solution (30mg/ml) administered twice daily for six weeks in subjects with moderate or" severe anxiety in addition to their normal treatment regimen"
NCT04269252	Terminated (recruitment challenges due to COVID)	CHI-907 CBD Extract and Experiences of Test Anxiety "randomized, placebo-controlled study examining the effects of CHI-907 (CBD extract) on test anxiety specifically, and state anxiety more broadly"
NCT04577612	Completed, no results posted	A Randomized Controlled Test of the Effects of CHI-554 on Fear "to reduce fear elicited via a safe, well-established, controlled, laboratory-based carbon dioxide (CO2)-enriched air biological challenge that causes abrupt increases in bodily arousal with 150-600 mg CBD in healthy volunteers"
NCT04726475	Recruiting	Use of CBD Oil in the Treatment of Panic Attack-Related Fear "whether 300 mg cannabidiol (CBD) (vs placebo) can interfere with the reconsolidation of naturally acquired pathological interoceptive fear memory in humans with DSM-5 panic disorder or subthreshold elevated concerns about having additional panic attacks"
NCT03549819	Not yet recruiting	Cannabidiol for the Treatment of Anxiety Disorders: An 8-Week Pilot Study "In DSM-5 anxiety disorders (GAD, SAD, PD or agoraphobia), 200 mg CBD- titrated as tolerated up to a maximum 2 capsules twice daily (200 mg- 800 mg total dose), or placebo"

Supplemental Table 5. Ongoing and incomplete studies
Fear learning, fear expression, anxiety symptoms

Study ID	Status	Title and brief description
2014-004094-17	Ongoing	Cannabidiol enhancement of exposure therapy in treatment refractory patients with phobias "To test the hypothesis that administration of cannabidiol as an augmentation step in combination with exposure therapy can strengthen treatment outcome in patients with phobic disorders (generalized social anxiety and panic disorder with agoraphobia) who do not respond satisfactorily to treatment as usual."
Pharmacokinetic outcomes		
NCT04790136	Completed, no results posted	Cannabidiol Bioavailability Trial With Oral Multiple Dose Administration "characterisation of maximum systemic exposure of CBD and its active metabolite 7-OH-CBD of the newly developed Test product in the estimated target effective dose for treatment of COVID-19 as well as the comparison of its systemic bioavailability to CBD administered as oily solution"
NCT04283019	Recruiting	The Pharmacokinetic and Pharmacodynamic Effects of Oral Cannabidiol (CBD) Under Acute and Chronic Exposure Conditions "This study will evaluate the pharmacokinetic and pharmacodynamic effects of oral Cannabidiol (with or without low levels of THC), under acute and chronic dosing conditions."

Supplemental Table 5. Ongoing and incomplete studies
Fear learning, fear expression, anxiety symptoms

Study ID	Status	Title and brief description
ACTRN12619000443190	Recruiting	A double-blind, randomized, two-period, two treatment, fixed sequence, crossover (fed versus fasted) study to evaluate the effect of food on pharmacokinetics of CBD with robust ECG monitoring in Healthy Volunteers (HVs) "A double-blind, randomized, two-period, two treatment, fixed sequence, crossover (fed versus fasted) study"
NCT04280289	Not yet recruiting	CBD Cannabis Extract: Pharmacokinetic Studies "The initial goal is to ascertain the pharmacokinetic (PK) profile of CBD (cannabidiol) after a single dose of CBDE (cannabidiol extract), although the plan is to extend these studies to multiple dose administrations in the future, since it is likely that (cannabidiol) and/or its metabolites will show some accumulation"
NCT03877991	Unknown	Bioequivalence Assessment of Cannabidiol (CBD) Administrated in Oral Formulations "to evaluate the bio-equivalence of CBD in the LNL product, compared to CBD in a sesame oil vehicle and CBD without any formulation, in powder form"
NCT04589455	Not yet recruiting	Pharmacokinetics of CBD From a Hennep Extract "To determine the pharmacokinetics of CBD following oral administration of a novel oil-based hemp extract containing 70 mg CBD and to study the effects of a high-fat meal on the oral bioavailability of CBD."
ACTRN12621000142831	Not yet recruiting	Tissue Levels of Delta-9-tetrahydrocannabinol (THC) and Cannabidiol (CBD) and Effects on Perception and Personality in healthy volunteers "hypothesise that THC will influence anxiety, autism and psychosis proneness relative to both before drug and to CBD, and that these effects will correlate with THC levels in blood but not saliva or urine"

Supplemental Table 5. Ongoing and incomplete studies
Fear learning, fear expression, anxiety symptoms

Study ID	Status	Title and brief description
NCT03164512	Completed, no results posted	The Pharmacokinetics and Pharmacodynamics of Oral and Vaporized Cannabidiol "evaluate the pharmacokinetics and pharmacodynamics of cannabidiol administered via inhalation and oral ingestion"
ACTRN12620000891921	Completed, no results posted	A phase I, open label, two-way crossover study to determine the pharmacokinetic effects, safety, and tolerability of single doses of sublingual cannabidiol wafers vs. cannabidiol oil in healthy volunteers "examine how much CBD is available in the blood when administered via a wafer dissolved under the tongue vs. oil that is swallowed"
NCT03471559	Terminated, ("two step study, step two was not feasible based on results from phase one")	Cannabidiol - an in Vivo Innovative Drug Delivery Study "a comparative bioavailability study will be conducted, comparing cannabidiol capsules (reference formulation) with an intranasal cannabidiol gel (test formulation)"

Supplemental Table 6. Included studies with anxiety outcomes

Study ID	Sample characteristics	Dose (timing, frequency)	Dissolving vehicle	Anxiety test	Outcome	PK outcomes	Safety outcomes
Almeida et al., 2013	Male Wistar rats, n = 10-12 (veh n = 10-12)	1-60 mg/kg i.p. 30 min before test	polyoxyethylenesorbitan monooleate (Tween 80) 2% in saline 0.9%	Social interaction test	Active social interaction time	NI	Motor activity and rearing in SIT
Aso et al., 2019	Male mice C57BL/6J, n = 7 (veh n = 7)	3 mg/kg i.p. 15 min before test	5% ethanol 5% tween 90% saline	Open field test	Time in centre	NI	Motor activity in OFT, object recognition memory in two-object recognition test
Assareh et al., 2020	Male mice C57BL/6J, n = 10-17 (veh n = 10-13)	1-100 mg/kg i.p., 60 min before test	ethanol, 0.90% NaCl, Tween 80 in a ratio of 1:1:18	Cued fear conditioning, contextual fear conditioning, light-dark test	% time freezing, % time in light compartment	NI	NI
Bergamaschi et al., 2011b	Male and female human subjects, n = 12 (PLB n = 12) with social anxiety disorder	600 mg p.o. 1 h 20 min before pre-stress measures	Dissolved in corn oil	Simulated public speaking	State anxiety, heart rate, blood pressure, electrodermal activity	NI	Self-report scale to measure drug effects
Breuer et al., 2016	Male Swiss mice, n=7-11 (veh n = 7-11)	15-60 mg/kg i.p.	dissolved in 2% Tween 80 in sterile saline	Marble burying test	Number of buried marbles	NI	NI
Casarotto et al., 2010	Male C57BL/6J mice, n = 5-8 (veh n = 7)	5-30 mg/kg i.p. 30 min before test/ 30 mg/kg i.p. for 7 days	Diluted in 2% tween 80 in sterile saline	Marble burying test	No of buried marbles	NI	Motor activity in OFT

Supplemental Table 6. Included studies with anxiety outcomes

Study ID	Sample characteristics	Dose (timing, frequency)	Dissolving vehicle	Anxiety test	Outcome	PK outcomes	Safety outcomes
Crippa et al., 2004	Male healthy human subjects, n = 10 (PLB n = 10)	400 mg p.o. 110 min before SPECT imaging	Dissolved in corn oil	SPECT scanning	State anxiety, neuronal activity	NI	Self-report scale to measure drug effects
Crippa et al., 2011	Male healthy human subjects, n = 10 (PLB n = 10)	400 mg p.o. 110 min before SPECT imaging	Dissolved in corn oil	SPECT scanning	State anxiety, neuronal activity	NI	Self-report scale to measure drug effects
Crippa et al., 2021	Male healthy human subjects, n = 15 (PLB n = 15)	150 mg p.o. 60 min before test	Powder/ dissolved in corn oil	Simulated public speaking	State anxiety, heart rate, blood pressure	Cmax	Self-report scale to measure drug effects, adverse effects reported
Deiana et al., 2012	Male Swiss mice, n = 15 (veh n = 15)	120 mg/kg p.o. 2-6 h before test	Suspended in cremophor EL/ethanol/saline in a ratio of 1:1:18	Marble burying test	No of buried marbles	NI	Side-effects reported
Espejo-Porras et al., 2013	Male Sprague-Dawley rats, n = 6-8 (veh n = 6-8)	20 mg/kg i.p. 30 min before test	Tween 80-saline, 1:16	Actitrack	Time spent in the central area of the actitrack	NI	Motor activity (vertical, horizontal) in actitrack
Florensa-Zanuy et al., 2021	Male NMRI mice, n = 6-9 (veh n = 6-9)	30 mg/kg i.p.12.5 hours before test	Dissolved in 2% Tween 80: 5% Propilenglycol: saline	Open field test	Time in centre	NI	Motor activity in OFT
Fusar-Poli et al., 2009	Male healthy human subjects, n = 15 (PLB n = 15)	600 mg p.o. 1 h before fMRI data acquisition	NI	fMRI paradigm: Viewing fearful faces with different intensity of fearfulness	Electrodermal activity, neuronal activity	1 and 2 h after drug administration	Self-report scales to measure drug effects, intoxication, psychotic symptoms

Supplemental Table 6. Included studies with anxiety outcomes

Study ID	Sample characteristics	Dose (timing, frequency)	Dissolving vehicle	Anxiety test	Outcome	PK outcomes	Safety outcomes
Fusar-Poli et al., 2010	Male healthy human subjects, n = 15 (PLB n = 15)	600 mg p.o. 1 h before fMRI data acquisition	NI	fMRI paradigm: Viewing fearful faces with different intensity of fearfulness	Neuronal activity	1 and 2 h after drug administration	See Fusar-Poli 2009
Gáll et al., 2020	Male Wistar rats, n = 8 (veh n = 8), arm subjected to chronic unpredictable mild stress	10 mg/kg i.p. 1 h before test/ daily for 32 days	Crystalline cannabidiol (99.5% purity from Trigal Pharma GmbH,Wien, Austria), dissolved in saline containing 4% of dimethyl sulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO, USA) and 1% of Polysorbate 80 (Sigma-Aldrich, Steinheim, Germany)	Open field test, elevated plus maze	centre time, % open arm entries	NI	Motor activity in EPM and OFT, change in body weight
Gazarini et al., 2014	Male Wistar rats, n = 10 (veh n = 9)	10 mg/kg i.p. after a 3 min context re-exposure	Dissolved in NaCl 0.9% containing 5% of Tween 80 (Vetec)	Contextual fear conditioning	% time freezing	NI	NI
Guimarães et al., 1990	Male Wistar rats, n = 10 (veh n = 10)	2.5-20 mg/kg i.p. 60 min before test	Dissolved in a solution of 10% proplyene glycol - 1% Tween 80 - saline	Elevated plus maze	% open arm entries	NI	Motor activity in EPM
Guimarães et al., 1994	Male Wistar rats, n = 5 (veh n = 22)	5 mg/kg i.p. 35 min before test	Dissolved in a 10% propylene glycol-1% Tween 80-saline solution	Elevated plus maze	% open arm entries	NI	Motor activity in EPM

Supplemental Table 6. Included studies with anxiety outcomes

Study ID	Sample characteristics	Dose (timing, frequency)	Dissolving vehicle	Anxiety test	Outcome	PK outcomes	Safety outcomes
Hložek et al., 2017	Male Wistar rats, n = 10 (veh n = 10)	10 mg/kg subcutaneous/oral, 5 mg pulmonary, 60/120/5 min before test	Dissolved in pharmaceutical grade sunflower oil (subcutaneous/oral); dissolved in 98% ethanol in a volume of 200 µl (pulmonary)	Open field test, startle	Time in centre, habituation of startle	NI	Motor activity in OFT
Jurkus et al., 2016	Male Lister-hooded rats, n = 10-11 (veh n = 10-11)	5 mg/kg i.p. 30 min before extinction training	Suspended in 2% Tween 80 and 0.9% sterile saline	Cued fear conditioning	% time freezing	NI	NI
Kajero et al., 2020	Male Wistar rats, n = 7 (veh n = 7)	5 mg/kg p.o. daily for 21 days	Dissolved in 70% ethanol, and diluted with distilled water.	Elevated plus maze	Time in open arms	NI	Motor activity in EPM and OFT, change in body weight, object recognition memory in object recognition test
Karniol et al., 1974	Male Wistar rats, n = 12 (veh n = 13)	10 mg/kg i.p. 45 min before test	0.6 % solution of tween-80 in saline	Open field test	No of fecal boluses	NI	NI
Kasten et al., 2019	Male and female C57BL/6J (B6) mice, single housed, n = 8 (veh n = 8)	5-20 mg/kg i.p. 30 min before test/ 20 mg/kg 8 injections within 3 weeks	Dissolved in a vehicle solution of 5% Tween80, 5% 100 proof ethanol, and 90% saline	Elevated plus maze, open field test	Time in open arms, % time in centre	NI	Motor activity in OFT, object recognition memory consolidation in novel object recognition test

Supplemental Table 6. Included studies with anxiety outcomes

Study ID	Sample characteristics	Dose (timing, frequency)	Dissolving vehicle	Anxiety test	Outcome	PK outcomes	Safety outcomes
Lemos et al., 2010	Male Wistar rats, n = 9 (veh n = 9)	10 mg/kg i.p. 30 min before context re-exposure	Dissolved in tween 80 1% and propilenoglycol 10%	Contextual fear conditioning	% time freezing, neuronal activity	NI	NI
Linares et al., 2019	Male healthy human subjects, n = 15 (PLB n = 15)	150-600 mg p.o. 90 min before test	Dissolved in corn oil	Simulated public speaking	State anxiety, blood pressure	NI	NI
Long et al., 2010	Male C57BL/6JArc mice, n = 7-10 (veh n = 7-10)	1-50 mg/kg i.p. daily, 1-18 days of treatment	Suspended in a 1:1:18 mixture of ethanol:Tween-80: saline	Open field test, light-dark test, social interaction test, startle	Distance ratio, total social interaction time, startle	NI	Tetrad testing
Long et al., 2012	Male wild-type mice, n = 14-17 (veh n = 14-17)	1-100 mg/kg i.p. daily for 21 days	Suspended in a 1:1:18 mixture of ethanol:Tween-80: saline	Open field test, light-dark test, social interaction test, startle	Distance ratio, total social interaction time, startle	Plasma levels 48h after last administration	Motor activity and exploration in OFT
Mahmud et al., 2017	Male Long Evans rats, n = 5 (veh n = 5)	10 mg/kg i.p. 1 h before test	Dissolved in a solution of DMSO 5%, chremophor 5%, and saline	Elevated plus maze	Time in open arms	NI	Motor activity in EPM
Malone et al., 2009	Male Sprague-Dawley rats, n = 6 (veh n = 6)	5-20 mg/kg i.p. 40 min before test	1:1:98 Tween 80: ethanol: saline	Social interaction test	Active social interaction time	NI	Motor activity in SIT

Supplemental Table 6. Included studies with anxiety outcomes

Study ID	Sample characteristics	Dose (timing, frequency)	Dissolving vehicle	Anxiety test	Outcome	PK outcomes	Safety outcomes
Martín-Gonzalez 2018	Male Wistar rats, n = 10 (veh n = 10), high drinkers in schedule induced polydipsia	1-3 mg/kg i.p. 30 min before test	Suspended in 2% Tween 80 and 0.9% saline	Schedule-induced polydipsia	Consumed water volume	NI	Motor activity in SIP
Moreira et al., 2006	Male Wistar rats, n = 7 (veh n = 6-7)	2,5-10 mg/kg i.p. 30 min before test	Suspended in polyoxyethylenesorbitan monooleate (Tween 80) 2% in saline (NaCl 9%)	Vogel conflict test	Number of punished licks	NI	NI
Murkar et al., 2019	Male Sprague-Dawley rats, n = 9 (veh n = 9)	50 mg/kg p.o. following 5 min context reexposure	Almond oil as vehicle	Contextual fear memory	% time freezing	NI	NI
Myers et al., 2019	Male C57Bl6 mice, n = 8 (veh n = 8)	5 mg/kg i.p. every 12 h for 4.5 days	Dissolved in 1:1:18 ethyl alcohol: cremophor:saline	Elevated plus maze	% time in open arms	NI	Cognitive functioning in conditional discrimination test and Barnes Maze
Nardo et al., 2014	Male swiss mice, n = 6-10 (veh n = 6-11)	5-30 mg/kg i.p. 30 min before test	2% Tween 80 in sterile saline	Marble burying test, open field test	Number of buried marbles, % time in centre	NI	Motor activity in OFT

Supplemental Table 6. Included studies with anxiety outcomes

Study ID	Sample characteristics	Dose (timing, frequency)	Dissolving vehicle	Anxiety test	Outcome	PK outcomes	Safety outcomes
Navarrete et al., 2018	Male C57BL/6J mice, n = 8 (veh n = 8)	5-20 mg/kg i.p. 90 min before test	ethanol : cremophor : saline (1:1:18)	Light-dark test	Time in light compartment	NI	Motor activity and withdrawal-related somatic signs (number of rearings, groomings, rubbings and jumpings)in OFT
O'Brien et al., 2013	Male Sprague Dawley rats, n = 8 (veh n = 8)	2.5 mg/kg, i.p. 30 min before test/ daily for 7 days/ daily for 14 days	VEH of 1:1:18 formulation of EtOH, Cremophor, and saline	Light-dark test	Time in light compartment	NI	Motor activity in LD test
Onaivi et al., 1990	Male ICR mice, n=10-12 (veh n = 10-12)	0.01-100 mg/kg i.p. 30 min before test	saline;ethanol;emulphor (1:1:18)	Elevated plus maze	% open arm entries	NI	Motor activity in EPM
Resstel et al., 2006	Male Wistar rats, n = 5 (veh n = 5)	10 mg/kg i.p. before context reexposure	Suspended in polyoxyethylenesorbitan monooleate (tween 80) 2%-saline	Contextual fear conditioning	% time freezing	NI	NI
Rock et al., 2017	Male Sprague-Dawley rats, n = 6-7 (veh n = 9-15), one arm exposed to foot shock	5 mg/kg i.p. 45 min before test	dissolved in ethanol; final concentration of drug (VEH) in a ratio of 1:9; Tween80:saline [SAL]) following evaporation of the ethanol	Light dark test	Mean time in light box	NI	NI

Supplemental Table 6. Included studies with anxiety outcomes

Study ID	Sample characteristics	Dose (timing, frequency)	Dissolving vehicle	Anxiety test	Outcome	PK outcomes	Safety outcomes
Schiavon et al., 2016	Male Swiss albino mice, n= 8-10 (veh n = 9)	3-30 mg/kg i.p. 1 hour before test/ daily for 15 days	0.9% NaCl with 1% Tween 80	Elevated plus maze	% open arm entries	NI	Motor activity in EPM
Shallcross et al., 2019	Male Sprague-Dawley rats, n = 8 (veh n = 8), stress-susceptible phenotype (in EPM and habituation of startle), and predator-stress scent exposed	5 mg/kg i.p. 20 min before extinction sessions/ 30 min before test	Dissolved in a mixture of 100% ethanol, Cremophor, and 0.9% NaCl	Contextual fear conditioning, light-dark test	Time freezing, latency to enter the light compartment	NI	NI
Shoval et al., 2016	Male Wistar rats, n = 11-16 (veh n = 11-16)	15-45 mg/kg p.o. 2 h before test	crystalline product, above 99% pure, vehicle of 100 microliter ethanol laced into high-fat diet pellet	Elevated plus maze, novel object exploration	Time spent in open arms, time spent near a novel object	NI	Motor activity in NOE test
Song et al., 2016	Male Lister hooded rats, n = 7-8 (veh n = 7-8)	10 mg/kg i.p. before extinction training	Dissolved in DMSO and diluted to a final vehicle of 20% DMSO in Saline with 0.1% Tween 80	Contextual fear conditioning	% time freezing	NI	NI
Stern et al., 2012	Male Wistar rats, n = 6-12 (veh n = 6-12)	3-30 mg/kg i.p. after memory retrieval	NaCl 0.9% containing 5% of polyoxyethylene sorbitan monooleate	Contextual fear conditioning	% time freezing during context reexposure	NI	NI

Supplemental Table 6. Included studies with anxiety outcomes

Study ID	Sample characteristics	Dose (timing, frequency)	Dissolving vehicle	Anxiety test	Outcome	PK outcomes	Safety outcomes
Stern et al., 2015	Male Wistar rats, n = 9 (veh n = 8)	1 mg/kg i.p. after memory retrieval	NaCl 0.9% containing 5% of Tween 80	Contextual fear conditioning	% time freezing during context reexposure	NI	NI
Todd and Arnold, 2016	Male C57BL/6 mice, n = 6 (veh n = 6)	10 mg/kg i.p. 30 min before test	Dissolved in a mixture of ethanol, Tween 80 and saline (1:1:18)	Open field test	% distance traveled in centre, neuronal activity	NI	Motor activity in OFT
Todd et al., 2017	Male C57Bl6 mice, n = 6-12 (veh n = 7-12)	10-50 mg/kg i.p. 1-15 doses	suspended in a 1:1:18 mixture of ethanol:Tween-80: saline	Open field test, social interaction test, light-dark test, startle	Distance ratio, social interaction time, latency to leave the hide box,	NI	Motor activity in OFT
Twardowschy et al., 2013	Male Swiss mice, n = 9-15 (veh n = 9-15)	3 mg/kg i.p. 30 min before test	Vehicle of saline solution at 0.9% and DMSO (1:1) 0.2 mL/kg	Predator exposure	behavioural index of defensive immobility outside the burrow	NI	NI
Uribe-Mariño et al., 2012	Male swiss mice, n= 11-12 (veh n = 11-12)	0.3 mg/kg i.p. 30 min before test	99.9% pure, dissolving vehicle not reported	Predator exposure	behavioural index of defensive immobility outside the burrow	NI	NI
Zagzoog et al., 2020	Male C57BL/6 mice, n = 6 (veh n = 6)	1 mg/kg i.p. 1 h before test	Dissolved in DMSO, final concentration of 0.1% in assay media	Open field test	Time in centre	NI	Tetrad testing

Supplemental Table 6. Included studies with anxiety outcomes

Study ID	Sample characteristics	Dose (timing, frequency)	Dissolving vehicle	Anxiety test	Outcome	PK outcomes	Safety outcomes
Zieba et al., 2019	Male C57BL/6J mice, n = 12 (veh n = 12)	5-20 mg/kg i.p. 30 min before test	Dissolved in equal amounts of Tween 80 (Sigma-Aldrich Co., St Louis, USA) and 100% ethanol and diluted with 0.9% sodium chloride to the appropriate concentration to a final ratio of 1:1:18	Open field test, elevated plus maze	% distance traveled in centre, % distance traveled in open arms	NI	Motor activity in OFT, learning and memory in Y-maze and passive avoidance test, sociability in social preference test
Zuardi et al., 1993	Male and female healthy human subjects, n = 10 (PLB n = 10)	300 mg p.o. 1 h 20 min before pre-stress measures	Dissolved in corn oil, 100 mg/ml	Simulated public speaking	State anxiety, heart rate, blood pressure	NI	Self-report scale to measure drug effects, psychomotor performance in digital-symbol substitution test
Zuardi et al., 2017	Male and female healthy human subjects, n = 11-12 (PLB = 11-12)	100-900 mg 153 min before speech	Dissolved in corn oil at doses of 100 and 200 mg/ml	Simulated public speaking	State anxiety, heart rate, blood pressure	NI	Self-report scale to measure drug effects

Legend

EPM	elevated pluz maze
fMRI	functional magnetic resonance imaging
i.p.	intraperitoneal
LD	light dark test
NI	no information
NOE	novel object exploration test
OFT	open field test
PLB	placebo
p.o.	per os
SIP	schedule induced polydipsia
SIT	social interaction test
SPECT	single photon emission computed tomography
veh	vehicle

Supplemental Table 7. Included studies with pharmacokinetic outcomes

Study ID	Sample characteristics	Dose (timing, frequency)	Dissolving vehicle	Outcome	Tmax	Food intake	Safety outcomes
Anderson et al., 2021	Male and female mice, n = 4-5	25 mg/kg p.o.	Hemp seed oil	Cmax	90 min	Ad libitum	NA
Atsmon et al., 2018	Male healthy human subjects, n = 14	10/100 mg p.o.	PTL101	Cmax, AUC to infinity	3/3,5h	Overnight fast followed by standard meal	Adverse events and vital signs recorded, physical and oral examination
Brzozowska et al., 2016	Male mice, n = 8	10 mg/kg subcutaneous	Dissolved in a mixture of ethanol, Tween 80, and saline (1:1:18)	Plasma levels at 1h	NA	Ad libitum	NA
Cherniakov et al., 2017	Male rats, n = 6 per group	15 mg/kg p.o.	CBD-Curcumin-PNL, CBD-Piperine-PNL and CBD-Resveratrol-PNL	Cmax, AUC to infinity	1.07-1.9h	Deprived from food	NA
Crippa et al., 2021	Male healthy human subjects, n = 15 per administration mode	150 mg p.o.	Powder/ dissolved in corn oil	Cmax	2,5h / 2h	Standardized breakfast	Self-report scales to measure drug effects, adverse effects reported by participants
Crocket et al., 2020	Male and female healthy human subjects, n = 30	750 mg p.o.	Epidiolex	Cmax, AUC to infinity	3-5h	fasted, low calorie, high calorie, whole milk	treatment emergent AE review, vital sign measurements, ECG, clinical lab evaluations, physical exams and CSSRS

Supplemental Table 7. Included studies with pharmacokinetic outcomes

Study ID	Sample characteristics	Dose (timing, frequency)	Dissolving vehicle	Outcome	Tmax	Food intake	Safety outcomes
Deiana et al., 2012	Male mice/rats, n = 4 per route	120 mg/kg i.p. p.o./ i.p.	Cremophor EL/ethanol/saline in a ratio of 1:1:18/ 30% Solutol HS 15	Cmax, AUC to infinity	0,5 - 6h	Food-deprived over night/ ad libitum	Side-effects reported
Feng et al., 2021	Male rats, n = 5-7 per formulation	12 mg/kg p.o.	Sesame oil, oleic acid, linoleic acid, 2-oleoylglycerol with oleic acid, oileic acid with glycerol, glycerol trioleate	Cmax	3-6h	Ad libitum	NA
Fusar-Poli et al., 2009	Male healthy human subjects, n = 15	600 mg p.o.	NI	Plasma levels at 2h	NA	8h fast, light breakfast 2h before experiment	Self-report scales to measure drug effects, intoxication, psychotic symptoms
Grimm et al., 2018	Male healthy human subjects, n = 16	600 mg p.o.	NI	Plasma levels at 208 min	NA	350 kcal sandwich	Self-report of adverse events
Hložek et al., 2017	Male rats, n = 6 per group	10 mg/kg subcutaneous/oral, 5 mg pulmonary	Dissolved in pharmaceutical grade sunflower oil (subcutaneous/p.o.); dissolved in 98% ethanol in a volume of 200 µl (pulmonary)	Cmax	0,9-2h	Pulmonary/subcutaneous: fed, p.o.: 12 hour fast	Motor activity in OFT

Supplemental Table 7. Included studies with pharmacokinetic outcomes

Study ID	Sample characteristics	Dose (timing, frequency)	Dissolving vehicle	Outcome	Tmax	Food intake	Safety outcomes
Hosseini et al., 2020	Male and female healthy humans, n = 12 (single dose), n = 3 (multiple dose)	25-50 mg p.o. single dose/ 2 daily doses during 5 days	WaferiX, MediCabilis	Cmax, AUC to infinity after single dose and after last dose	3,3-5,2h	Fasted	AEs recorded throughout the study, ECG, vital signs and urinalysis at different time points up to 24h after dose, CSSRS at end of each treatment.
Izgelov et al., 2020a	Male healthy humans, n = 12 for each formulation	90 mg p.o.	CBD-SNEDDS, CBD-sesame oil, CBD-powder	Cmax, AUC to infinity	2-8,4h	Certain food types/beverages not allowed within the 14 days prior to first day of the trial and throughout the study.	Adverse events documented by investigator through question-naires/interview and physical exams
Izgelov et al., 2020b	Male rats, n = 4-6	15 mg/kg p.o.	CBD-PNL, CBD-piperine-PNL	Cmax, AUC to infinity	1.2-2h	Ad libitum	NA
Izgelov et al., 2020c	Male rats, n = 5 per formulation	15 mg/kg p.o.	SNEDDS/Sesame oil	Cmax, AUC to infinity	1,5h/ 4h	Ad libitum	NA

Supplemental Table 7. Included studies with pharmacokinetic outcomes

Study ID	Sample characteristics	Dose (timing, frequency)	Dissolving vehicle	Outcome	Tmax	Food intake	Safety outcomes
Izgelov et al., 2020d	Male rats, in cocoa butter and tricaprin n = 3, in sesame oil and MIGLYOL 812 n = 6	16 mg/kg p.o.	MCT-SNEDDS in cocoa butter and tricaprin, LCT-SNEDDS in cocoa butter and tricaprin, LCT-SNEDDS in sesame oil and MIGLYOL 812 N, MCT-SNEDDS in sesame oil and MIGLYOL 812 N	Cmax, AUC to infinity	1-6h	Ad libitum	NA
Javadi-Paydar et al., 2019	Male (n = 8) and female (n = 8) rats	10-30 mg/kg i.p.	1:1:8 ratio of eth-anol:cremulphor:saline	Plasma levels at 35 min	NA	Ad libitum	Body temperature and motor activity
Knaub et al., 2019	Male and female healthy human subjects, n = 16 (M+F) for each formulation	25 mg p.o.	Food emulsifiers, vegetable oils and fatty acids diluted in water (1:99)/ MCT oil	Cmax, AUC 0-24h	1/3h	Fasted	Self-rating of tolerability and adverse events during the intervention,
Majimbi et al., 2021	Female mice, n = 3-4 per administration mode	5 mg/kg p.o.	CBD capsules/ CBD capsules + DCA capsules /naked CBD oil	Cmax	0,3h/ 1h / 3h	Fasted	NA

Supplemental Table 7. Included studies with pharmacokinetic outcomes

Study ID	Sample characteristics	Dose (timing, frequency)	Dissolving vehicle	Outcome	Tmax	Food intake	Safety outcomes
Martín-Santos et al., 2012	Male healthy humans, n = 16	600 mg p.o.	NI	Plasma levels at 3h	NA	Standardized light breakfast	Self-report scales to measure drug effects and psychotic symptoms
Nagao et al., 2020	Male rats, n = 4 per dose	5-50 mg/kg p.o.	Nanoemulsion	Cmax, AUC to infinity	1,5-2,75h	Ad libitum	NA
Pang et al., 2021	Male and female mice, n = 6 per route	10-30 mg/kg oral, 30 mg/kg nasal single dose	0.5% (w/v) CMC-Na solution/ CBD temperature sensitive hydrogel	Cmax, AUC 0-t	1h/ 0.29h	Ad libitum	Motor activity in OFT
Patrician et al., 2019	Male healthy human subjects, n = 12 for each formulation	45/90 mg p.o.	TurboCBD or hemp oil	Cmax, AUC to infinity	110-130 min	Allowed a light, low-fat breakfast	

Supplemental Table 7. Included studies with pharmacokinetic outcomes

Study ID	Sample characteristics	Dose (timing, frequency)	Dissolving vehicle	Outcome	Tmax	Food intake	Safety outcomes
Perkins et al., 2020	Male and female healthy humans, n = 6 per dose	5-20 mg/kg p.o.	Lipid-based oral solution (100 g/L) comprising Super Refined soybean oil (to improve oxidative stability) and flavouring agents (to ensure palatability)	Cmax, AUC to infinity	3,76-4,06h	Consumption of grapefruit, grapefruit juice or Seville oranges within 1 week of administration not allowed	Collection of data relating to adverse events, vital signs, clinical laboratory assessments, 12-lead ECGs, physical examinations and concomitant medications
Paudel et al., 2010	Male and female rats, n = 3 per group/ guinea pigs, n = 6 (without enhancer), n = 3 (with enhancer)	200 µg/kg intranasal/ 18 mg transdermal	PEG 400-ES 50:35:15 (v/v) PEG:saline:ethanol solvent system + 1% DM-β-CD solution with permeation enhancer/ 18 mg CBD/ml, 80:20 PG:nanopure water, 2% gelling agent (hydroxyethyl cellulose), 500 µl per application, 2 patches per guinea pig	Cmax, AUC	0.33-0.35h/ 31.2-38.4h	NI	NA

Supplemental Table 7. Included studies with pharmacokinetic outcomes

Study ID	Sample characteristics	Dose (timing, frequency)	Dissolving vehicle	Outcome	Tmax	Food intake	Safety outcomes
Schoedel et al., 2018	Male and female healthy human subjects, n = 41	750-4500 mg p.o.	Epidiolex	Cmax, AUC to infinity	4,07-6,13h	fasted	Recording of the incidence, frequency, and severity of AEs; and regular assessments of vital signs, clinical laboratory assessments (hematology, biochemistry, urinalysis), 12-lead electrocardiogram (ECG), physical examinations, "Since Last Visit" Columbi

Supplemental Table 7. Included studies with pharmacokinetic outcomes

Study ID	Sample characteristics	Dose (timing, frequency)	Dissolving vehicle	Outcome	Tmax	Food intake	Safety outcomes
Taylor et al., 2018	Male and female healthy human subjects, n = 6 (SAD arm), n = 9 (MD arm), n = 12 (food effect arm)	750-6000 mg p.o. single dose/ doses twice daily for 6 days, last dose on day 7	Epidiolex	Cmax, AUC to infinity/ over a dosing interval at day 1 am and pm, and day 7 am	2,5-5h	Fasted/high-fat breakfast	Recording the incidence and severity of AEs throughout the trial, review of clinical laboratory tests, vital signs, electrocardiogram, physical examination, sleep disruption scale, sleepiness scale and other self-report scales
Taylor et al., 2019	Male and female healthy human subjects, n = 8	200 mg p.o.	Epidiolex	Cmax, AUC to infinity	2,3h	Standardized low-protein breakfast	Blood pressure and heart rate at multiple time points during each visit, blood and urine tests, tests ofcognitive functioning
Tayo et al., 2019	Male healthy human subjects, n = 8	200 mg p.o.	Epidiolex	Cmax, AUC to infinity	2,5h	Standardized low-protein breakfast	Adverse events recorded, clinical laboratory tests, vital signs, 12-lead electrocardiography (ECG), and physical examinations

Supplemental Table 7. Included studies with pharmacokinetic outcomes

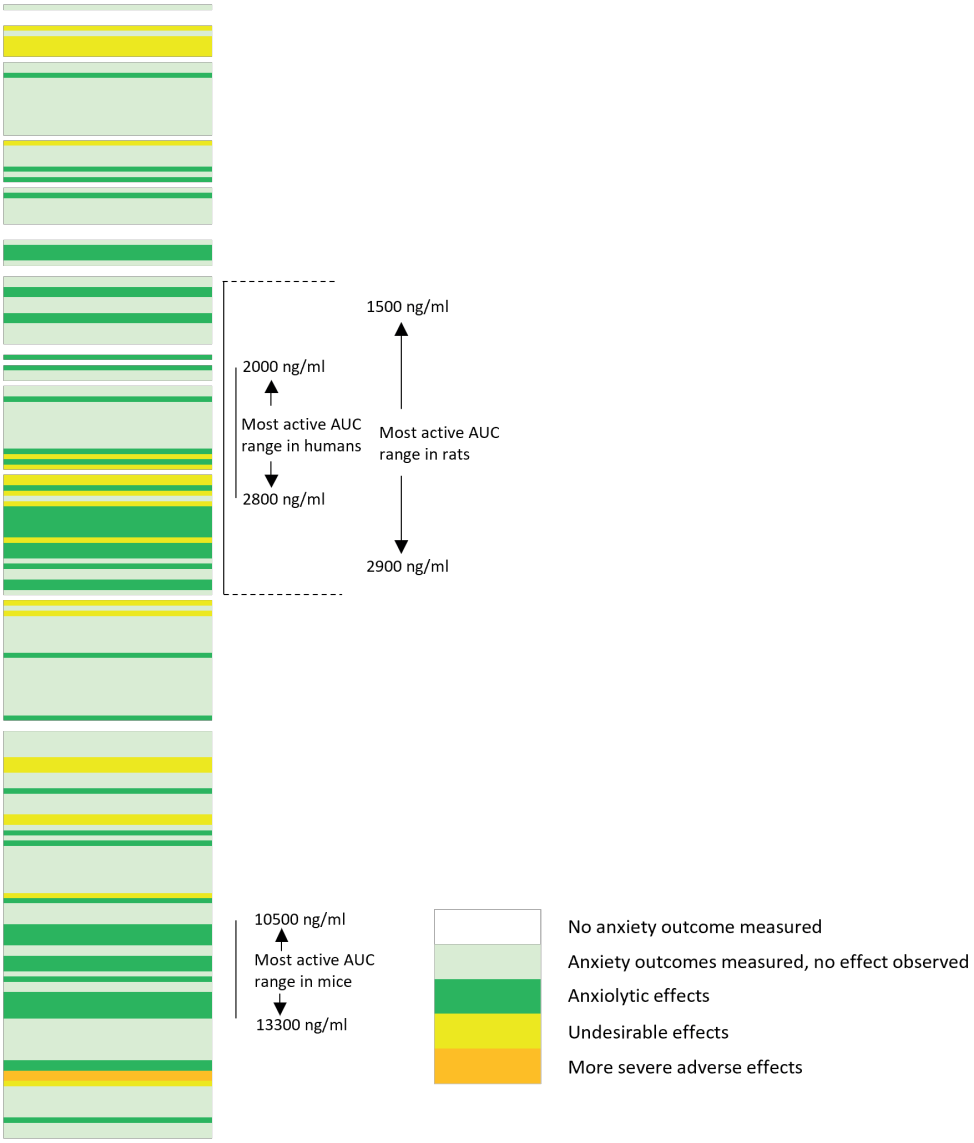
Study ID	Sample characteristics	Dose (timing, frequency)	Dissolving vehicle	Outcome	Tmax	Food intake	Safety outcomes
Verrico et al., 2021	Human subjects, NI about sex, n = 5 per administration mode	10 mg p.o.	CBD naked/ liposomal	Plasma levels at 1h	NA	fasted	NA
Williams et al., 2020	Male and female healthy humans, n = 8-15 per formulation	30 mg p.o.	CBD Tincture Base, CBD Powder in Water, 20% CBD Concentration Liquid, 5% CBD Concentration Powder, 5% CBD Concentration Liquid	Cmax, AUC to infinity	0,7/3,39h	Fasted	Heart rate, heart rate variability and blood pressure recorded
Xu et al., 2019	Male rats, n = 3 per group	20 mg/kg p.o.	Soybean oil and fat emulsion, at 3.5 mg CBD per ml	Cmax	2h	Ad libitum	NA
Zgair et al., 2016	Male rats, n = 4 per group	12 mg/kg p.o.	Dissolved in LCT (sesame oil) to prepare 20 mg/mL solution	Cmax	3h	Ad libitum	NA

Supplemental Table 7. Included studies with pharmacokinetic outcomes

Study ID	Sample characteristics	Dose (timing, frequency)	Dissolving vehicle	Outcome	Tmax	Food intake	Safety outcomes
Zieba et al., 2019	Male C57BL/6J mice, n = 6 per dose	5-20 mg/kg i.p.	Dissolved in equal amounts of Tween 80 (Sigma-Aldrich Co., St Louis, USA) and 100% ethanol and diluted with 0.9% sodium chloride to the appropriate concentration to a final ratio of 1:1:18	Plasma levels at 30 min	NA	Ad libitum	Motor activity in OFT, learning and memory in Y-maze and passive avoidance test, sociability in social preference test

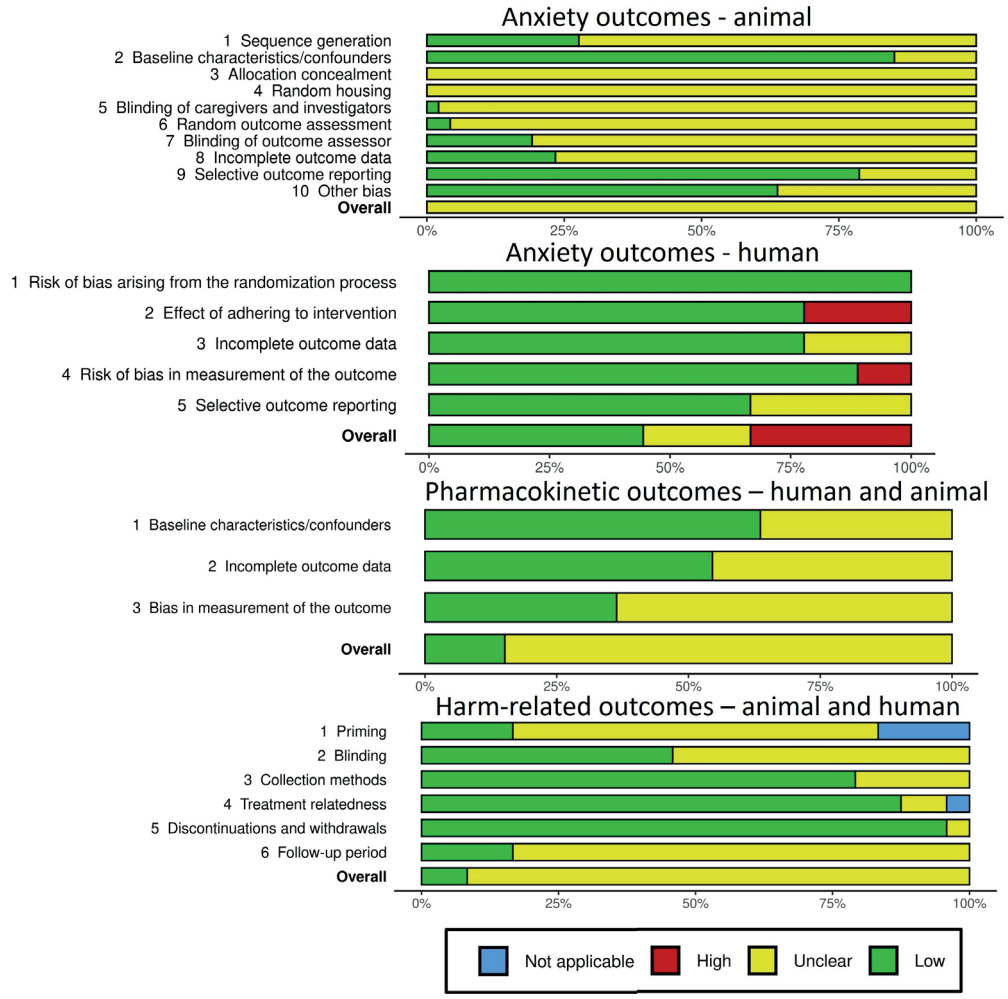
Legend

CBD	cannabidiol
i.p.	intraperitoneal
MD	multiple dose
NA	not applicable
NI	no information
p.o.	oral
SAD	single ascending dose



Supplemental Table 8. Overview of anxiety-reducing effects (dark green), null effects on anxiety (light green), undesirable effects (yellow), and more severe adverse effects (orange) of CBD across species, sorted by area under the curve (AUC)

Note: The interested reader is referred to the online supplementary materials (https://doi.org/10.1177/0269881122112479) for the complete table and overviews of effects within species.



Supplemental Figure 5. Summary risk of bias plot for anxiety outcomes (animal, human), pharmacokinetic outcomes and harm-related outcomes.

3.S.2 Amendments to the information provided in the protocol

AUC was added as a second primary outcome next to Cmax.

Assessment of risk of bias for harm-related outcome was based on the CONSORT extension on reporting of HARMS (Ioannidis et al., 2004) rather than SYRCLE's RoB (Hooijmans et al., 2014) or Cochrane RoB 2.0 (Sterne et al., 2019), because these tools are primarily aimed at efficacy outcomes.

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Part II

Current clinical research with cannabidiol in anxiety disorders

Chapter 4

Cannabidiol enhancement of exposure therapy in treatment refractory patients with phobias: study protocol of a randomized controlled trial

Febe E. van der Flier
Caroline M.B. Kwee
Danielle C. Cath
Neeltje M. Batelaan
Lucianne Groenink
Puck Duits
Date C. van der Veen
Anton J.L.M. van Balkom
Johanna M.P. Baas

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JB (PI), DC, AvB, LG and NB obtained funding for this study. All authors contributed to the design of the study. FvdF and CK coordinated the recruitment of patients and the data collection. JB, DC, AvB and NB are responsible for the overall design and supervision. FvdF wrote the manuscript. All authors (FvdF, JB, DC, PD, DvdV, AvB, LG, NB, CK) read, contributed and approved the final manuscript.

Abstract

Background:

Phobic anxiety disorders are among the most prevalent psychiatric disorders and are burdensome in terms of loss of quality of life and work productivity. Evidence-based treatments are relatively successful in the majority of patients, especially exposure therapy. However, a substantial subset of patients fails to achieve or stay in remission. Preclinical and genetic research have yielded evidence that the cannabinoid system is involved in the extinction of fear, presumed to underlie the beneficial effects of exposure therapy in phobic disorders. A cannabinoid constituent that may enhance endocannabinoid signaling is cannabidiol (CBD), a non-psychoactive component of cannabis. Hence, the addition of CBD to exposure therapy is expected to strengthen effects of treatment. To determine the added benefit of CBD on exposure therapy, we conduct a randomized controlled trial, in which patients in whom previous treatment as usual has not yielded sufficient response receive either CBD or placebo preceding 8 exposure sessions in a double-blind fashion. A subsidiary aim is to explore which (combination of) clinical, behavioral and genetic profiles of patients are related to treatment response.

Methods/design:

This is an 8-week multicenter, randomized, double-blind, placebo-controlled trial. Seventy-two patients with social phobia or panic disorder with agoraphobia with incomplete response to earlier treatment will be included from outpatient clinics in the Netherlands. Patients are randomized to augmentation of exposure therapy with 300 mg CBD or placebo. The study medication is administered orally, 2 h preceding each of the eight 90 min exposure sessions. Measurements will take place at baseline, first administration of medication, every session, mid-treatment, last administration of medication, post-treatment and at 3 and 6 months' follow-up. The primary outcome measure is the score on the Fear Questionnaire (FQ). In addition, determinants of the expected treatment enhancing effect of CBD will be explored.

Discussion:

This is the first trial to investigate whether the addition of CBD to exposure therapy is effective in reducing phobic symptoms in treatment refractory patients with social phobia or panic disorder with agoraphobia.

Trial registration:

Netherlands Trial Register NTR5100. Registered 13 March 2015. Protocol version: issue date 17 Jan 2018, protocol amendment number 7.

4.1 Background

Phobic disorders (e.g. social anxiety disorder, panic disorder with agoraphobia) are among the most prevalent disorders according to the World Health Organization's World Mental Health Survey Initiative (Kessler et al., 2005). These and other anxiety disorders have major impact on health, individual suffering and societal costs (Alonso et al., 2004). The estimated societal costs in Europe as a result of anxiety disorders were 74.4 billion Euros in 2010, affecting more than 69 million Europeans (Gustavsson et al., 2010). Anxiety disorders often co-occur with other mental health disorders (Klein Hofmeijer-Sevink et al., 2012; Souêtre et al., 1994), and are associated with an increased risk of suicide (Sareen et al., 2005). Spontaneous recovery from these disorders is uncommon; if left untreated, phobias typically follow a chronic course, with low remission and high relapse rates (Hendriks et al., 2013).

The current evidence-based treatment entails exposure with response prevention therapy, either alone or in combination with serotonin reuptake inhibitors (SSRIs). Exposure therapy is relatively successful, with improvement in up to 60% of patients. However, only 30 to 50% of phobic patients achieves full remission (Gloster et al., 2013). Likewise, treatment with SSRIs is relatively effective, however, many patients experience relapse after discontinuing SSRI treatment (Batelaan et al., 2017; Donovan et al., 2010), while the effects of successful exposure treatment seem to be more sustainable (Feske and Chambless, 1995). Considering the high prevalence of anxiety disorders and the large number of patients for whom the anxiety symptoms remain refractory after (repeated) gold-standard treatments, new approaches to the treatment of anxiety are urgently needed (Hofmann, 2008a; Singewald et al., 2015). Preclinical as well as clinical studies have pointed to the relevance of utilizing fear learning paradigms for a deeper understanding of the neurocircuitry and neurochemistry of the fear system involved in anxiety disorders (Hofmann, 2008b). Specifically, patients with anxiety disorders show stronger fear responses during extinction than comparison subjects (Duits et al., 2015), and poor fear extinction is predictive of poor outcome in exposure therapy (Duits et al., 2018).

A potential novel target for the facilitation of fear extinction has been derived from preclinical research. The crucial involvement of the cannabinoid system in fear extinction was first shown by Marsicano et al. (Marsicano et al., 2002). The results show that (genetic or pharmaco-

logical) blockage of transmission at the cannabinoid receptor 1 (CB1) inhibits extinction of fear in mice. This is not surprising given the fact the CB1 receptors are richly expressed in memory-related brain areas such as hippocampus and prefrontal cortex, and as such can modulate (fear) memory (Riedel and Davies, 2005). In the last 15 years many studies have extended this finding using both animal and human subjects (for reviews see (Singewald et al., 2015) or (Berardi et al., 2016)). Animal research has shown that facilitation of the endocannabinoid system (ECS) enhances extinction, whereas blocking or deletion of CB1 receptors impairs extinction. In healthy human subjects we have demonstrated that genetic variation in a CB1 polymorphism significantly affected extinction learning (Heitland et al., 2012). Furthermore, the administration of cannabinoids in humans has shown to strengthen extinction and protect against reinstatement of fear (Das et al., 2013; Rabinak et al., 2013; 2014). In summary, previous research clearly points to the ECS as a promising candidate for extinction enhancement. Until now, studies in humans have mainly investigated the effects of delta-9-tetrahydrocannabinol (THC), which has been shown to decrease physiological measures of fear during extinction (Klumpers et al., 2012) and recall (Rabinak et al., 2013). However, THC is not suitable for phobic patients given the diversity of psychoactive effects caused by THC, among which the high that recreational users of cannabis seek.

In the meantime, studies have demonstrated the potential benefit of another important ingredient of cannabis: cannabidiol (CBD, for a review see (Blessing et al., 2015)). CBD interacts with several receptors in the brain including cannabinoid receptors 1 and 2, transient receptor potential vanilloid type 1 (TRVP1) and serotonin 1A (5-HT1A) receptor, and inhibits or in other ways negatively affects the function of the enzyme that degrades endogenously released cannabinoid neurotransmitters (fatty acid amine hydrolase; FAAH (Campos et al., 2012)). In line with FAAH's function in degrading anandamide (Ahn et al., 2008), inhibition of FAAH has been shown to increase levels of anandamide. Preclinical research indicates that CBD enhances fear extinction and reconsolidation, and co-administrating CB1 antagonists block such effects suggesting that they are exerted via modulation of the ECS (Bitencourt et al., 2008; Stern et al., 2012). Extinction of conditioned fear is proposed to underlie the effect of exposure therapy (Hofmann, 2008b). Hence, the finding that CBD specifically affects (the consolidation of) extinction suggests a potential use of CBD in augmenting the effect of exposure therapy. This leads to the hypothesis that administration of CBD during sessions of exposure therapy is expected to specifically enhance the extinction of pathological fears. The advantage of this application is that CBD needs to be administered occasionally, i.e. preceding exposure sessions only.

We aim to take this previous research to the next level by conducting the first randomized controlled trial with CBD versus placebo, administered in a double-blind fashion, for the

augmentation of exposure treatment in patients with social phobia or panic disorder with agoraphobia. Also, we aim to specifically target patients who have already received one of the gold-standard treatments without responding sufficiently or having relapsed, because this group needs additional approaches most.

The main study aim is to test whether administration of CBD as an augmentation step in exposure therapy can strengthen treatment outcome in patients with phobic disorders who have previously failed to respond satisfactorily to evidence-based treatment. Clinical measurements are used to investigate whether the effect of CBD on exposure is quicker, stronger, or longer-lasting than regular exposure therapy only. Additionally, there are various exploratory subsidiary aims in this study. First, a fear conditioning and extinction task is applied at baseline. This task has shown enhanced fear responses in patients with anxiety disorders as opposed to healthy comparison subjects (Duits et al., 2017). This task also revealed different extinction trajectories, with patients being overrepresented in a poor extinction profile (Duits et al., 2018). These profiles have also shown to be sensitive to differences between patients who will benefit from exposure treatment and those who will not. A re-extinction assessment is done after the first medication administration. The aim of this task is to explore a) whether patients with a specific profile can particularly benefit from CBD augmentation during exposure, and b) the acute effects of CBD intake on fear extinction. Second, we aim to explore the interactions between specific genetic variation and CBD administration on treatment effect. We are particularly interested in studying whether variants within the cannabinoid receptor 1 gene are involved in a differential response to CBD augmented exposure therapy, including rs2180619 identified in our previous study in healthy individuals associated with impaired spontaneous extinction of conditioned fear (Heitland et al., 2012). Additionally, impact of genetic polymorphisms within the FAAH gene (Dincheva et al., 2015) and genetic polymorphisms identified as being related to treatment response in anxiety disorders (Lester and Eley, 2013) will be explored. Similarly, clinical predictors of treatment response will be assessed to determine which sort of patients might benefit most from this augmented treatment. Lastly, we aim to assess cost-effectiveness of CBD enhancement of exposure treatment.

4.2 Methods

4.2.1 Study design

The study encompasses a multi-site randomized, double-blind, placebo-controlled fixed dose clinical trial for patients with treatment resistant social phobia or panic disorder with agorapho-

bia. Either placebo (N=36) or 300 mg cannabidiol (N=36) will be administered 8 times as an adjunct to 8 weekly 90 minute sessions consisting of standardized exposure therapy. The study has been approved by the Medical Ethics Committee of the University Medical Centre Utrecht. Written informed consent will be obtained from all participants. The enrollment of the first participant was on 15 February 2016, recruitment is ongoing at the time of submission. Figure 1 displays a flowchart of the study.

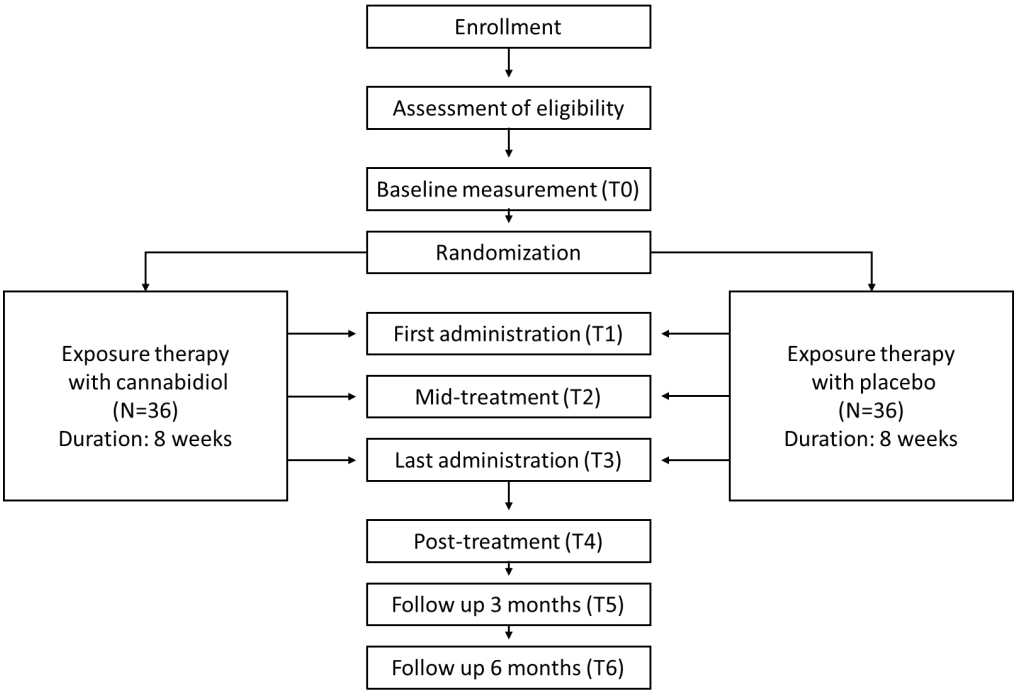

Figure 1. Flowchart of the study design.

Data is collected both during T0-T6 measurements and therapy sessions, see Table 1 for a complete overview.

4.2.2 Participants

Inclusion criteria

- Adult patients between 18 and 65 years with a phobic disorder (social phobia or panic disorder with agoraphobia), diagnosed with the Structured Clinical Interview on DSM-IV disorders (SCID; (First et al., 2002))
- At least one attempt to treat the disorder, according to guidelines, either by means of psychotherapy or with the use of serotonergic antidepressants, has induced insufficient clin-

ically relevant response during or after treatment

Exclusion criteria

- Co-morbid psychiatric disorders, i.e. current severe major depressive or bipolar disorder, psychosis, dependence on alcohol and drugs, as determined by the SCID
- Mental deficiency (IQ<80, as determined by the Nederlandse Leestest voor Volwassenen (NLV; (Schmand et al., 1992)))
- Autism traits (AQ>32, as determined by the Autism Spectrum Quotient (AQ; (Hoekstra et al., 2008)))
- Inadequate proficiency in Dutch, both verbal and written
- (A history of) epilepsy, brain damage, cardiac, renal or liver abnormalities
- History of allergies to medication (adverse reactions or rash)
- Use of antipsychotic medication
- Regular daytime use of benzodiazepines, since use concomitant to exposure has been shown to hamper the treatment effect (Rothbaum et al., 2014)
- Changes in dosing regimen of serotonergic antidepressants shorter than 4 weeks prior to study entry (i.e. use of serotonergic antidepressants at a stable regimen throughout the study is permitted)
- Use of recreational drugs (among others THC, XTC, cocaine) from 2 months preceding study entry
- Pregnancy or breastfeeding

4.2.3 Sample size

The CBD and placebo groups will each include 36 patients. The sample size is aimed at detection of a Cohen's D effect size of 0.6, based on effect sizes found in previous published studies on the augmentation of exposure with d-cycloserine (Norberg et al., 2008). This sample size has been calculated using G*power version 3.0.10 (Faul et al., 2007), with a repeated measures design for two groups with two measurements, an envisioned effect size of 0.6 Cohen's D, error probability of 0.05, power of 0.8 and correlation among repeated measurements of 0.6 based on previous clinical data.

4.2.4 Recruitment

Patients will be recruited at anxiety outpatient clinics of specialized mental health care centers in the Netherlands (Altrecht, GGZinGeest and UCP). Before including patients in the study, they first undergo an intake interview by an experienced therapist. Eligible patients are informed about the study and are invited to a screening and diagnostic interview (SCID) by the researcher or a trained research assistant to confirm in- and exclusion criteria. Patients receive the information brochure and informed consent form if they are eligible and interested in participating. Informed consent is obtained by the researcher or a trained research assistant before the start of the baseline assessment. Additionally, participants can opt to consent to the use of their genetic material in larger international databases.

4.2.5 Randomization and blinding

The randomization (CBD or placebo) is conducted by an independent statistician using a computer algorithm, stratifying for study location and diagnosis (panic disorder with agoraphobia or social phobia). Patients are allocated to one of the medication groups after baseline measurements according to the order of patients in the stratum. Investigators, research assistants, therapists and participants will be blinded with respect to randomization. The capsules containing the different medications are identical in appearance except for filling which is either CBD or lactose (placebo). An independent data manager can break the randomization code in case of pregnancy, allergic reactions or any severe inexplicable symptoms. Apart from these circumstances, unblinding will not be done until after the last patient has completed the last follow up measurement.

4.2.6 Intervention

Eight 90-min exposure sessions will be carried out by therapists who are well trained in cognitive behavioral therapy (CBT), including exposure exercises, and in the current study protocol. Protocols in this study are based on standardized protocols of exposure with response prevention in social phobia (Hofmann and DiBartolo, 2010) and in panic disorder with agoraphobia (Kampman et al., 2012). The protocols consist of therapist-assisted exposure in vivo to fear-provoking thoughts and situations, coupled with response prevention treatment (e.g. not leaving the feared situation or using safety behaviors), tailored to idiosyncratic symptoms of the patients. After every therapy session homework is given, resulting in patients doing at least 8 exposure exercises per week.

Two hours prior to the exposure treatment sessions the study medication is administered. Timing of administration is based on a study by Englund et al. (Englund et al., 2013), indicating Tmax at 3 h 45 min after administration with high plasma levels from 2 h onwards. Therefore, taking the medication 2 h before the start of the session results in relatively stable CBD levels during the entire session.

The eight sessions that are part of the study protocol are not expected to be sufficient for most patients to achieve remission, but this allows sufficient room to investigate whether CBD strengthens therapy response relative to placebo. After the eight sessions in the study protocol patients can continue treatment as needed without further administration of study medication.

4.2.7 Assessments

Response to treatment will be assessed at baseline (T0), at mid-treatment (T2), post-treatment (T4) and at 3 and 6 months' follow-up (T5 and T6 respectively). During treatment, a short assessment is done at each therapy session. Table 1 provides an overview of the measures that are used at each time point. The primary endpoint of this study is the clinical outcome post-treatment (at assessment T4). The other measurements are aimed at the time course of the effect. The mid-treatment and per session assessments are specifically aimed at examining the possibility of a quicker and/or stronger effect of exposure with CBD as opposed to placebo, whereas the follow up measurements allow evaluation of potential long term beneficial effects of CBD. Furthermore, preceding the first and last treatment session with medication administration (T1 and T3 respectively) several secondary measures will be used to study the mechanism underlying acute effects of CBD. Also, blood samples from these assessments will be used to determine CBD plasma levels.

4.2.8 Outcome measures

Primary outcome

The primary outcome measure is the Fear Questionnaire (FQ; (Marks and Mathews, 1979)) which will be administered at every time point (T0-T6) and at every treatment session.

The *FQ* is a part of a standard self-report questionnaire measuring avoidance, the complete form also includes one specific main target phobia, a global phobia rating, and five associated anxiety and depression symptoms (not included in this study). The version of the FQ employed here consists of 15 items asking about the most common phobias rating avoidance using a nine-point scale from '0: would not avoid it' to '8: always avoid it'. The score reflects the level of avoidance, with a total score range from 0 to 120. Three subscores can also be derived using the

sum of 5 items, concerning Agoraphobia, Blood injury phobia and Social phobia.

Secondary outcomes

Clinical questionnaires

Various secondary outcome measures are used to further explore the effect of CBD augmentation on general clinical and specific disorder-related symptoms. Baseline scores on these questionnaires will be used to develop clinical determinants of the effect from augmentation with CBD. All secondary clinical questionnaires are administered at baseline, mid- and post-treatment and follow up assessments.

The *Beck Anxiety Inventory* (BAI; (Beck et al., 1988)) is a 21-item self-report instrument that assesses the overall severity of anxiety. Respondents rate how much each symptom bothered them the past week on a 4-point scale, ranging from 0 (not at all) to 3 (severely, I could barely stand it). The BAI is scored by summing the ratings for all the 21 symptoms to obtain a total score ranging from 0 to 63. Whereas avoidance (measured using the FQ) is a highly relevant clinical construct, restricting analysis to just this aspect may overlook impact on other symptoms of anxiety, such as physiological changes, that may not have a direct effect on behavior as measured by the FQ. Therefore, we have chosen to use the BAI as most important secondary outcome, which is why it is also administered at every treatment session with the FQ.

The *Beck Depression Inventory-II* (BDI-II; (Beck et al., 1996)) is a 21-item self-report instrument that is the most widely used to assess the presence and/or intensity of depressive symptoms. Similar to the BAI, symptoms are scored on a 4-point scale resulting in total scores ranging from 0 to 63.

The *Body Sensations Questionnaire* (BSQ (Chambless et al., 1984)) is a 17-item self-report instrument assessing fear for bodily sensations associated with autonomic arousal. Items are rated on a 5-point scale, total scores range from 17 to 85.

The *Social Phobia and Anxiety Inventory* (SPAI; (Turner et al., 1989)) is used to assess specific somatic symptoms, cognitions and behavior across a range of potentially fear-producing situations. The original SPAI has two subscales, Social phobia (32 items) and Agoraphobia (13 items). A shorter SPAI-18 has been developed assessing only the Social Phobia scale (de Vente et al., 2014). In this study the SPAI-18 is combined with the original Agoraphobia subscale, resulting in 31 items. Thirteen items require separate ratings concerning either four different social situations or physiological and cognitive questions. Mean scores are calculated for these items based on the 7-point scale ranging from 1 (never) to 7 (always). To obtain the score the number of items is subtracted from the summed item scores. The maximum score for the SPAI-18 is 108, and for the Agoraphobia scale 78.

Only during the treatment sessions the *Clinical Global Impression* (CGI; (Busner and Tar-

gum, 2007)) and *Subjective Units of Distress* (SUDS; (Wolpe, 1969)) are administered. The CGI consists of 2 items, measuring illness severity and improvement. The items are rated on a 7-point scale by the therapist, with the severity scale ranging from 1 (normal) to 7 (amongst the most severely ill patients), and the improvement scale ranging from 1 (very much improved) to 7 (very much worse). Each component is rated separately, there is no total score (Guy, 1976). The SUDS are used during exposure to measure within-session extinction. Before and after the exposure in vivo exercise percentage of fear and credibility of thoughts about the exercise are rated by the patient (Benito and Walther, 2015).

Besides the broader clinical questionnaires, diagnosis-specific questionnaires are only administered to patients with the diagnosis in question. Questionnaires pertaining to the diagnosis of panic disorder with agoraphobia:

The *Panic Disorder Severity Scale* (PDSS; (Shear et al., 1997)) is a 7-item clinician-administered instrument assessing severity of panic disorder and monitoring treatment outcome. Items are rated on a 5-point scale which ranges from 0 to 4, total scores are calculated by summing the scores for the items resulting in a range of 0 to 28.

The *Mobility Inventory* (MI; (Chambless et al., 1985)) is a 27-item self-report instrument for the measurement of agoraphobic avoidance behavior in specific situations. These situations are rated both when patients are accompanied and when they are alone. Items are rated on a 5-point scale which ranges from 1 (never) to 5 (always), the score is calculated by averaging the items.

The *Agoraphobic Cognitions Questionnaire* (ACQ; (Chambless et al., 1984)) is a 14-item self-report instrument assessing thought concerning negative consequences of experiencing anxiety. Each item is rated on a 5-point scale ranging from 1 (never occurs) to 5 (always occurs), total scores are calculated by averaging the items. Specifically, catastrophic thoughts typically noted during exposure to anxiety-provoking experiences are used, making it highly relevant for the assessment of therapy success.

Questionnaire pertaining to the diagnosis of social phobia:

The *Liebowitz Social Anxiety Scale* (LSAS; (Mennin et al., 2002)) is a self-report instrument with 24 items measuring both fear and avoidance across a number of social situations. Fear scale ratings range from 0 (no fear) to 3 (severe fear), avoidance ratings also range from 0 to 3 and are based on percent of time avoiding the situation (0=never, 1=occasionally (10%), 2=often (33-67%), and 3=usually (67-100%). The LSAS is divided in two subscales, related to performance anxiety (11 items) and social interaction (13 items).

All clinical questionnaires have been shown to have adequate reliability and validity (ACQ (Bouman, 1995), BAI (Steer et al., 1993), BDI (van der Does, 2002), BSQ (Bouman, 1998), FQ

(van Zuuren, 1988), LSAS (Baker et al., 2002), MI (Chambless et al., 1985), PDSS (Shear et al., 2001), SPAI (de Vente et al., 2014)), except for the CGI (Forkmann et al., 2011) which is advised to be used in accordance with other validated questionnaires, which are used in this study.

Table 1 Overview of assessments

Measure	Assessment	T0	T1	T2	T3	T4	T5	T6	Treatments
SCID	Diagnosis	x							
General patient characteristics	Demographic information	x							
Clinical background	Therapy history	x							
CTQ	Childhood trauma	x							
AQ	Autism quotient	x							
FQ	Presence and severity phobic symptoms	x		x		x	x	x	x
BAI	Anxiety severity	x		x		x	x	x	x
CGI	Clinical global impression								x
SUDS	Degree of habituation and extinction								x
BDI	Depression	x		x		x	x	x	
BSQ	Somatic symptoms	x		x		x	x	x	
EQ5D	Quality of life	x		x		x	x	x	
Tic-P	Loss of work and productivity plus health care costs	x		x		x	x	x	
SPAI	Social phobia and anxiety severity	x		x		x	x	x	
LSAS	Social anxiety severity (SOC PHOB)	x		x		x	x	x	
PDSS	Panic disorder severity (PD+AGO)	x		x		x	x	x	
MI	Mobility inventory (PD+AGO)	x		x		x	x	x	
ACQ	Agoraphobia severity (PD+AGO)	x		x		x	x	x	
Fear conditioning task	Acquisition and extinction of fear learning	x	x		x				
Blood	CBD level, DNA, epigenetics		x		x				

Note: Questionnaires that are only assessed for a specific diagnosis are specified between brackets (panic disorder with agoraphobia = PD+AGO, social phobia = SOC PHOB). T0 = Baseline, T1 = First medication administration, T2 = Mid treatment, T3 = Last medication administration, T4 = Post treatment, T5 = Follow up (3 months), T6 = Follow up (6 months), Treatments = All 8 therapy sessions. *SCID* Structured Clinical Interview for DSM disorders axis I, *CTQ* Childhood Trauma Questionnaire, *AQ* Autism spectrum Quotient, *FQ* Fear Questionnaire, *BAI* Beck Anxiety Inventory, *CGI* Clinical Global Impression, *SUDS* Subjective Units of Distress Scale, *BDI* Beck Depression Inventory, *BSQ* Bodily Sensations Questionnaire, *EQ5D* EuroQol, *Tic-P* Trimbos and iMTA questionnaire on Costs associated with Psychiatric illness, *SPAI* Social Phobia and Anxiety Inventory, *LSAS* Liebowitz Social Anxiety Scale, *PDSS* Panic Disorder Severity Scale, *MI* Mobility Inventory, *ACQ* Agoraphobic Cognitions Questionnaire

4.2.8.1 General patient characteristics

Demographic information such as age, gender, education, employment, nationality and marital status will be collected using a general demographic questionnaire at baseline. Current use of drugs and medication is assessed with a short questionnaire. Additional questions are asked concerning the clinical background, e.g. treatment history, and the Childhood Trauma Questionnaire (Bernstein and Fink, 1998) is administered.

4.2.8.2 Experimental assessment of fear learning

To explore whether capacity for fear and extinction learning at baseline impacts the effect of CBD, and whether treatment with CBD has impact on improvements in extinction learning after treatment, an experimental fear conditioning and extinction task will be used to assess the capacity to acquire and extinguish conditioned fear (Duits et al., 2017). At baseline, this task will investigate the acute effect of CBD on extinction learning, a second extinction phase with the same conditioned stimuli as at baseline is administered 2 h after the first ingestion of the medication. This additional fear extinction phase is administered 1 to 2 weeks after administration of the baseline fear conditioning task. Finally, post-treatment the same fear conditioning task will be administered, with minor adaptations to minimize previous learning effects (e.g. with different conditioned stimuli). With this post-treatment task, changes in rate of extinction due to treatment is compared between the CBD and placebo groups.

4.2.8.3 Genetics

Profiling of phobic patients based on genetic variance will be done to examine potential factors that have impact on the effect of exposure therapy and on the effect of CBD augmentation. In general, we expect more benefit of CBD augmentation for individuals with genetic profiles associated with lack of spontaneous extinction. More specifically, for the impact on CBD augmentation, genetic variance in CNR1 (Heitland et al., 2012), FAAH (Dincheva et al., 2015; Leweke et al., 2012) and genes related to treatment response in phobic disorders (Lester and Eley, 2013), will be analyzed.

4.2.8.4 Cost effectiveness

The documentation of (non-)medical costs and productivity loss will be collected to assess cost-effectiveness of CBD-augmented psychotherapy. Both cost effectiveness-questionnaires are administered at baseline, mid- and post-treatment and follow up assessments.

The *Treatment inventory of costs in Psychiatric patients* (Tic-P (Bouwmans et al., 2013)) is a self-report questionnaire consisting of two parts, medical resource, including volume of mental and general health care utilization (direct medical costs), travel to and from health care providers (non-medical costs), and productivity loss, generated by absence from paid work (indirect costs). Corresponding costs are calculated by multiplying the volumes by the corresponding reference unit prices (Hakkaart-van Roijen et al., 2015).

The *EuroQol five dimensions* (EQ5D (EuroQolGroup, 1990)) is a 5-item self-report instrument which is the most commonly used generic health status measurement. The items have five response categories from no problems to incapacity/extreme problems. Additionally, a visual analogue scale (VAS) is used to rate their health on a scale ranging from 0 (worst possible health) to 100 (best possible health).

4.2.9 Statistical analysis

4.2.9.1 Treatment augmentation

Data concerning the primary and secondary outcome measures will be analyzed by comparing the scores on the measurement scales using mixed modeling, with medication (CBD vs. placebo) and time (time points: baseline, mid-treatment, post-treatment and follow-ups). Analyses are conducted according to the intention-to-treat principle, i.e. all patients who have completed the baseline assessment are included in the analyses. Furthermore, also a 'completers only' analysis will be done including just the participants who have completed the treatment and participated in all measurements (T0-T6).

4.2.9.2 Patient profiling

To determine which patient characteristics may predict additional benefit of CBD augmentation, explorative multilevel analyses with treatment success as dependent variable will be performed, with the following independent variables (among others); medication (CBD or placebo), diagnosis (panic disorder with agoraphobia or social phobia), fear learning (response during extinction, and reduction of fear from acquisition to extinction), cannabinoid system genetics (using a candidate gene approach focused on CNR1 and FAAH), prior treatment history (failed CBT, SSRI, or both), clinical state at baseline and demographic variables (gender, age).

4.2.9.3 Fear learning

Acute effect of CBD on fear learning is analyzed with retention of conditioned fear, and rate of extinction in this re-extinction phase as outcome variables, and medication as independent variable. Impact of CBD-augmented exposure therapy on changes in rate of extinction from baseline between the CBD and placebo groups is examined by comparing extinction before and after treatment.

4.2.9.4 Cost effectiveness

Costs of illness and intervention is measured using resource utilization which will be valued with unit costs based on standardized real cost price calculations. The economic evaluation is primarily designed as exploratory cost-effectiveness analyses.

4.2.10 Data management and dissemination

To improve data completeness we have developed a study specific digital file to store personal information and to get reminders for upcoming assessments and missing data, which can be accessed by the researchers per participating center. Actual data are not collected in this file, but stored digitally in a database on the servers of GGZinGeest, separately from personal information of the participants. To ensure data quality and reliability, questionnaires are administered online and saved digitally, together with and data from interviews. Data from treatment sessions is collected and entered into the study data base and subsequently checked by research assistants. Data management and monitoring is conducted by data managers from GGZinGeest. Study conduct is reported and audited in interim, with final reports to the funding agency. The procedures comply with Dutch data privacy laws.

If participants wish to withdraw from the intervention, their participation in the post-treatment and follow up assessments are encouraged. Unless participants have withdrawn consent for follow-up, repeated attempts are made to contact participants. In a step-wise manner, this will involve sending emails and calling the individual on contact numbers provided on various days of the week and at different times. As much information as possible will be collected from protocol non-adherers.

Adverse events occurring after entry into the study are recorded. Investigators will determine relatedness of an event to the study drug based among others on temporal relationship and the subject's clinical course.

Any modifications to the protocol which may impact the conduct of the study, potential benefit of the patient or may affect patient safety, including changes of study objectives, study

design, patient population, sample sizes, study procedures, or significant administrative aspects will require a formal amendment to the protocol. Such amendment will be approved by the Ethics committee prior to implementation and information on the Trial register website will be updated to ensure transparency.

There are no interim analyses planned. The final trial dataset will be accessible to the researchers and data managers. Results of the analyses will be published in scientific journals and presented on scientific conferences by the researchers, regardless of the outcome. A summary report of trial results written in lay language will be sent to study participants and other people who have expressed interest.

4.3 Discussion

Phobic disorders are among the most prevalent disorders and have a major impact on the life of patients and society as a whole resulting in suffering and associated costs. Evidence-based treatments of these disorders, while effective for a large number of patients, are not adequate for a substantial group who are not sufficiently relieved from their anxiety symptoms. One strategy may be to boost the effectiveness of current treatments. Enhancing exposure therapy with pharmacological agents that affect the neurological processes involved in the extinction of fear is an avenue that has been explored with augmentation using d-cycloserine, with mixed success (Hofmann, 2016). Since an enhancer of exposure therapy is needed but the compounds so far have not proven to be sufficiently effective, we have opted to use a new strategy using the modulation of the endocannabinoid system. This study will be the first clinical trial in which cannabidiol is used to augment exposure therapy for phobic patients.

It is important to note that this study is investigator initiated, and independent from pharmaceutical or other industry interests. Findings will be submitted to a peer reviewed scientific journal for publication.

This study is based on the preclinical evidence that ECS manipulations can be used to enhance (the retention of) fear extinction. However, acute anxiolytic effects of cannabidiol have also been reported. One study reported anxiolysis during a public speaking challenge, which resembles the type of challenges that patients with phobia are faced with during exposure therapy (Bergamaschi et al., 2011). Hence, an additional possible outcome of the study is that cannabidiol reduces fear and anxiety acutely during the treatment sessions, making the treatments easier to tolerate. Despite the conviction based on other anxiolytic treatments that anxiolysis during exposure reduces effectiveness (Foa et al., 1986), the expectation is that cannabidiol may combine acute anxiolysis with enhanced retention of treatment effects.

A strong feature of this study is the exploratory assessment of genetic, experimental and clinical differences between patients related to extinction and subsequent treatment response. The results of this study might give rise to new insights into the possibility of personalized treatment, by exploring whether this strategy is best, specifically for patients with certain characteristics.

Abbreviations

5-HT1A	Serotonin 1A
ACQ	Agoraphobic Cognitions Questionnaire
AQ	Autism spectrum quotient
BAI	Beck Anxiety Inventory
BDI	Beck Depression Inventory
BSQ	Bodily Sensations Questionnaire
CB1	Cannabinoid 1
CBD	Cannabidiol
CBT	Cognitive behavioral therapy
CGI	Clinical Global Impression
CTQ	Childhood Trauma Questionnaire
ECS	Endocannabinoid system
EQ5D	EuroQol 5D
FAAH	Fatty acid amide hydrolase
FQ	Fear Questionnaire
LSAS	Liebowitz Social Anxiety Scale
MI	Mobility Inventory
NLV	Nederlandse leestest voor volwassenen
PDSS	Panic Disorder Severity Scale
SCID	Structured clinical interview on DSM-IV disorders
SPAI	Social Phobia and Anxiety Inventory
SSRI	Selective serotonin re-uptake inhibitor
SUDS	Subjective Units of Distress Scale
THC	Delta-9-tetrahydrocannabinol
Tic-P	Trimbos and iMTA questionnaire on Costs associated with Psychiatric illness
TRPV1	Transient receptor potential vanilloid type 1
VAS	Visual analogue scale

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and will not be involved in data collection and analysis, decision to publish or preparation of the manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study from patients consenting to sharing their data are available from the corresponding author on reasonable request.

Roles and responsibilities

Trial sponsor: Department of Experimental Psychology, Helmholtz Institute, Utrecht University

Representative: Prof. dr. J.L. Kenemans, j.l.kenemans@uu.nl

The sponsor has no role in the design of this study and will not have any role during its execution, analyses, interpretation of the data, or decision to submit results.

User committee

- Agreement of demands for patients
- Agreement of patient recruitment procedure, information letter and consent form

Data management

- Maintenance of trial IT system and data entry

Ethics approval and consent to participate

The study has been approved by the Medical Ethics Committee of the University Medical Centre Utrecht (reference number: NL50898.041.15). Written informed consent will be obtained from all participants.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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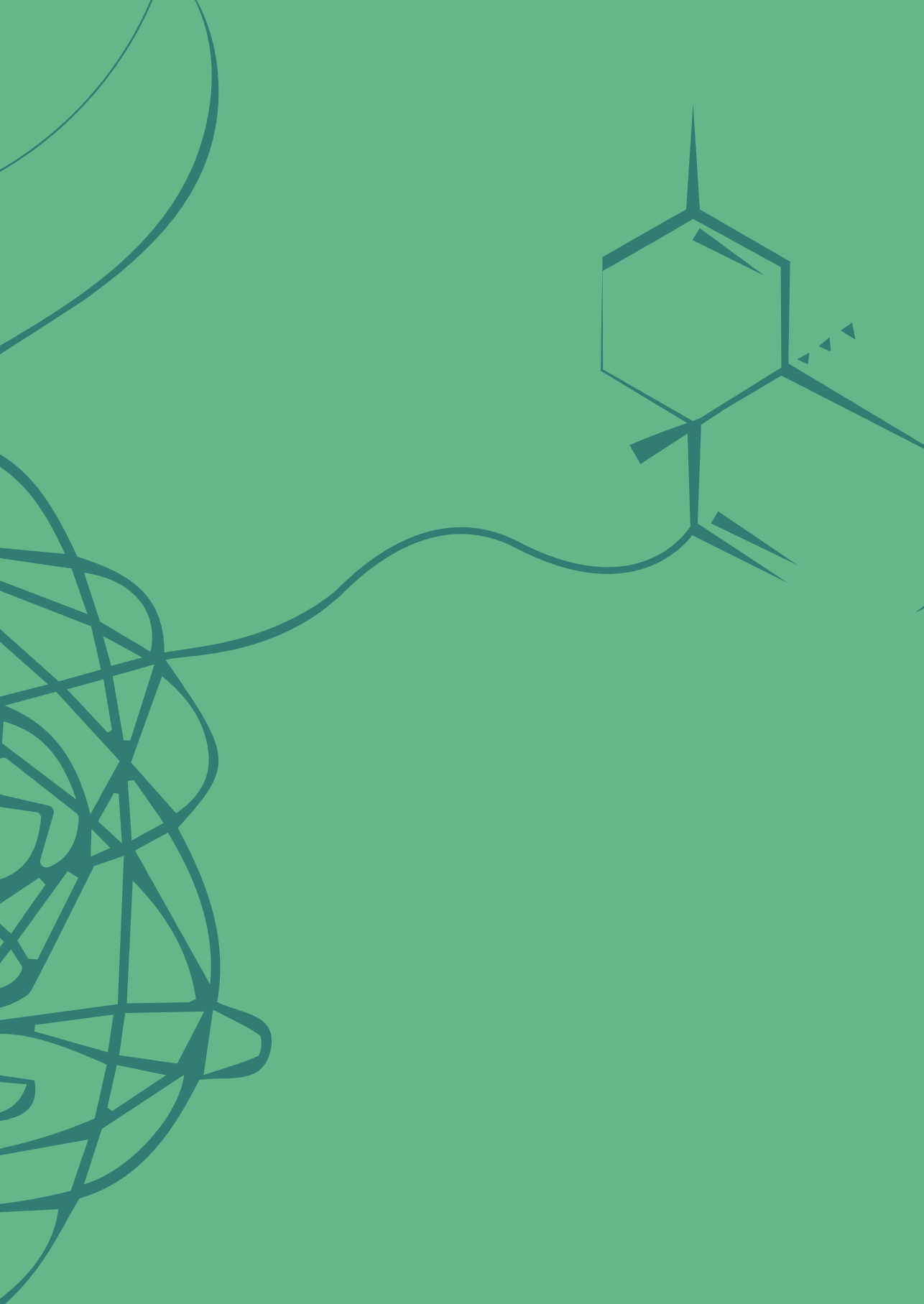

Chapter 5

Cannabidiol enhancement of exposure therapy in treatment refractory patients with social anxiety disorder and panic disorder with agoraphobia: A randomized controlled trial

Caroline M.B. Kwee
Johanna M.P. Baas
Febe E. van der Flier
Lucianne Groenink
Puck Duits
Merijn Eikelenboom
Date C. van der Veen
Mirjam Moerbeek
Neeltje M. Batelaan
Anton J.L.M. van Balkom
Danielle C. Cath

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JB (PI), DC, AvB, LG and NB obtained funding for this study. All authors contributed to the design of the study. FvdF and CK coordinated the recruitment of patients and the data collection. CK, JB, and ME have accessed and verified the data. CK and MM did the statistical analyses. JB, DC, AvB and NB are responsible for the overall design and supervision. CK wrote the manuscript. All authors (FvdF, JB, DC, PD, DvdV, AvB, LG, NB, CK, MM, ME) read, contributed to and approved the final manuscript.

Abstract

Preclinical research suggests that enhancing CB1 receptor agonism may improve fear extinction. In order to translate this knowledge into a clinical application we examined whether cannabidiol (CBD), a hydrolysis inhibitor of the endogenous CB1 receptor agonist anandamide (AEA), would enhance the effects of exposure therapy in treatment refractory patients with anxiety disorders. Patients with panic disorder with agoraphobia or social anxiety disorder were recruited for a double-blind parallel randomized controlled trial at three mental health care centers in the Netherlands. Eight therapist-assisted exposure in vivo sessions (weekly, outpatient) were augmented with 300 mg oral CBD ($n = 39$) or placebo ($n = 41$). The Fear Questionnaire (FQ) was assessed at baseline, mid- and post-treatment, and at 3 and 6 months follow-up. Primary analyses were on an intent-to-treat basis. No differences were found in treatment outcome over time between CBD and placebo on FQ scores, neither across ($\beta = 0.32$, 95% CI [-0.60; 1.25]) nor within diagnosis groups ($\beta = -0.11$, 95% CI [-1.62; 1.40]). In contrast to our hypotheses, CBD augmentation did not enhance early treatment response, within-session fear extinction or extinction learning. Incidence of adverse effects was equal in the CBD ($n = 4$, 10.3%) and placebo condition ($n = 6$, 15.4%). In this first clinical trial examining CBD as an adjunctive therapy in anxiety disorders, CBD did not improve treatment outcome. Future clinical trials may investigate different dosage regimens.

5.1 Introduction

Cognitive behavioral therapy including exposure therapy is the first line evidence based treatment for anxiety disorders (van Dis et al., 2020). Effect sizes are small to medium at six to twelve months of follow-up for panic disorder and social anxiety disorder. Notwithstanding its effectiveness at a group level, around one third of patients are non-responders (Taylor et al., 2012). In addition, relapse rates in anxiety disorders are high, with recurrence rates up to 23.5% in patients with a remitted anxiety disorder within two years (Scholten et al., 2012).

Fear extinction, which occurs when a conditioned stimulus is repeatedly presented in the absence of the associated aversive event, is presumed to underlie the effect of exposure therapy (Craske et al., 2014). Marsicano et al. (2002) were the first to show the central function of cannabinoid type 1 (CB1) receptors in fear extinction in rodents. They demonstrated that fear extinction was impaired after genetically or pharmacologically blocking of CB1 receptors (Marsicano et al., 2002), which are expressed in the prefrontal cortex, hippocampus, and amygdala (Herkenham et al., 1991; Moldrich and Wenger, 2000).

Direct evidence of involvement of the human endogenous cannabinoid system in fear extinction stems from a study in healthy human subjects (n = 150) who underwent a fear conditioning and extinction procedure in a virtual reality environment (Heitland et al., 2012). Participants were genotyped for two polymorphisms located within the promoter (rs2180619) and coding region (rs1049353) of the CB1 receptor. Whereas both homozygote (G/G, n = 23) and heterozygote (A/G, n = 68) G-allele carriers of rs2180619 displayed robust extinction of fear, extinction of fear-potentiated startle was absent in A/A homozygotes (n = 51). This resistance to extinguish fear resulted in increased levels of fear-potentiated startle at the end of the extinction training within the group of A/A carriers (Heitland et al., 2012).

Human studies have investigated exocannabinoids in relation to fear extinction. Of these, Δ^9-tetrahydrocannabinol (THC) may be less suitable for clinical applications given the induction of psychotic and anxiety symptoms in some individuals (Bhattacharyya et al., 2010). In contrast, cannabidiol (CBD) seems to exert an anxiolytic effect in animal experimental (Almeida et al., 2013; Campos et al., 2012; Moreira et al., 2006) and human studies (Crippa et al., 2011; Fusar-Poli et al., 2009; Zuardi et al., 2017) without these psychotropic effects, coupled with a favorable safety profile (Bergamaschi et al., 2011a) and low abuse potential (Parker et al., 2004). Contrary to direct CB1 receptor agonists (like THC), CBD does not induce psychomotor impairment and does not lead to feelings of "high" (Dalton et al., 1976). CBD increases availability of the endogenous cannabinoid anandamide (AEA; Kano et al., 2009), which binds to CB1 receptors (Devane et al., 1992). The main metabolic enzyme of AEA, fatty acid amide hydrolase

(FAAH), catalyses its' hydrolysis (Ueda et al., 2000).

The role of AEA signalling in anxiety has been underpinned by genetic data. Non-patient carriers (n = 31) of the FAAH polymorphism C385A (rs324420), which has been associated with low expression of FAAH in human blood T-lymphocytes (Chiang et al., 2004) and elevated plasma AEA levels (Sipe et al., 2010), exhibited greater amygdala-habituation during repeated viewing of threatening faces compared to other genotypes (n = 50; Gunduz-Cinar et al., 2013). In addition, homozygous C385A carriers (n = 48) had lower trait stress reactivity scores than other genotypes (n = 833; Gunduz-Cinar et al., 2013). These findings suggest that pharmacological augmentation of AEA signaling may be a promising avenue for reducing anxiety. Here, CBD, an AEA reuptake and hydrolysis inhibitor (Bisogno et al., 2001), comes into play.

There is substantial -although not entirely unambiguous- evidence from animal studies that CBD may be used as an adjunct to exposure therapy and help to alleviate anxiety through enhancement of fear memory extinction learning (Bitencourt et al., 2008; Do Monte et al., 2013; Lemos et al., 2010; Resstel et al., 2006; Song et al., 2016). In addition, CBD may also exert more global anxiolytic effects apart from extinction learning, which may not in all experiments be distinguishable from effects on fear extinction (Lemos et al., 2010; Resstel et al., 2006).

Two studies demonstrated lower freezing during context re-exposure in fear conditioned rats treated intraperitoneally with 10 mg/kg CBD, compared to vehicle-treated animals. In non-conditioned rats, CBD had no effects on freezing (Lemos et al., 2010; Resstel et al., 2006). In another study, freezing during context re-exposure was lower in rats who received CBD prior to extinction training than in rats who received vehicle (Song et al., 2016). This effect was reversed when rats were conditioned with two foot shocks (Song et al., 2016).

Further, rats treated with high dosages of 2 μg intracerebroventricular CBD prior to extinction training displayed lower freezing time compared to the vehicle-treated group (Bitencourt et al., 2008). This effect, which only occurred at this high dose, persisted after one day of CBD washout, which suggests long-term facilitation of extinction (Bitencourt et al., 2008). Another study with a similar set-up found effects during extinction training with their highest dose of 0.4 μg CBD injected into each side of the infralimbic cortex, that persisted until the drug-free test (Do Monte et al., 2013). These effects were blocked by administering a CB1 receptor antagonist, which suggests mediation of extinction learning by CB1 receptor activation (Do Monte et al., 2013).

The effect on extinction learning of 32 mg vaporized CBD or placebo before or after extinction training was studied in healthy human volunteers (n = 48; Das et al., 2013). Administration after extinction training led to lower shock expectancy upon presentation of the conditioned stimulus 24 h after conditioning compared to placebo. Null effects were found when CBD was

administered prior to extinction training. The authors argued that ceiling-level extinction might have obscured potential differences between drug conditions (Das et al., 2013). More room for improvement would be expected in patients than in healthy subjects (Duits et al., 2015).

There is a paucity of clinical studies with CBD in patients with anxiety disorders. We identified three studies with a randomized controlled design in patients with social anxiety disorder (Bergamaschi et al., 2011b; Crippa et al., 2011; Masataka, 2019). In the first single dosage study, 24 participants were subjected to a simulated public speaking task 80 min after ingesting 600 mg oral CBD dissolved in corn oil, or placebo (Bergamaschi et al., 2011b). In the second single dosage study, 10 subjects ingested 400 mg CBD or placebo 110 min before SPECT neuroimaging (Crippa et al., 2011). Subjective state anxiety and functional activity of temporo-limbic and paralimbic regions were measured (Crippa et al., 2011). In these works, CBD exerted beneficial effects on measures of subjective anxiety (Bergamaschi et al., 2011b; Crippa et al., 2011), and led to functional activity changes that were in line with these effects (Crippa et al., 2011). In a third randomized controlled trial 300 mg CBD ingested daily for four weeks ($n = 17$) by teenagers decreased severity of social anxiety disorder compared to placebo ($n = 20$; Masataka, 2019). In this study no exposure-based CBT was added during the study period.

In conclusion, preclinical animal and human research suggests a critical role of the endocannabinoid system in fear extinction and, possibly, extinction consolidation. In addition, evidence exists for a general anxiolytic effect of CBD. In order to bridge the gap between these promising results and application in the clinic we conducted a randomized controlled trial in patients with panic disorder with agoraphobia or social anxiety disorder. Our main research question was whether augmentation of exposure therapy with 300 mg oral CBD would lead to stronger or faster improvement of anxiety symptoms. In addition, we explored whether CBD would enhance extinction within treatment sessions and/or would reduce fear acutely. Furthermore, considering potential effects on extinction consolidation, we tested whether an effect of CBD on symptom severity would be moderated by within-session extinction learning. Assuming that CBD would enhance the consolidation of adaptive learning during treatment sessions, we expected a beneficial effect from CBD on symptom severity only when fear and/or credibility of the feared outcome at the end of the treatment session were low.

5.2 Experimental procedures

5.2.1 Study design

Patients with treatment refractory social anxiety disorder or panic disorder with agoraphobia participated in this randomized, double-blinded, parallel, placebo-controlled fixed dose clinical multicenter trial. Patients were considered to be treatment refractory when they either had not profited from at least one previous state of the art pharmacological and/or psychological treatment, or when they experienced a relapse after previous successful treatment. The study was approved by the Medical Ethics Committee of the University Medical Center Utrecht (protocol number 40-41,200-98-9269). The study protocol has been published (`https://link.springer.com/article/10.1186/s12888-019-2022-x`), and the trial is registered on EU Clinical Trials Register (2014-004094-17).

5.2.2 Participants

At one of the three participating mental health care centers, participants received an explanation of study procedures and were given the opportunity to ask questions before providing written informed consent. A screening interview and Structured Clinical Interview on DSM-IV disorders (SCID; First et al., 2002) was conducted to check in- and exclusion criteria (full criteria in study protocol; van der Flier et al., 2019):

Inclusion criteria.

- Patients between 18 and 65 years with a primary diagnosis of social anxiety disorder or panic disorder with agoraphobia according to DSM-IV criteria.

Exclusion criteria.

- Co-morbid psychiatric disorders, i.e. current severe major depressive (BDI > 40) or bipolar disorder, psychosis, dependence of alcohol and drugs;
- Use of antipsychotic medication;
- Regular daytime use of benzodiazepines;
- Changes in dosing regimen of serotonergic antidepressants < 4 weeks prior to study entry;

- Use of recreational drugs < 2 months preceding study entry (alcohol and tobacco were permitted);
- Pregnancy or breastfeeding.

5.2.3 Randomization and masking

The randomization (CBD or placebo with a 1:1 allocation ratio) was conducted by an independent data manager using block randomization, stratifying for study center and primary diagnosis. Patients were allocated to one of the medication groups after enrollment according to the order in the stratum. The capsules containing CBD and placebo were identical in appearance.

Investigators, research assistants, therapists, and participants were blinded with respect to randomization. For one patient the randomization code was broken and participation discontinued because of an unplanned pregnancy. Data for the remaining patients were unblinded after the last post-treatment measurement (December 3, 2019) to allow timely reporting to the trial funder. Research assistants who remained blinded collected remaining follow-up measurements. Patients were unblinded after the last follow up measurement. In the eighth treatment session and at post-treatment, therapists and patients were asked to speculate whether the patient had received CBD or placebo. Judgements of therapists and patients were mostly based on expected and observed adverse or anxiolytic effects, and independent of actual drug conditions (for therapists $p = 0.851$; for patients, $p = 0.110$), indicating that blinding was successful.

5.2.4 Procedures

Eight 90-min therapist-assisted augmented exposure in vivo sessions were delivered by psychologists trained in cognitive behavioral therapy (CBT) and in the standardized protocols for exposure therapy in the current study.

In the introductory session patients received the treatment rationale and –explanations, and baseline assessments. Synthetic CBD in powder form (purity > 99.9 %) was manufactured by STI pharmaceuticals (UK) and THC Pharm (Germany) and encapsuled by ACE Pharmaceuticals in compliance with Good Manufacturing Practice (GMP). Approximately 2 h before exposure treatment sessions 1 to 8 patients ingested 300 mg CBD or placebo (lactose). Timing of administration was aimed at achieving peak plasma levels during the treatment session (Englund et al., 2013). In order to ensure dosage in the effective and safe range we employed dosages of 300 mg, in line with previous work (Zuardi et al., 2017, 1993).

Outcome measures were assessed at baseline (T0), at mid-treatment (T1), post-treatment (T2) and at 3 and 6 month follow-up (T3 and T4). During treatment, a short assessment includ-

ing the primary outcome measure was done at each therapy session (S0 to S8). In order to check compliance, blood samples to assess CBD plasma levels were collected preceding the first (S1) and last (S8) treatment session, 2 h after medication administration.

5.2.5 Outcomes

The primary outcome measure comprised the Fear Questionnaire (FQ; Marks and Mathews, 1979), which measures level of avoidance as a result of the anxiety disorder. Overall severity of anxiety, as measured by the Beck Anxiety Inventory (BAI; Beck et al., 1988), was our most important secondary outcome measure (for all clinical outcome measures, see Table S1). The primary endpoint of this study was the clinical change until post-treatment (at assessment T2) measured with the FQ. Therapists asked their patients in each treatment session (S0 to S8) whether any negative effect had occurred that could be related to the study medication. In addition, patients' spontaneous reports of adverse events were collected. Grouping of adverse events into the categories "none", "potential", "probable", and "definite" related to the study medication was based on therapists' and researchers' judgement .

5.2.6 Statistical analysis

Sample size calculation, based on a repeated measures design for two groups with two measurements, and an envisioned effect size of 0.6 Cohen's *d* yielded groups sized of 36 patients per treatment arm for a power of 0.8, with $\alpha = 0.05$.

We used multilevel regression analyses with factors time (linear and quadratic trends), drug, and diagnosis to investigate 1) whether CBD augmentation would be associated with better clinical outcome at post-treatment (T2) and a more favorable time course of the treatment effect. Further, we assessed 2) whether clinical improvement in the CBD condition was more enduring (from post-treatment (T2) to follow-up at 6 months (T4) and 3) whether improvement occurred faster (from the introductory (S0) to the last drug augmented treatment session S8) compared to the placebo condition. In order to reduce unexplained error variance, the following covariates were investigated (and discarded from the final models if they did not significantly affect outcome): Use of antidepressant medication, stability or change in medication dosing regimen during the follow-up period, and number of treatment sessions until the last follow-up assessment.

With ancillary multilevel discrete-time (since treatment response was measured per session rather than continuously) survival analyses (Hox et al., 2018) we investigated 4) early treatment response by CBD, defined as a 25% or greater symptom reduction (Taylor et al., 2012;

Hofmeijer-Sevink et al., 2017) on FQ and BAI from the introductory session to session 8.

We also examined 5) direct CBD effects on extinction learning or anxiolysis occurring within treatment sessions, as measured with subjective units of distress (SUDs) scores prior and directly after exposure exercises. Finally, to explore whether CBD may enhance consolidation of (mal)adaptive learning during exposure therapy sessions we tested, in line with a previous method (Hofmeijer-Sevink et al., 2017; Smits et al., 2013) whether 6) low SUDs scores at the end of treatment sessions moderated effects of CBD on FQ, BAI, CGI, LSAS, or MI "alone" score at the next session.

In the analyses on assessments from baseline to second follow-up (T0-T4), full information maximum likelihood (FIML) estimates for model parameters were calculated using all available data of 78 patients. Two patients failed to fill in any of the questionnaires, so they were not included in these analyses. Missing values in the per session assessments were handled by multiple imputation.

Elaborate report of methodology in Supplemental experimental procedures, section S1.

5.3 Results

5.3.1 Study population

Patients were randomly assigned between June 2016 and August 2019. The last follow-up assessment took place in May 2020. Treatment discontinuation rates did not differ significantly between the CBD (n = 7, 17.9%) and placebo condition (n =12 , 29.3%), χ^2 = 1.41, p = 0.23. The flow of participants through the study is displayed in a CONSORT diagram (Fig. S1). Participants' baseline characteristics are provided in Table 1.

Table 1 Sociodemographic and clinical characteristics at baseline, split by drug condition.

	Total sample		Placebo		Cannabidiol	
	(n = 80)		(n = 41)		(n = 39)	
Sociodemographics						
Age at study entry	36.7	(10.5)	38.3	(11.3)	34.9	(9.3)
Female sex	32	(40.0)	15	(36.6)	17	(43.6)
Double nationality	4	(7.7)	2	(7.7)	2	(7.7)
Married or cohabiting	27	(50.9)	15	(55.6)	12	(46.2)
Post-high school education	35	(68.6)	17	(65.4)	18	(72.0)
Currently employed	43	(65.2)	19	(59.4)	24	(70.6)
Clinical varables						
Having received previous treatment	49	(65.3)	26	(66.7)	23	(63.9)
Use of antidepressant medication	34	(42.5)	16	(39)	18	(46.2)
Primary diagnosis social anxiety disorder	37	(46.3)	19	(46.3)	18	(46.2)
Primary diagnosis panic disorder with agoraphobia	43	(53.8)	22	(53.7)	21	(53.8)
FQ, mean	51.9	(19.8)	54.2	(19.4)	49.5	(20.2)
BAI, mean	27.9	(10)	29.3	(9.1)	26.4	(10.8)
CGI severity, mean	4.8	(1.0)	4.7	(1.0)	4.9	(1.1)
BDI-II	22.9	(12.1)	22.1	(12.3)	23.6	(12.0)
SPAI-18 Social phobia subscale	60.2	(21.2)	61.8	(20.9)	58.7	(21.6)
BSQ	2.3	(0.7)	2.4	(0.8)	2.2	(0.7)
Panic disorder specific questionnaires (n=43)						
ACQ	2.2	(0.6)	2.1	(0.6)	2.2	(0.7)
MI "alone"	3.1	(0.9)	3.3	(0.9)	2.9	(1.0)
MI "accompanied"	2.4	(0.9)	2.4	(0.9)	2.4	(0.9)
PDSS	15.5	(5.0)	15.5	(5.3)	15.5	(4.7)
Social anxiety disorder specific questionnaires (n=37)						
LSAS	78.7	(29.8)	84.3	(27.4)	74.1	(32.1)

Note: Data are mean (SD) or n (%).

5.3.2 Intent-to-treat analysis

The multilevel analyses to answer objectives 1), 2), and 3) described under 'Statistical analysis' were performed on the intent-to-treat sample. Results from the analyses on assessments from baseline (T0) to follow-up (T4) to assess clinical change until post-treatment (T0 to T2; objective 1) and long-term treatment effect (T2 to T4; objective 2) are summarized in Tables 2 and 3. As shown in Table 2, a two-level regression analysis with factors time (linear and quadratic trends), drug, and diagnosis revealed main effects of time on level of avoidance, measured by the FQ, with patients showing on average a decrease in avoidance over time. Most importantly, the linear and quadratic time by drug and linear and quadratic time by drug by diagnosis interactions were not significant. This indicates that there were no significant differences between placebo and CBD condition in FQ scores over the course of the study, in both diagnostic groups. Numerically, it seemed that the placebo condition unexpectedly improved more than the CBD condition (Fig. S2), suggesting that lack of power was not the reason for these non-significant results.

On overall severity of anxiety, measured by the BAI, the multilevel analysis yielded main linear and quadratic time effects (Table 2). Time by drug and time by drug by diagnosis interactions were not significant. This indicates that there were no significant between-group differences: both groups improved, but CBD did not lead to stronger or more enduring effects compared to placebo, regardless of diagnosis (objectives 1 and 2). Again, the fact that the placebo condition seemingly improved more than the CBD condition (Fig. S3) suggests that lack of power was not the reason for these non-significant results.

Further, multilevel regression analyses on the assessments taken in every treatment session (S0 to S8) yielded no significant time by drug or time by drug by diagnosis interactions, indicating that CBD did not lead to faster improvement compared to placebo, irrespective of diagnosis (objective 3).

5.3.3 Secondary outcomes and completers analysis

The multilevel analyses to answer objectives 1), 2), and 3) described under 'Statistical analysis' were also performed with secondary outcomes and on the completers sample. Overall, secondary outcomes (see Tables 2 and 3) and results of completers analyses (see Tables S4, S5, and Fig. S4) corroborated the primary outcomes. That is, CBD did not lead to stronger, faster, and/or more enduring clinical improvement on these outcome measures, neither in patients with panic disorder with agoraphobia nor in those with social anxiety disorder.

5.3.4 Exploratory analysis

Additional exploratory analyses to answer objectives 4), 5), and 6) described under 'Statistical analysis' did not yield significant effects of CBD either. This included multilevel discrete-time survival analyses with response defined as 25% reduction in FQ and BAI scores (objective 4). This implies that probability of response was equal for the CBD and placebo condition in each treatment session, $ps \geq 0.089$ (Figures S5-S6). Further, analyses of within session improvement using within session SUD scores (objective 5) showed no significant enhancement of within-session extinction learning (Supplemental results Section 2.1, Table S8, Figures S7-S8) nor enhancement of SUDS within session fear or credibility scores at the end of treatment sessions by CBD augmentation (objective 6, Tables S9-S12).

Table 2 Predictors of primary and secondary outcomes in multilevel regression analyses on assessments from baseline to second follow-up (T0-T4) in the intent-to-treat sample (placebo n = 39; cannabidiol n = 39).

	FQ (primary outcome)	BAI	BDI-II	BSQ	SPAI-18
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Time level					
Time	-1.82*(-2.51; -1.14)	-0.65*(-1.07; -0.22)	-0.44*(-0.73; -0.15)	-0.056*(-0.084; -0.029)	-0.52*(-0.74;-0.30)
Time^2	0.027*(-0.13; 0.042)	0.0098 (-0.00040; 0.020)	0.0069 (0.00010; 0.014)	0.00089* (0.00036;0.0014)	NA
Patient level					
Intercept	55.59*(46.49; 64.69)	22.86*(18.52; 27.19)	22.32*(17.26; 27.39)	2.38*(2.05; 2.71)	51.79*(41.31; 62.26)
Drug (PLB; CBD)	-8.88 (-22.42; 4.66)	5.25 (-0.99; 11.48)	1.53 (-4.61; 7.68)	0.027 (-0.38; 0.43)	-5.48 (-18.91; 7.95)
Diagnosis (PD; SOC)	-11.20 (-23.38; 0.98)	-3.97 (-11.04; 3.09)	1.08 (-6.55; 8.71)	-0.17 (-0.66; 0.32)	13.95*(1.81; 26.09)
Drug x diagnosis	9.57 (-9.12; 28.26)	-5.73 (-15.13; 3.67)	-0.53 (-10.54; 9.48)	-0.26 (-0.86; 0.34)	5.52 (-11.20; 22.25)
Antidep (no; yes)	NA	NA	-4.77*(-8.74; -0.80)	NA	NA
Time interaction variables					
Time x drug	0.32 (-0.60; 1.25)	-0.32 (-0.87; 0.23)	-0.11 (-0.48; 0.26)	0.013 (-0.022; 0.048)	0.22 (-0.047; 0.50)
Time x diagnosis	0.26 (-0.66; 1.18)	0.053 (-0.51; 0.61)	0.0042 (-0.50; 0.49)	0.011 (-0.032; 0.055)	0.088 (-0.21; 0.38)
Time x drug x diagnosis	-0.11 (-1.62; 1.40)	0.56 (-0.20; 1.33)	0.065 (-0.54; 0.67)	0.0074 (-0.045; 0.060)	0.017 (-0.35; 0.39)
Time^2 x drug	-0.00067 (-0.021; 0.020)	0.0095 (-0.0044; 0.023)	0.0037 (-0.0055; 0.013)	-0.00023 (-0.00096; 0.00050)	NA
Time^2 x diagnosis	0.00092 (-0.019; 0.021)	0.0016 (-0.012; 0.015)	-0.0018 (-0.013; 0.0092)	-0.000074 (-0.00098; 0.00083)	NA
Time^2 x drug x diagnosis	0.0026 (-0.033; 0.038)	-0.013 (-0.033; 0.0072)	0.0038 (-0.011; 0.018)	-0.000054 (-0.0013; 0.0011)	NA

Note: FQ=Fear Questionnaire; BAI=Beck Anxiety Inventory; BDI-II=Beck Depression Inventory-II; BSQ=Bodily Sensations Questionnaire; SPAI-18=Social Phobia and Anxiety Inventory-18; PLB=placebo; CBD=cannabidiol; PD=panic disorder with agoraphobia; SOC=social anxiety disorder; Antidep=antidepressant; CI=confidence interval; NA=not applicable: Covariates which did not significantly predict outcome were omitted from the final models. Confidence intervals based on robust standard errors. * $p < 0.05$.

5.3.5 Compliance check

Overall, significant, albeit variable CBD plasma concentrations were measured for participants in the CBD condition preceding the first (S1) (mean = 19.23 ng/ml; SD = 24.28; n = 38) and the last (S8) treatment session (mean = 21.44 ng/ml; SD = 32.19; n = 30). Thus, CBD ingestion resulted in marked increases in plasma concentrations of CBD on at least one measurement occasion. For all participants in the placebo condition CBD concentrations were below the detection threshold of the assay (<0.10 ng/ml) preceding S1 (n = 36) and S8 (n = 28). For four participants in the CBD condition, CBD concentrations were below the detection threshold at S1, when drug intake was monitored by a research assistant (n = 3), and at S8 (n=1). Taken together, these results suggest that overall, trial participants adhered to the assigned drug treatments.

5.3.6 Adverse events

No serious adverse events occurred. Adverse events were judged by blinded therapists and researchers as 'not related' or 'possibly related' to the study medication. After unblinding they appeared evenly distributed between the CBD (n = 4) and the placebo condition (n = 6), p = 0.40 (see Table 4). Suicidal thoughts occurred in one patient in the placebo condition, leading to treatment discontinuation. The patient stabilized after a period of crisis intervention.

5.3.7 Sensitivity analysis

Outcomes of sensitivity analyses, in which we excluded patients in treatment trajectories with low protocol adherence (including one patient who displayed an undetectable CBD level despite allocation to the CBD condition) and excluding patients who needed intensive treatment programs after the per-protocol sessions, corroborated the outcomes of primary analyses.

Detailed results are provided in Supplemental results, Tables S2-S17, Figs. S2-S8.

Table 3 Predictors of primary and secondary disorder-specific outcomes in multilevel regression analyses on assessments from baseline to second follow-up (T0-T4) in the intent-to-treat sample.

	LSAS	MI "alone"	MI "accompanied"	ACQ	PDSS
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Time level					
Time	-2.48*(-3.86; -1.10)	-0.035*(-0.071; 0.0019)	-0.020*(-0.029; -0.011)	-0.039*(-0.068; -0.010)	-0.41*(-0.61; -0.21)
Time^2	0.052*(0.022; 0.083)	0.00047 (-0.00031; 0.0013)	NA	0.00057 (-0.000024; 0.0012)	0.0053*(0.0015; 0.0090)
Session	NA	-0.016*(-0.018; -0.014)	NA	NA	-0.037*(-0.059; -0.014)
Patient level					
Intercept	71.99*(56.40; 87.58)	3.20* (2.82; 3.58)	2.34*(1.97; 2.72)	2.12*(1.83; 2.40)	15.42*(13.31; 17.52)
Drug (PLB; CBD)	0.40 (-20.69; 21.49)	-0.32 (-0.84; 0.20)	-0.11 (-0.59; 0.37)	0.062 (-0.32; 0.44)	0.65 (-2.28; 3.57)
Time interaction variables					
Time x drug	1.40 (-0.76; 3.56)	-0.0053 (-0.051; 0.040)	0.0072 (-0.0038; 0.018)	0.0059 (-0.028; 0.039)	-0.082 (-0.37; 0.20)

Note: LSAS=Liebowitz Social Anxiety Scale; MI=Mobility Inventory; ACQ=Agoraphobic Cognitions Questionnaire; PDSS=Panic Disorder Severity Scale; PLB=placebo; CBD=cannabidiol; CI=confidence interval; NA=not applicable: Covariates which did not significantly predict outcome were omitted from the final models. LSAS was filled in by 37 patients with social anxiety disorder (placebo n = 19; cannabidiol n = 18), MI, ACQ and PDSS by 41 patients with panic disorder with agoraphobia (placebo n = 20; cannabidiol n = 21). Confidence intervals based on robust standard errors. Time2 x drug interactions were not significant and are not displayed in the table. * $p < 0.05$.

Table 4 Adverse events.

	Placebo (n = 39)	Cannabidiol (n = 39)
Isolated	sweating, hot flushes, nausea, blurred vision, and a bad taste in the mouth (n = 1)	dizziness (n = 1)
	the flu and gout attacks (n = 1)	drowsiness (n = 1)
	suicidal thoughts (n = 1)	
Recurrent	tiredness (n = 1)	tiredness (n = 1)
	drowsiness (n = 1)	feeling of a strong blood flow (n = 1)
	headaches (n = 1)	

5.4 Discussion

In this clinical trial we examined whether the AEA hydrolysis inhibitor CBD would improve exposure-based in vivo treatment outcome in social anxiety and panic disorder patients with agoraphobia. Results indicated that 300 mg CBD prior to 8 exposure in vivo treatment sessions did not lead to stronger, more enduring, or faster symptom improvement compared to placebo, nor did cannabidiol augmentation increase the probability of early treatment response. In addition, CBD did not improve within-session fear extinction, nor did it affect extinction learning consolidation. These negative findings applied to patients with panic disorder with agoraphobia and those with social anxiety disorder. Although we found no benefits of CBD as an adjunctive therapy, no side effects were detected either.

With respect to representativeness of our sample, on average, patients in the current study presented with severe symptoms, characteristic for a population in need of an adjunctive therapy. Recruitment at multiple treatment centers and with two different diagnoses increased generalizability of findings. The gender distribution in our study sample, with somewhat more men (60%) than women, was somewhat deviant from anxiety disordered populations in general, as anxiety disorders occur more frequently in women. However, the gender imbalance is smaller for social anxiety compared to panic disorder with agoraphobia (Wittchen et al., 2011). Moreover, our results were not affected by participants' gender.

The present findings contrast with earlier research that suggested acute fear extinction enhancement by CBD in rodents (Bitencourt et al., 2008; Do Monte et al., 2013; Lemos et al., 2010; Resstel et al., 2006; Song et al., 2016) and extinction learning consolidation in humans (Das et al., 2013), with lasting effects (Bitencourt et al., 2008; Do Monte et al., 2013). Absence of CBD effects on fear extinction, however, has also been reported in experimental rodent studies. Intraperitoneal administration of CBD (5, 10 or 20 mg/kg) prior to extinction training had no effect on time freezing upon re-exposure to the conditioned cue (Jurkus et al., 2016). When tested in stress-susceptible rats exposed to predator odor, CBD (5 mg/kg) administered prior to extinction training had no effect on contextual conditioned fear (Shallcross et al., 2019).

The strengths of this study include the use of both patient and clinician rated outcome measures, and both disorder specific and generic outcome measures. We included a six month follow-up and accounted for (changes in) antidepressant treatment and /or number of treatment sessions. Within the active treatment phase, per session measures allowed to investigate drug induced acceleration of treatment response. Notwithstanding the richness of our dataset, multiple analyses on these data increased the probability of incorrectly rejecting the null hypothesis. However, none of our analyses indicated superiority of CBD to placebo.

Some limitations deserve discussion. First, although we opted for a 300 mg dose, which exerted anxiolytic effects without being sedative in healthy human subjects (Zuardi et al., 2017, 1993), patients may have received a suboptimal dose. In rats, an effect during extinction training was observed with 20 mg/kg intraperitoneally administered CBD, but not with any lower dosages (Jurkus et al., 2016). Further, dose-dependent effects have been reported with respect to within-session extinction (Do Monte et al., 2013; Lemos et al., 2010) and extinction retention (Do Monte et al., 2013) following intracerebral CBD administration in rats (Do Monte et al., 2013; Lemos et al., 2010). Beneficial effects were found only with a dose of 0.4 μg/side of the infralimbic cortex, but not with a lower dose (Do Monte et al., 2013). Further, there are some indications for an inverted U-shaped dose-response curve of CBD in anxiety. In an intermediate dose of 30 nmol, CBD enhanced extinction in rats, whereas lower and higher doses sorted no effects (Lemos et al., 2010). In healthy humans subjected to a public speaking task, only an intermediate dose of 300 mg oral CBD elicited anxiolytic effects, but lower and higher doses did not (Linares et al., 2019; Zuardi et al., 2017).

These findings from the only two studies that compared multiple doses in humans so far corroborate our choice for 300 mg. Nevertheless, the lack of clarity regarding the therapeutic window of oral CBD is an impediment to the advancement of the field. We are currently integrating data from (pre)clinical research to estimate the effective dose ranges in humans (van Gerven and Cohen, 2018). This knowledge will be imperative for future studies of the effects of CBD in anxiety.

A second limitation is that we focused on the partly theoretical potential of CBD to enhance extinction learning. By exclusively dosing preceding exposure treatment sessions, and not also during homework assignments, we may have missed the potential of a general anxiolytic effect of CBD in anxiety disordered patients. Recently, general anxiolytic effects of CBD in a continuous dosing scheme (300 mg CBD ingested daily for four weeks) were reported by patients with social anxiety disorder (Masataka, 2019) and frontline health care professionals working with patients with COVID-19 (Crippa et al., 2021). This is supported by preclinical research in rats, in which differences between CBD and vehicle groups occurred only preceding, and in the first block of extinction training, but not in subsequent blocks (Jurkus et al., 2016).

In the present study, significant CBD plasma levels were observed in the CBD treated versus the placebo treated group, which are comparable to those previously reported (Fusar-Poli et al., 2009). However, our timing of administration, which was based on time to reach peak plasma levels in a published study (Englund et al., 2013), may have been suboptimal. The time course of plasma levels heavily depends on (the absence/presence of) the dissolving vehicle (Izgelov et al., 2020). Additionally, in line with previous pharmacokinetic results (Fusar-Poli et al., 2009;

Izgelov et al., 2020) inter-subject variability of the CBD plasma levels was high. Future work might consider using a dissolving vehicle rather than administering CBD in powder form, in order to minimize this variability (Izgelov et al., 2020).

Although our study was adequately powered for our main analyses, sample sizes were still relatively small for analyses on secondary disorder specific outcome measures and survival analyses, with the risk of type II error. Furthermore, results with respect to a lack of within-session extinction learning enhancement should be replicated in future research.

In conclusion, augmentation of therapist-assisted exposure in vivo treatment with CBD administered preceding exposure therapy sessions did not have added value in a relatively large group of anxiety disordered patients. Overall, studies directly examining efficacy of CBD in patients are scarce. Whether the combination of continuous administration of CBD with exposure therapy may elicit better effects, is as of yet unknown. Future work may expand on the effects of CBD using continuous administration, and examine its added effect when combined with exposure therapy. Further, different dosing regimens should be explored.

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Declaration of competing interest

All authors report no biomedical financial interests or potential conflicts of interest.

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5.S Supplemental information

Contents

5.S.1 Supplemental experimental procedures

5.S.1.1 Protocols for exposure therapy

The study protocols were based on standardized protocols of exposure therapy in social anxiety disorder (Hofmann and DiBartolo, 2010) and in panic disorder with agoraphobia (Kampman et al., 2012). After every therapy session homework was given, including at least eight home exposure exercises per week tailored to patients' idiosyncratic symptoms.

5.S.1.2 Choice of study dose

Timing and dosage of administration were based on a study by Englund et al. (2013), which indicated high plasma levels from 2 h after administration onwards and mean Tmax at 3 h 45 min after administration. Results provided by Zuardi et al. (2017) showed anxiolytic effects of CBD in a dose of 300 mg per administration, without noticeable side effects. Up to 600 mg, CBD was demonstrated to be safe.

5.S.1.3 Methods for extraction of CBD and analysis of CBD levels

Extraction of CBD from human K2-EDTA samples was performed by Ardena Bioanalytical Laboratory (Assen, the Netherlands) using Liquid Liquid Extraction (LLE), followed by derivatization, subsequent off-line Solid Phase Extraction (SPE) and analysis using liquid chromatography-tandem mass spectrometry (API 6500+ LC-MS/MS). The analyses will be performed in accordance with the method described in Analytical Working Instruction (AWI) 4365 (de Boer, 2020). The method has been validated in Ardena validation study 15148

with regard to response function, selectivity (including blank matrix evaluation, matrix effect, haemolyzed plasma effect, lipemic plasma effect, mutual interference), carryover, precision, accuracy, recovery, stability (including bench-top stability, re-injection stability, processed sample stability, freeze/thaw stability, stock/spike solution stability, whole blood stability, long-term frozen sample storage stability), dilution (10 and 100 times), and batch size determination (Ardena Bioanalytical Laboratory, 2021). The isotopically labelled derivative cannabidiol-d3 (CBD-d3) was used as internal standard for CBD (de Leenheer et al., 1985). Concentrations that fell above the detection threshold of the assay (> 20.0 ng/ml) were extrapolated. CBD concentrations that fell below the detection threshold of the assay (< 0.10 ng/ml) were set at 0.10 ng/ml for calculation of descriptive statistics.

5.S.1.4 Outcomes

The primary outcome measure was the Fear Questionnaire (FQ) (Marks and Mathews, 1979) which was administered at every time point (T0-T4) and at every treatment session. The FQ is part of a standard self-report questionnaire measuring avoidance of potentially fear-inducing situations, the complete form also includes one specific main target phobia, a global phobia rating, and five associated anxiety and depression symptoms (not included in this study). The version of the FQ employed here consists of 15 items asking about the most common phobias rating avoidance using a nine-point scale from '0: would not avoid it' to '8: always avoid it'. The score reflects the level of avoidance, with a total score range from 0 to 120.

The Beck Anxiety Inventory (BAI) (Beck et al., 1988) is a thoroughly validated 21-item self-report instrument that assesses the overall severity of anxiety at various domains including physiological changes, anxiety symptoms and avoidance. Respondents rate how much each symptom bothered them the past week on a 4-point scale, ranging from 0 (not at all) to 3 (severely, I could barely stand it). The BAI is scored by summing the ratings for all the 21 symptoms to obtain a total score ranging from 0 to 63. Being the most important secondary outcome, the BAI was administered at every time point (T0-T4) and at every treatment session, together with the FQ.

Characteristics of the FQ and BAI are listed together with other clinical outcome measures in Supplemental Table 1. All questionnaires have been shown to have adequate reliability and validity (Baker et al., 2002; Bouman, 1995, 1998; Chambless et al., 1985; de Vente et al., 2014; Shear et al., 2001; Steer et al., 1993; Van der Does, 2002; Van Zuuren, 1988) except for the CGI (Forkmann et al., 2011), which is advised to be used in accordance with other validated questionnaires, which are used in this study.

Supplemental Table 1 Clinical outcome measures.

Instrument	Measurement aim	No. of items	Range scale	Assessments
Fear Questionnaire (FQ)	Level of avoidance relating to the most common phobias	15	0 to 8	T0-T4, S0-S8
Beck Anxiety Inventory (BAI)	Overall severity of anxiety	21	0 to 3	T0-T4, S0-S8
Beck Depression Inventory-II (BDI-II)	Presence and/or intensity of depressive symptoms	21	0 to 3	T0-T4
Body Sensations Questionnaire (BSQ)	Fear for bodily sensations associated with autonomic arousal	17	1 to 5	T0-T4
The Social Phobia and Anxiety Inventory-18 (SPAI-18)	Somatic symptoms, cognitions and behavior in/concerning potentially fear-producing social situations	18	1 to 7	T0-T4
Clinical Global Impression severity scale (CGI)	Therapist-rated illness severity	1	1 to 7	S0-S8
Subjective Units of Distress (SUDS)	Within-session extinction (pre- and post-exposure fear and credibility of negative expectations)	4	0 to 100	S0-S8
Panic Disorder Severity Scale (PDSS)	Panic disorder severity	7	0 to 4	T0-T4, in PD + AGO
Mobility Inventory (MI)	Agoraphobic avoidance behavior in specific situations (alone/accompanied)	27	1 to 5	T0-T4, in PD + AGO
Agoraphobic Cognitions Questionnaire (ACQ)	Thoughts concerning negative consequences of experiencing anxiety	14	1 to 5	T0-T4, in PD + AGO
Liebowitz Social Anxiety Scale (LSAS)	Fear and avoidance across a number of social situations	24	0 to 3	T0-T4, in SOC

Note: FQ (Marks and Mathews, 1979), BAI (Beck et al., 1988), BDI-II (Van der Does, 2002), BSQ (Chambless et al.,1984), SPAI-18 (De Vente et al., 2014), CGI (Busnerand Targum, 2007); SUDS (Wolpe, 1969), PDSS (Shear et al., 2001), MI(Chambless et al., 1985), ACQ (Chambless et al.,1984), LSAS (Mennin et al., 2002). PD + AGO: Panic disorder and agoraphobia; SOC: Social anxiety disorder. Diagnosis-specific questionnaires were only administered to patients with the diagnosis in question. The PDSS was administered as an interview by trained research assistants; the other instruments were self-report questionnaires. Higher scores reflect higher symptom severity.

5.S.1.5 Statistical analysis

Due to insights gained while refining our data analysis plan we analyzed our data using multilevel models, which outperform traditional methods by avoiding spurious positive results (Hox et al., 2018). Keeping in mind the advantage that multilevel longitudinal analyses have over repeated measures analyses in terms of power (Fan, 2003) and that measurements were taken at more time points than the two the sample size calculation was based on, with the recruited sample a power of at least 0.8 is obtained.

Baseline characteristics were compared between treatment groups by means of independent samples t-tests for continuous variables and chi-square or Fisher's exact tests for categorical variables, using SPSS version 25 (IBM Corp., 2017).

With our main analyses we investigated whether CBD augmentation would be associated with 1) better clinical outcome post-treatment (T2) and a more favorable time course of the treatment effect. That is, a clinical effect that was 2) more enduring (T2 to T4) or 3) quicker (S0 to S8) compared to the placebo condition. Treatment effect was measured with the instruments listed in Table 1. Explanatory variables were drug (CBD vs placebo), diagnosis (panic disorder with agoraphobia vs social anxiety disorder), the drug by diagnosis interaction term, polynomial(s) for time and interactions of the other explanatory variables with time.

The explanatory variables describe differences between patients, who make up the second level in the multilevel models we used because we expected dependence of observations within patients treated by the same therapists. Either three levels (for time, patients and therapists) or two levels (for time and patients) were modeled, dependent on whether variance of the outcome at the therapist level was significantly larger than zero. At the first level, the trends in outcomes over time were modeled by first degree (linear) polynomials. Higher polynomials were added if the relationship between the outcome and time was non-linear, which we assessed by curve-fitting.

With the analyses including the baseline (T0), mid- (T1), post- (T2), and follow-up assessments (T3 and T4) we investigated whether CBD would lead to 1) stronger and/or 2) more enduring treatment effects. The elapsed time between assessments (T0-T4) varied between patients. We therefore used elapsed time in weeks until the assessment took place. As covariate we included number of received treatment sessions (8 per-protocol treatment sessions and any additional sessions during the follow-up period, after the end of the protocol). Interactions of the explanatory variables with elapsed time in weeks and/or number of treatment sessions were examined, depending on which factor(s) significantly predicted outcome. Other covariates were use of antidepressant medication and an indicator variable for stability or change in medication

dosing regimen during the follow-up period.

With the analyses including the per session assessments (S0-S8) we investigated whether 3) CBD would lead to quicker treatment effects. Again, use of antidepressant medication was included as a covariate. In case the covariates did not significantly influenced outcome they were discarded from the final analyses.

Intent-to-treat, completers, and sensitivity analyses (excluding low protocol adherence trajectories and patients who continued treatment after the per-protocol sessions in intensive programs) were conducted. Only interactions of drug with time were interpreted (main effects of drug were not interpreted), since we hypothesized CBD enhancement of exposure therapy effects, which would occur over the course of treatment. Moreover, as CBD was administered from session 1 onwards and not yet in session 0, any acute drug effects would be evident by an interaction with time.

Concerning our hypothesis of a quicker treatment effect, and because it is not yet known how CBD effects on experimental extinction in rodents may translate to the clinical level, we explored, next to our main analysis, in ancillary analyses (4) early treatment response and (5) within-session extinction.

For (4) we used multilevel discrete-time survival analysis to calculate the probability that treatment response, defined as 25% or greater symptom reduction on FQ and BAI, had occurred in each treatment session. We expected that treatment response would occur earlier in the CBD condition compared to the placebo condition. Time was modeled with indicator variables for each treatment session (S1-S8). Explanatory variables were drug, diagnosis, drug by diagnosis and interactions between these variables and time indicators for S0 to S7 (we would run into estimation problems by including interactions with all the time indicators, so we excluded S8). If significant, covariates for baseline symptom severity and use of antidepressants were included in the models, which was especially important as this type of models does not include a random error term.

For (5) we calculated within-session extinction by subtracting SUD fear and credibility (of the feared outcome) scores from respective SUD begin scores for treatment session 2 to session 8 (in which exposure exercises took place). With multilevel models we tested whether (the course over treatment of) within-session extinction differed between drug conditions. To rule out the possibility that an acute anxiolytic effect would attenuate a decrease in SUD fear scores in the CBD condition, we tested whether SUD begin scores were lower in the CBD compared to the placebo condition.

Assuming that CBD may enhance consolidation of (mal)adaptive learning during exposure therapy we explored in ancillary analysis (6) moderation of CBD effects by within-session

extinction learning: More specifically, whether adaptive learning, operationalized as low end session SUD scores as a result of exposure therapy, moderated an effect of CBD on FQ, BAI, CGI, LSAS, or MI "alone" score at the next session.

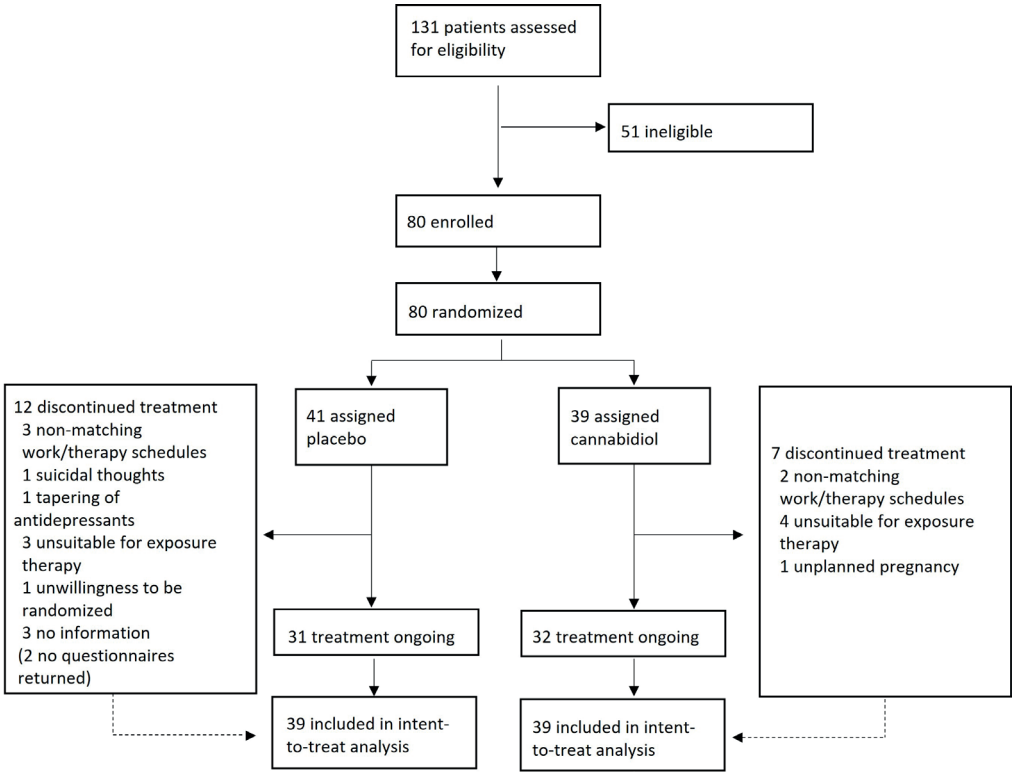
Multilevel analyses were conducted in HLM 6.0 (Raudenbush and Bryk, 2002) and multilevel survival analyses in R version 3.6.32 (R Core Team, 2019) package lme4 (Bolker, 2020). For ancillary analyses and models without multilevel structure, SPSS version 25 was used (IBM Corp, 2017).

In the main analyses on baseline to follow-up assessments (T0-T4) full information maximum likelihood (FIML) estimates for model parameters were calculated using all available data of 78 patients. Two patients failed to fill in any of the questionnaires, so they were not included in these analyses. Missing values in the per session assessments (30% of FQ, 34% of BAI, and 20% of CGI items, and 50% of SUDs) were handled by multiple imputation, thereby creating 20 imputed datasets of which pooled results were used in the analyses (Van Buuren, 2018). A proportion of missing values of at most 40% was given as a rule of thumb for the use of multiple imputation in randomized clinical trials (Jakobsen et al., 2017). The results of the ancillary analyses (5) and (6) with SUD scores should therefore be interpreted as hypothesis generating. Included in the multiple imputation models were number of treatment sessions, explanatory variables from the multilevel analyses and outcome measures highly correlated with the imputed variables.

Two research assistants assessed 10% randomly selected audiotapes of the treatment sessions on therapists' protocol adherence. Each treatment trajectory was categorized by the degree to which exposure exercises actually took place: low adherence (exposure in 0-3 sessions), medium adherence (exposure in 4-6 sessions), high adherence (exposure in 7-8 sessions). By this definition adherence was low in 19 trajectories, medium in 31 trajectories, and high in 30 trajectories.

In general, patients adhered to the study medication regimen. Intake of the CBD or placebo pill was later than prescribed for 58 sessions, with a delay up until one hour preceding the session on most occasions. Given the variability in kinetics of CBD (Atsmon et al., 2018) these sessions were included in per-protocol analyses, whereas self-reported failures to ingest the study medication preceding a session ($n = 5$) and/or low adherence in terms of frequency of in session exposure exercises were not. We additionally excluded three participants who had CBD plasma concentrations of < 0.10 ng/ml, either preceding treatment sessions S1 ($n = 2$) or preceding S8 ($n = 1$).

5.S.2 Supplemental results

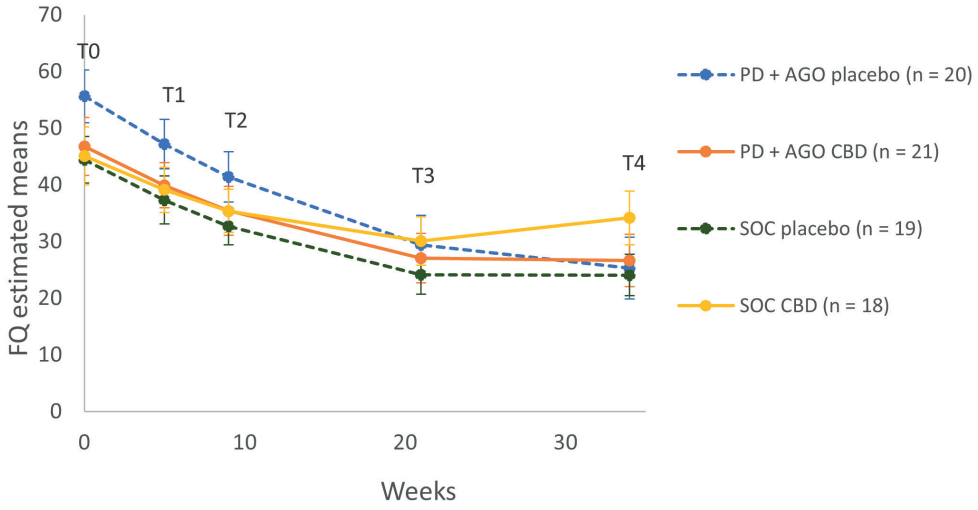


Supplemental Figure 1 CONSORT diagram.

Supplemental Table 2 Estimated mean scores and within-group effect sizes at different times for the Fear Questionnaire (FQ) outcome measure, based on the intent-to-treat analysis.

Placebo		T0	T1	T2	T3	T4
PD + AGO	Estimate	55.59	47.15	41.4	29.39	25.3
(n = 20)	(95% CI)	(46.49; 64.69)	(38.66; 55.66)	(32.69; 50.11)	(19.30; 39.49)	(14.59; 36.00)
	p for within-group effect	Reference	0.000008	0.000006	0.000001	p <0.0001
	Within-group ES	Reference	0.56	0.9	1.48	1.86
SOC	Estimate	44.39	37.29	32.63	24.09	24.04
(n = 19)	(95% CI)	(36.29; 52.49)	(29.02; 45.56)	(26.34; 38.92)	(17.38; 30.79)	(16.90; 31.19)
	p for within-group effect	Reference	0.000018	0.000016	0.00001	0.000008
	Within-group ES	reference	0.48	0.74	1.15	1.25
CBD						
PD + AGO	Estimate	46.71	39.88	35.38	27.01	26.64
(n = 21)	(95% CI)	(36.69; 56.74)	(32.10; 47.66)	(26.90; 43.85)	(18.45; 35.56)	(17.59; 35.68)
	p for within-group effect	Reference	0.000031	0.000024	0.00001	0.000005
	Within-group ES	Reference	0.46	0.72	1.12	1.23
SOC	Estimate	45.09	39.09	35.38	30.06	34.15
(n = 18)	(95% CI)	(35.07; 55.11)	(31.29; 46.89)	(27.88; 42.87)	(21.61; 38.51)	(24.83; 43.46)
	p for within-group effect	Reference	0.008	0.0072	0.0041	0.0025
	Within-group ES	Reference	0.4	0.61	0.85	0.67

Note: Explanatory variables in the analysis are drug (cannabidiol (CBD) or placebo), diagnosis (panic disorder with agoraphobia (PD +AGO) or social anxiety disorder (SOC)), Drug by diagnosis, and the interactions of these variables with linear and quadratic time. Higher scores reflect higher symptom severity.



Supplemental Figure 2 Estimated means and error bars indicating standard errors for the Fear Questionnaire (FQ) outcome measure, based on the intent-to-treat analysis.

Note: Explanatory variables drug (cannabidiol (CBD) or placebo), diagnosis (panic disorder with agoraphobia (PD +AGO) or social phobia (SOC)), drug by diagnosis, and the interactions of these variables with linear and quadratic time. Higher scores reflect higher symptom severity. On the x-axis is time in weeks. Data is displayed for each assessment point: T0 (0 weeks), T1 (5 weeks), T2 (9 weeks), T3 (21 weeks), and T4 (34 weeks). These planned assessment times were not identical to actual assessment times (the latter were modelled in the analyses). Scores did not significantly change from T3 to T4, all $p \geq 0.18$.

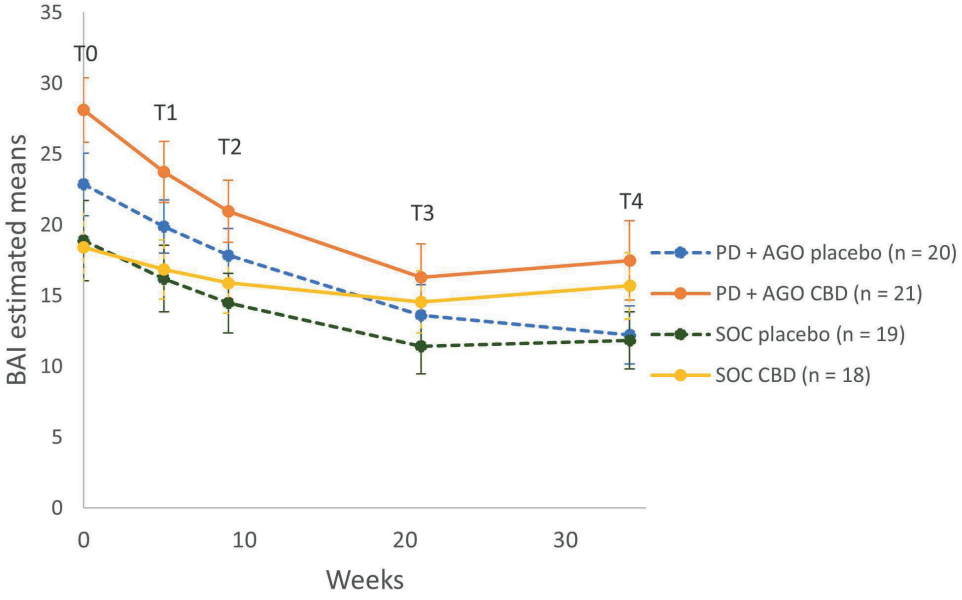
Supplemental Table 3 Estimated mean scores and within-group effect sizes at different times for the Beck Anxiety Inventory (BAI) outcome measure, based on the intent-to-treat analysis.

Placebo		T0	T1	T2	T3	T4
PD + AGO	Estimate	22.86	19.87	17.84	13.61	12.21
(n = 20)	(95% CI)	(18.51; 27.19)	(16.16; 23.58)	(14.10; 21.58)	(9.35; 17.87)	(8.20; 16.14)
	p for within-group effect	reference	0.0024	0.0016	0.00031	0.000014
	Within-group ES	reference	0.4	0.63	1.02	1.23
SOC	Estimate	18.88	16.2	14.46	11.43	11.82
(n = 19)	(95% CI)	(13.31; 24.46)	(11.62; 20.78)	(10.31; 18.61)	(7.59; 15.26)	(7.88; 15.77)
	p for within-group effect	reference	0.0018	0.0016	0.00096	0.00069
	Within-group ES	reference	0.36	0.55	0.82	0.82

Supplemental Table 3 Estimated mean scores and within-group effect sizes at different times for the Beck Anxiety Inventory (BAI) outcome measure, based on the intent-to-treat analysis.

CBD						
PD + AGO (n = 21)	Estimate (95% CI)	28.1 (23.63; 32.57)	23.75 (19.52; 27.97)	20.95 (16.65; 25.26)	16.28 (11.61; 20.96)	17.48 (12.00; 22.97)
	p for within-group effect	Reference	0.000005	0.000004	0.000003	0.00013
	Within-group ES	reference	0.58	0.89	1.31	1.23
SOC (n = 18)	Estimate (95% CI)	18.4 (13.66; 54.47)	16.85 (12.77; 20.93)	15.89 (11.67; 20.10)	14.54 (10.25; 18.84)	15.68 (11.10; 20.27)
	p for within-group effect	reference	0.056	0.045	0.0094	0.0042
	Within-group ES	reference	0.2	0.31	0.43	0.32

Note: Explanatory variables in the analysis are drug (cannabidiol (CBD) or placebo), diagnosis (panic disorder with agoraphobia (PD +AGO) or social anxiety disorder (SOC)), drug by diagnosis, and the interactions of these variables with linear and quadratic time. Higher scores reflect higher symptom severity.



Supplemental Figure 3 Estimated means and error bars indicating standard errors for the Beck Anxiety Inventory (BAI) outcome measure, based on the intent-to-treat analysis

Note: Explanatory variables drug (cannabidiol (CBD) or placebo), diagnosis (panic disorder with agoraphobia (PD +AGO) or social anxiety disorder (SOC)), drug by diagnosis, and the interactions of these variables with linear and quadratic time. Higher scores reflect higher symptom severity. On the x-axis is time in weeks. Data is displayed for each assessment point: T0 (0 weeks), T1 (5 weeks), T2 (9 weeks), T3 (21 weeks), and T4 (34 weeks). These planned assessment times were not identical to actual assessment times (the latter were modelled in the analyses). BAI scores did not significantly change from T3 to T4, $ps \geq 0.22$.

Supplemental Table 4 Predictors of primary and secondary outcomes in the multilevel analyses on assessments from baseline to second follow-up (T0-T4) in the completers sample (placebo $n = 29$; cannabidiol $n = 32$).

	FQ		BAI		BDI		BSQ		SPAI Social phobia	
	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI
Time level										
Time	-2.01*	(-2.78; -1.24)	-0.56*	(-1.04; -0.080)	-0.41*	(-0.65; -0.16)	-0.066*	(-0.096; -0.035)	-1.33*	(-1.98; -0.68)
Time^2	0.031*	(0.014; 0.048)	0.0078	(-0.0037; 0.019)	0.0059	(-0.00056; 0.012)	0.0011*	(0.00047; 0.0017)	0.020*	(0.0058; 0.033)
Patient level										
Intercept	53.82*	(44.24; 63.39)	20.62*	(16.24; 25.00)	19.52*	(15.08; 23.96)	2.47*	(2.049; 2.89)	52.94*	(42.99; 62.89)
Antidepressant (no; yes)	NA	NA	NA	NA	-3.97	(-7.88; -0.065)	NA	NA	NA	NA
Drug (placebo; cannabidiol)	-8.2	(-23.38; 6.98)	6.92*	(0.17; 13.66)	3.27	(-2.33; 8.87)	-0.13	(-0.56; 0.31)	5.23	(-19.31; 8.85)
Diagnosis (PD + AGO; SOC)	-11.67	(-24.65; 1.31)	-0.18	(-8.99; 8.63)	5.64	(-3.06; 14.34)	-0.25	(-0.78; 0.27)	14.83*	(1.64; 28.02)
Drug x diagnosis	13.36	(-8.19; 34.92)	-9.29	(-20.63; 2.05)	-4.22	(-14.63; 6.19)	-0.044	(-0.71; 0.62)	9.2	(-10.62; 29.03)
Time interaction variables										
Time x drug	0.6	(-0.44; 1.64)	-0.31	(-0.84; 0.22)	-0.15	(-0.48; 0.19)	0.026	(-0.0099; 0.061)	0.87*	(0.056; 1.68)
Time x diagnosis	0.67	(-0.41; 1.76)	0.033	(-0.66; 0.73)	-0.0076	(-0.41; 0.39)	0.024	(-0.011; 0.058)	0.58	(-0.31; 1.47)
Time x drug x diagnosis	-0.94	(-2.77; 0.89)	0.53	(-0.34; 1.40)	-0.051	(-0.64; 0.53)	-0.017	(-0.069; 0.035)	-1.66*	(-3.30; -0.031)

Supplemental Table 4 Predictors of primary and secondary outcomes in the multilevel analyses on assessments from baseline to second follow-up (T0-T4) in the completers sample (placebo $n = 29$; cannabidiol $n = 32$).

Time^2 x drug	-0.0061	(-0.029; 0.017)	0.01	(-0.0026; 0.023)	0.0051	(-0.0042; 0.014)	-0.00045	(-0.0012; 0.00030)	-0.015	(-0.032; 0.0017)
Time^2 x diagnosis	-0.0057	(-0.030; 0.018)	0.0023	(-0.014; 0.019)	-0.0013	(-0.010; 0.0076)	-0.0003	(-0.00099; 0.00038)	-0.012	(-0.031; 0.0082)
Time^2 x drug x diagnosis	0.02	(-0.024; 0.064)	-0.013	(-0.035; 0.0095)	0.0052	(-0.0093; 0.020)	0.00051	(-0.00084; 0.0019)	0.045*	(0.0047; 0.084)

Note: FQ=Fear Questionnaire; BAI=Beck Anxiety Inventory; BDI-II=Beck Depression Inventory-II; BSQ=Bodily Sensations Questionnaire; SPAI-18=Social Phobia and Anxiety Inventory-18; CI=confidence interval; PD + AGO=panic disorder with agoraphobia; SOC=social anxiety disorder; NA=not applicable (non-significant predictor, not included in the final model). Confidence intervals based on robust standard errors.
* $p < 0.05$.

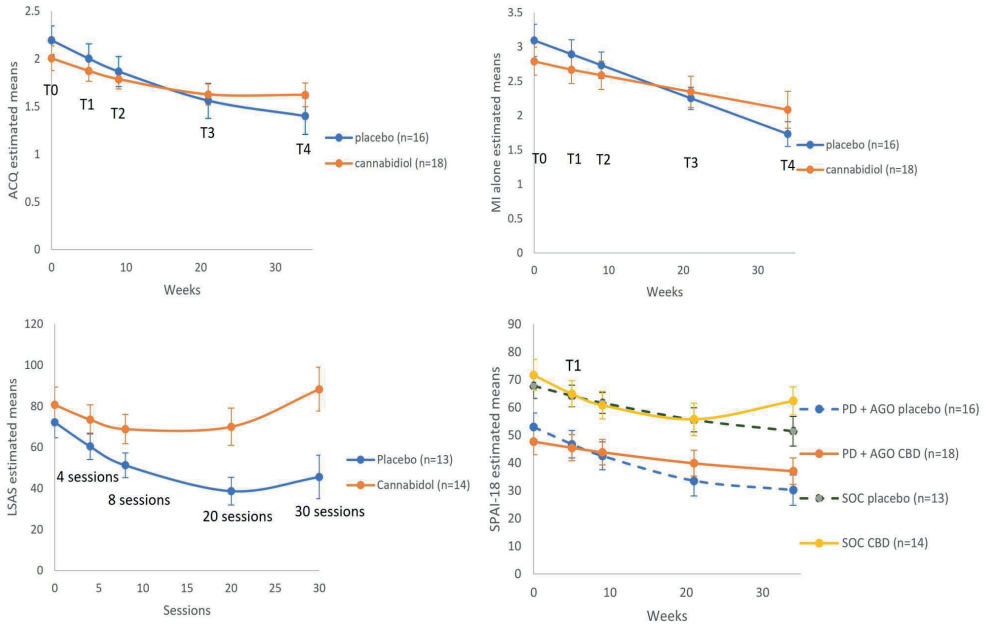
Supplemental Table 5 Predictors of secondary disorder-specific outcomes on assessments from baseline to second follow-up (T0-T4) in multilevel regression analyses in the completers sample.

	LSAS		MI "alone"		MI "accompanied"		ACQ		PDSS	
	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI
Time level										
Time	NA	NA	-0.04*	(-0.054; -0.026)	-0.055*	(-0.089; -0.020)	-0.041*	(-0.065; -0.018)	-0.46*	(-0.63; -0.29)
Time^2	NA	NA	NA	NA	0.00096*	(0.00022; 0.0017)	0.00052*	(0.00014; 0.00091)	0.0057*	(0.0024; 0.0091)
Session	-3.25*	(-4.74; -1.76)	NA	NA	NA	NA	NA	NA	NA	NA
Session^2	0.079*	(0.025; 0.13)	NA	NA	NA	NA	NA	NA	NA	NA

Supplemental Table 5 Predictors of secondary disorder-specific outcomes on assessments from baseline to second follow-up (T0-T4) in multilevel regression analyses in the completers sample.

Patient level										
Intercept	72.26*	(57.28; 87.25)	3.10*	(2.63; 3.56)	2.45*	(1.98; 2.92)	2.19*	(1.87; 2.52)	15.35*	(12.88; 17.82)
Drug (placebo; cannabidiol)	8.48	(-2.63; 19.59)	-0.32	(-0.94; 0.29)	0.012	(-0.58; 0.61)	-0.19	(-0.51; 0.13)	-1.74*	(-3.34; -0.14)
Time interaction variables										
Time x drug	NA	NA	0.020*	(0.0021; 0.038)	0.011	(-0.029; 0.051)	0.012*	(0.0019; 0.022)	0.58	(-1.71; 2.87)
Time^2 x drug	NA	NA	NA	NA	-0.00016	(-0.0010; 0.00068)	NA	NA	-0.016	(-0.10; 0.072)
Session x drug	1.14*	(0.37; 1.91)	NA	NA	NA	NA	NA	NA	NA	NA
Session^2 x drug	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Note: LSAS=Liebowitz Social Anxiety Scale; MI=Mobility Inventory; ACQ=Agoraphobic Cognitions Questionnaire; PDSS=Panic Disorder Severity Scale; CI=confidence interval; PD + AGO=panic disorder with agoraphobia; SOC=social anxiety disorder; NA=not applicable (non-significant predictors/no significant slope variance; predictors not included in the final model). LSAS was filled in by 27 patients with social anxiety disorder (placebo n = 13; cannabidiol n = 14), MI, ACQ and PDSS by 34 patients with panic disorder with agoraphobia (placebo n = 16; cannabidiol n = 18). Confidence intervals based on robust standard errors.
* $p < 0.05$.



Supplemental Figure 4 Estimated means and error bars indicating standard errors for the Liebowitz Social Anxiety Scale (LSAS), Agoraphobic Cognitions Questionnaire (ACQ), Mobility Inventory (MI) "alone", and Social Phobia and Anxiety Inventory-18 (SPAI-18) outcome measures, based on the completers analyses.

Note: Explanatory variable drug (cannabidiol (CBD) or placebo) and the interaction of drug with linear time. For the SPAI-18 outcome, administered to both patients with panic disorder with agoraphobia (PD +AGO) and social anxiety disorder (SOC), diagnosis, drug by diagnosis, and the interactions of these variables with linear and quadratic time were modelled as well. For the LSAS we used no. of treatment sessions instead of time in weeks, as the former but not the latter predicted outcome. For the other outcome measures, data is displayed for each assessment point: T0 (0 weeks), T1 (5 weeks), T2 (9 weeks), T3 (21 weeks), and T4 (34 weeks). These planned assessment times were not identical to actual assessment times (the latter were modelled in the analyses).

Supplemental Table 6 Predictors of FQ, BAI and CGI (treatment session S0-8) in multilevel regression analyses in the intent-to-treat sample (placebo $n = 41$; cannabidiol $n = 39$).

	FQ (primary outcome)		BAI		CGI	
	β	95% CI	β	95% CI	β	95% CI
Time level						
Session	-2.66*	(-3.42; -1.90)	-1.04*	(-1.51; -0.57)	-0.18*	(-0.22; 0.13)
Patient level						
Intercept	55.08*	(47.20; 62.97)	30.17*	(27.01; 33.33)	4.95*	(4.55; 5.34)
Drug (placebo; cannabidiol)	-8.12	(-19.40; 3.16)	-2.37	(-7.37; 2.62)	-0.1	(-0.37; 0.17)
Diagnosis (PD + AGO; SOC)	-13.35*	(-24.92; -1.77)	-5.65*	(-9.88; -1.42)	-0.52*	(-0.87; -0.16)
Drug x diagnosis	10.79	(-5.80; 27.37)	-1.74	(-8.60; 5.12)	0.69*	(0.094; 1.29)
Time interaction variables						
Session x drug	0.31	(-0.78; 1.40)	0.059	(-0.60; 0.72)	0.034	(0.037; 0.11)
Session x diagnosis	0.43	(0.69; 1.54)	-0.028	(-0.62; 0.57)	0.056	(0.047; 0.16)
Session x drug x diagnosis	-0.37	(-1.97; 1.23)	0.47	(-0.39; 1.33)	-0.031	(-0.18; 0.12)

Note: FQ=Fear Questionnaire; BAI=Beck Anxiety Inventory; CGI=Clinical Global Impression; CI=confidence interval; PD + AGO=panic disorder with agoraphobia; SOC=social anxiety disorder. Confidence intervals based on robust standard errors.

* $p < 0.05$.

Supplemental Table 7 Predictors of FQ, BAI and CGI (treatment session S0-8) in multilevel regression analyses in the completers sample (placebo $n = 29$; cannabidiol $n = 32$).

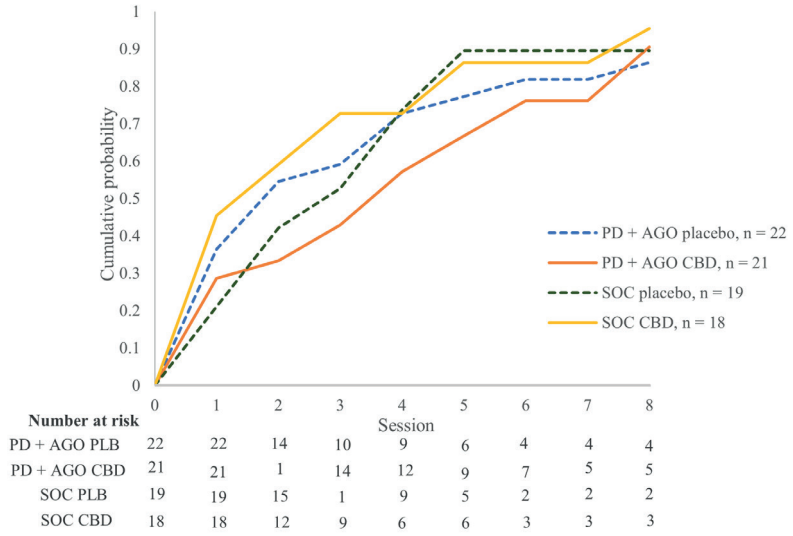
	FQ		BAI		CGI	
	β	95% CI	β	95% CI	β	95% CI
Time level						
Session	-3.14*	(-4.09; -2.18)	-1.10*	(-1.67; -0.54)	-0.18*	(-0.23; -0.13)
Patient level						
Intercept	56.50*	(46.13; 66.87)	29.87*	(25.99; 33.75)	4.83*	(4.36; 5.30)
Drug (placebo; cannabidiol)	-8.96	(-23.82; 5.90)	-1.59	(-7.48; 4.31)	0.13	(-0.23; 0.49)
Diagnosis (PD + AGO; SOC)	-13.96*	(-27.34; -0.58)	-4.74	(-10.10; 0.61)	-0.13	(-0.49; 0.24)

Supplemental Table 7 Predictors of FQ, BAI and CGI (treatment session S0-8) in multilevel regression analyses in the completers sample (placebo $n = 29$; cannabidiol $n = 32$).

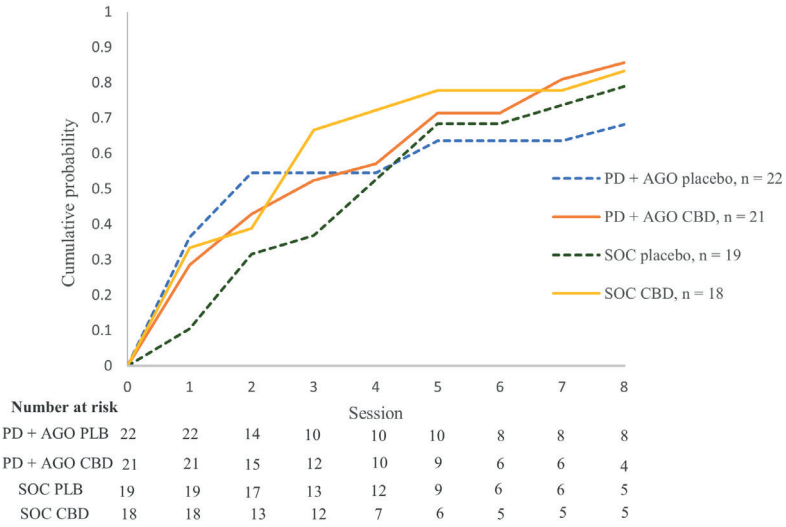
Drug x diagnosis	11.45	(-8.73; 31.62)	-2.15	(-10.37; 6.07)	0.33	(-0.36; 1.03)
Time interaction variables						
Session x drug	0.67	(-0.54; 1.88)	0.041	(-0.73; 0.82)	0.02	(-0.057; 0.098)
Session x diagnosis	0.7	(-0.65; 2.05)	-0.13	(-0.87; 0.61)	0.026	(-0.082; 0.13)
Session x drug x diagnosis	-0.68	(-2.57; 1.21)	0.51	(-0.51; 1.54)	0.00015	(-0.17; 0.17)

Note: FQ=Fear Questionnaire; BAI=Beck Anxiety Inventory; CGI=Clinical Global Impression; CI=confidence interval; PD +AGO=panic disorder with agoraphobia; SOC=social anxiety disorder. Confidence intervals based on robust standard errors.

* $p < 0.05$.



Supplemental Figure 5 Cumulative probability functions describing the observed cumulative probability that treatment response on the FQ has occurred at each treatment session. Drug condition (cannabidiol; placebo) was not associated with probability of treatment response.



Supplemental Figure 6 Cumulative probability functions describing the observed cumulative probability that treatment response on the BAI has occurred at each treatment session. Drug condition (cannabidiol, placebo) was not associated with probability of treatment response.

5.S.2.1 Ancillary analysis 5) on within-session extinction or acute anxiolysis

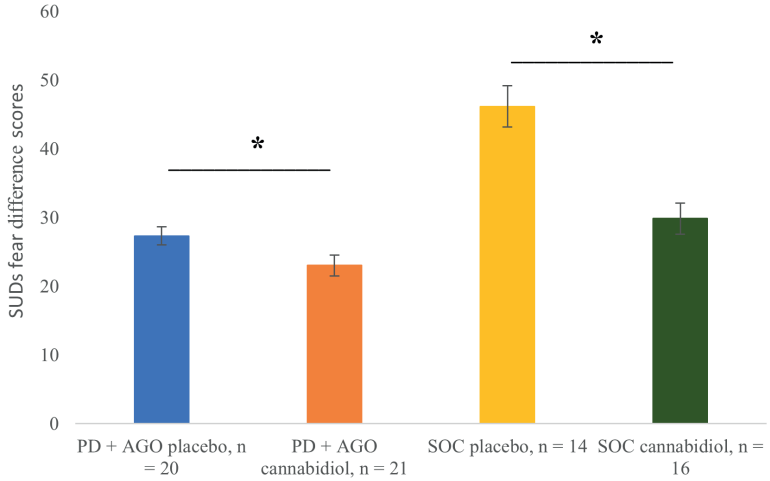
We found a significant main effect of drug on within-session change in SUDs fear levels, β = -4.31 [95% CI -7.63 to -0.99], and, additionally, a significant interaction with diagnosis, β = -12.01 [95% CI -19.81 to -4.22]. Visualization of this interaction revealed superiority of placebo compared to CBD in both diagnostic groups, with overlapping confidence intervals for panic disorder with agoraphobia, placebo β = 27.34 [95% CI 24.82 to 29.86], CBD β = 23.03 [95% CI 20.06 to 26.00]. For patients with social anxiety disorder within-session change in SUDs fear scores appeared larger in the placebo condition (β = 46.18 [95% CI 40.30 to 52.06]) compared to the CBD condition (β = 29.85 [95% CI 25.43 to 34.27]), see Supplemental Figure 7. A significant main effect of drug on within-session change in SUDs credibility of the feared outcome was found, suggesting superiority of CBD over placebo, β = 5.31 [95% CI 2.48 to 8.13]. However, despite the statistical significance of this difference, relevance of this finding seems low as confidence intervals are largely overlapping; placebo β = 24.99 [95% CI 22.34 to 27.64], CBD β = 27.73 [95% CI 24.36 to 31.11]. Drug conditions did not differ with respect to SUDs fear or credibility scores at the beginning of treatment sessions, which suggests that CBD did not have acute anxiolytic effects (details are shown in Supplemental Table 8 and Supplemental Figures 7 and 8).

Supplemental Table 8 Results from ancillary analysis 5) on within-session extinction and acute anxiolysis (placebo $n = 34$; cannabidiol $n = 37$).

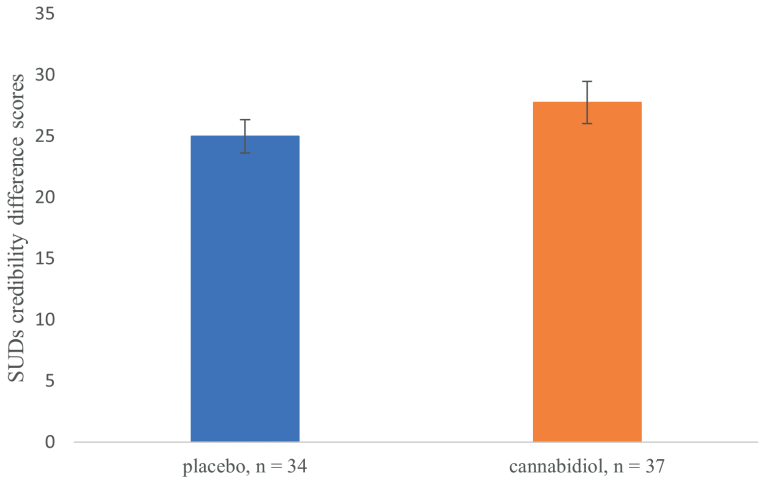
	SUD begin - end fear		SUD begin - end credibility		SUD begin fear		SUD begin credibility	
	β	95% CI	β	95% CI	β	95% CI	β	95% CI
Time level								
Session	-2.40*	(-3.24; -1.56)	NA	NA	4.4	(-4.73; 13.53)	-1.73*	(-2.54; -0.92)
Session^2	NA	NA	NA	NA	-0.72	(-1.59; 0.15)	NA	NA
Patient level								
Intercept	38.97*	(33.77; 44.17)	18.16*	(15.58; 20.74)	63.92*	(42.51; 85.33)	74.73*	(68.99; 80.47)
Drug (placebo; cannabidiol)	-4.31*	(-7.63; -0.99)	5.31*	(2.48; 8.13)	11.7	(-17.50; 40.90)	-2.24	(-10.23; 5.76)
Diagnosis (PD + AGO; SOC)	18.84*	(13.24; 24.43)	16.18*	(12.12; 20.23)	-2.27	(-35.52; 30.97)	6.96*	(2.03; 11.90)
Drug x diagnosis	-12.01*	(-19.81; -4.22)	-6.07	(-12.28; 0.14)	-22.52	(-67.46; 22.3)	-3.48	(-14.48; 7.52)
Time interaction variables								
Session x drug	NA	NA	NA	NA	-6.88	(-19.40; 5.63)	NA	NA
Session x diagnosis	NA	NA	NA	NA	2.05	(-11.91; 16.01)	NA	NA
Session x drug x diagnosis	NA	NA	NA	NA	9.98	(-9.02; 28.98)	NA	NA

Note: SUD=Subjective Unit of Distress; CI=confidence interval; PD + AGO=panic disorder with agoraphobia; SOC=social anxiety disorder; NA=not applicable (non-significant predictors/no significant slope variance; predictors not included in the final model). Confidence intervals based on robust standard errors. There were no significant predictors of SUD begin – end credibility difference scores at the time level. Session2 x drug, session2 x diagnosis, and session2 x drug x diagnosis were not significant and are not displayed in the table.

* $p < 0.05$.



Supplemental Figure 7 Mean SUDs fear difference scores across treatment sessions split by diagnosis and drug condition.



Supplemental Figure 8 Mean SUDs credibility difference scores across treatment sessions split by drug condition.

Supplemental Table 9 Results from ancillary analysis 6) on moderation by within-session extinction learning of an effect of CBD on illness severity in the next session.

	FQ session 3 and 8		BAI session 3 and 8		CGI session 3 and 8	
	β	95% CI	β	95% CI	β	95% CI
Time level						
SUD begin fear previous session	-0.019	(-0.19; 0.15)	-0.055	(-0.16; 0.051)	-0.0049	(-0.014; 0.0043)
SUD end fear previous session	0.1	(-0.073; 0.27)	0.14*	(0.027; 0.25)	0.0038	(-0.0043; 0.012)
Illness severity in previous session	0.77*	(0.64; 0.89)	0.78*	(0.62; 0.94)	0.86*	(0.74; 0.98)
Patient level						
Intercept	-3.16	(-14.06; 7.73)	3.06	(-3.97; 10.09)	0.71*	(0.17; 1.25)
Illness severity at baseline	0.17*	(0.045; 0.29)	0.016	(-0.13; 0.17)	-0.025	(-0.16; 0.11)
Drug (placebo; cannabidiol)	0.52	(-14.65; 15.69)	0.89	(-8.85; 10.64)	-0.81*	(-1.60; -0.018)
Time interaction variables						
SUD begin fear previous session x drug	-0.018	(-0.30; 0.26)	0.0075	(-0.17; 0.18)	0.017*	(0.0016; 0.031)
SUD end fear previous session x drug	-0.031	(-0.26; 0.20)	-0.084	(-0.23; 0.064)	-0.01	(-0.022; 0.0018)

Note: FQ=Fear Questionnaire; BAI=Beck Anxiety Inventory; CGI=Clinical Global Impression; T1=mid-treatment; CI=confidence interval; SUD=Subjective Unit of Distress. Confidence intervals based on robust standard errors. FQ, BAI and CGI data for 66 patients were used (placebo n = 32; cannabidiol n = 34). Following Hofmeijer-Sevink et al. (2017) and Smits et al. (2013), illness severity measured with FQ, BAI, or CGI, was regressed on SUDs scores in the previous treatment session.

Supplemental Table 10 Results from ancillary analysis 6) on moderation by within-session extinction learning of an effect of CBD on illness severity in the next session.

	MI "alone" at T1		LSAS at T1	
	β	95% CI	β	95% CI
Time level				
SUD begin fear previous session	-0.015	(-0.037; 0.0078)	0.44	(-0.24; 1.12)
SUD end fear previous session	0.007	(-0.019; 0.033)	0.49*	(0.26; 0.73)
Patient level				
Intercept	1.07	(-0.41; 2.55)	-35.94	(-80.37; 8.49)
Illness severity at baseline	0.73*	(0.45; 1.01)	0.62*	(0.36; 0.88)
Drug (placebo; cannabidiol)	-1.99*	(-3.72; -0.26)	-16.25	(-71.11; 38.62)
Time interaction variables				
SUD begin fear previous session x drug	0.040*	(0.0066; 0.074)	0.5	(-0.40; 1.40)
SUD end fear previous session x drug	-0.012	(-0.043; 0.019)	-0.29	(-1.07; 0.49)

Note: MI=Mobility Inventory; LSAS=Liebowitz Social Anxiety Scale; T1=mid-treatment; CI=confidence interval; SUD=Subjective Unit of Distress. Confidence intervals based on robust standard errors. LSAS at T0 and T1 was available for 20 patients with social anxiety disorder (placebo n = 9; cannabidiol n = 11), MI "alone" was available for 21 patients with panic disorder with agoraphobia (placebo n = 11; cannabidiol n = 10). As the variance for MI "alone" at the patient level was not significant, a linear regression without multilevel structure was fit. Following Hofmeijer-Sevink et al. (2017) and Smits et al. (2013) illness severity measured with MI "alone" or LSAS, was regressed on SUDs scores in the previous treatment session.

Supplemental Table 11 Results from ancillary analysis 6) on moderation by within-session extinction learning of an effect of CBD on illness severity in the next session.

	FQ session 3 and 8		BAI session 3 and 8		CGI session 3 and 8	
	β	95% CI	β	95% CI	β	95% CI
Time level						
SUD begin credibility previous session	0.024	(-0.13; 0.17)	-0.03	(-0.13; 0.072)	-0.0014	(-0.011; 0.0084)
SUD end credibility previous session	0.098	(-0.067; 0.26)	0.13*	(0.021; 0.24)	0.005	(-0.0056; 0.016)
Illness severity in previous session	0.74*	(0.61; 0.86)	0.77*	(0.61; 0.94)	0.83*	(0.69; 0.96)
Patient level						
Intercept	-7.86	(-18.69; 2.97)	-0.94	(-8.48; 6.60)	0.6	(-0.27; 1.46)
Illness severity at baseline	0.20*	(0.074; 0.32)	0.045	(-0.11; 0.20)	-0.045	(-0.19; 0.10)
Drug (placebo; cannabidiol)	9.15	(-4.06; 22.35)	4.26	(-3.24; 11.76)	-0.14	(-0.99; 0.71)

Supplemental Table 11 Results from ancillary analysis 6) on moderation by within-session extinction learning of an effect of CBD on illness severity in the next session.

Time interaction variables						
SUD begin credibility previous session x drug	-0.16	(-0.37; 0.058)	-0.016	(-0.16; 0.12)	-0.0019	(-0.015; 0.012)
SUD end credibility previous session x drug	0.019	(-0.19; 0.23)	-0.082	(-0.22; 0.056)	0.0068	(-0.0067; 0.020)

Note: FQ=Fear Questionnaire; BAI=Beck Anxiety Inventory; CGI=Clinical Global Impression; T1=mid-treatment; CI=confidence interval; SUD=Subjective Unit of Distress. Confidence intervals based on robust standard errors. FQ, BAI and CGI data for 66 patients were used (placebo n = 32; cannabidiol n = 34). Following Hofmeijer-Sevink et al. (2017) and Smits et al. (2013) illness severity measured with FQ, BAI, or CGI was regressed on SUDs scores in the previous treatment session.

Supplemental Table 12 Results from ancillary analysis 6) on moderation by within-session extinction learning of an effect of CBD on illness severity in the next session.

	MI "alone" at T1		**LSAS at T1**	
	β	**95% CI**	**β**	**95% CI**
Time level				
SUD begin credibility previous session	0.007	(-0.021; 0.035)	0.27	(-0.41; 0.96)
SUD end credibility previous session	-0.011	(-0.041; 0.018)	0.6	(0.0044; 1.19)
Patient level				
Intercept	0.2	(-1.30; 1.71)	-35.67	(-79.63; 8.28)
Illness severity at baseline	0.83*	(0.55; 1.10)	0.64*	(0.32; 0.96)
Drug (placebo; cannabidiol)	-1.36	(-2.72; 0.0039)	-7.55	(-61.70; 46.59)
Time interaction variables				
SUD begin credibility previous session x drug	0.023	(-0.011; 0.057)	0.53	(-0.23; 1.29)
SUD end credibility previous session x drug	0.0003	(-0.036; 0.035)	-0.35	(-1.22; 0.52)

Note: MI=Mobility Inventory; LSAS=Liebowitz Social Anxiety Scale; T1=mid-treatment; CI=confidence interval; SUD=Subjective Unit of Distress. Confidence intervals based on robust standard errors. Following Hofmeijer-Sevink et al. (2017) and Smits et al. (2013) illness severity measured with MI "alone" or LSAS was regressed on SUDs scores in the previous treatment session. LSAS at T0 and T1 was available for 20 patients with social anxiety disorder (placebo n = 9; cannabidiol n = 11), MI "alone" was available for 21 patients with panic disorder with agoraphobia (placebo n = 11; cannabidiol n = 10). As the variance for MI "alone" at the patient level was not significant, a linear regression without multilevel structure was fit.

Supplemental Table 13 Predictors of primary and secondary outcomes in the multilevel analyses on assessments from baseline to second follow-up (T0-T4) in the intent-to-treat sample, excluding those patients who participated in intensive treatment programs during the follow-up period (between T2 to T4). Placebo group $n = 37$; cannabidiol group $n = 37$.

	FQ		BAI		BDI-II		BSQ		SPAI-18	
	β	95% CI	*β*	95% CI	*β*	95% CI	*β*	95% CI	*β*	95% CI
Time level										
Time	NA	NA	-0.75*	(-1.18; -0.31)	-0.48*	(-0.67; -0.28)	-0.042*	(-0.055; -0.029)	NA	NA
Time^2	NA	NA	0.012*	(0.0023; 0.023)	0.0085*	(0.0035; 0.013)	0.00052*	(0.00026; 0.00079)	NA	NA
Session	-2.56*	(-3.20; -1.93)	NA	NA	NA	NA	NA	NA	-1.61*	(-2.18; -1.04)
Session^2	0.069*	(0.047; 0.090)	NA	NA	NA	NA	NA	NA	0.034*	(0.011; 0.057)
Patient level										
Intercept	56.99*	(48.95; 65.03)	24.65*	(20.52; 28.78)	24.24*	(19.48; 28.99)	2.36*	(2.10; 2.61)	59.05*	(49.83; 68.28)
Antidepressant (no; yes)	NA	NA	NA	NA	-4.87*	(-8.38; -1.36)	NA	NA	NA	NA
Drug (placebo; cannabidiol)	-10.08	(-22.37; 2.21)	3.36	(-2.92; 9.64)	-1.12	(-6.16; 3.92)	0.0055	(-0.30; 0.31)	-9.21	(-21.78; 3.36)
Diagnosis (PD + AGO; SOC)	-13.72*	(-23.93; -3.50)	-5.78	(-12.70; 1.15)	-0.46	(-8.44; 7.52)	-0.17	(-0.51; 0.17)	9.09	(-2.22; 20.40)
Drug x diagnosis	15.6	(-0.70; 31.91)	-3.82	(-13.36; 5.72)	1.7	(-6.52; 9.92)	-0.13	(-0.59; 0.34)	10.61	(-5.59; 26.80)
Time interaction variables										
Time x drug	NA	NA	-0.23	(-0.80; 0.35)	0.027	(-0.25; 0.30)	NA	NA	NA	NA
Time x diagnosis	NA	NA	0.16	(-0.41; 0.73)	0.035	(-0.28; 0.35)	NA	NA	NA	NA
Time x drug x diagnosis	NA	NA	0.5	(-0.29; 1.28)	-0.048	(-0.44; 0.35)	NA	NA	NA	NA

Note: FQ=Fear Questionnaire; BAI=Beck Anxiety Inventory; BDI-II=Beck Depression Inventory-II; BSQ=Bodily Sensations Questionnaire; SPAI-18=Social Phobia and Anxiety Inventory-18; CI=confidence interval; PD + AGO=panic disorder with agoraphobia; SOC=social anxiety disorder; NA=not applicable (non-significant predictors/no significant slope variance; predictors not included in the final model). Confidence intervals based on robust standard errors. Interactions time2 x drug, time2 x diagnosis, and time2 x drug x diagnosis were not significant and are not displayed in the table. * $p < 0.05$.

Supplemental Table 14 Predictors of secondary disorder-specific outcomes in the multilevel analyses on assessments from baseline to second follow-up (T0-T4) in the intent-to-treat sample, excluding those patients who participated in intensive treatment programs during the follow-up period (between T2 to T4).

	LSAS		MI "alone"		MI "accompanied"		ACQ		PDSS	
	β	95% CI	*β*	95% CI	*β*	95% CI	*β*	95% CI	*β*	95% CI
Time level										
Time	-0.37	(-0.81; 0.081)	NA	NA	-0.048*	(-0.084; -0.012)		(-0.029; -0.0092)	-0.25*	(-0.34; -0.16)
Time^2	NA	NA	NA	NA	0.00080*	(0.000047; 0.0016)	NA	NA	NA	NA
Session	NA	NA	-0.10*	(-0.15; -0.056)	NA	NA	NA	NA	NA	NA
Session^2	NA	NA	0.0019*	(0.00097; 0.0029)	NA	NA	NA	NA	NA	NA
Patient level										
Intercept	60.86*	(48.50; 73.22)	3.28*	(2.90; 3.66)	2.56*	(2.13; 2.98)	2.23*	(1.88; 2.57)	15.14*	(12.95; 17.33)
Antidepressant (no; yes)	NA	NA	NA	NA	NA	NA	-0.35*	(-0.65; -0.052)	NA	NA
Drug (placebo; cannabidiol)	7.85	(-9.82; 25.51)	0.0019	(0.00097; 0.0029)	-0.26	(-0.80; 0.28)	0.0083	(-0.37; 0.38)	-0.24	(-3.13; 2.64)
Time interaction variables										
Time x drug	0.28	(-0.37; 0.92)	NA	NA	0.008	(-0.032; 0.048)	0.0063	(-0.0070; 0.020)	-0.047	(-0.17; 0.073)
Session x drug	NA	NA	0.014	(-0.031; 0.059)	NA	NA	NA	NA	NA	NA

Note: LSAS=Liebowitz Social Anxiety Scale; MI=Mobility Inventory; ACQ =Agoraphobic Cognitions Questionnaire; PDSS=Panic Disorder Severity Scale; CI=confidence interval; PD + AGO=panic disorder with agoraphobia; SOC=social anxiety disorder; NA=not applicable (non-significant predictors; not included in the final model). LSAS was filled in by 36 patients with social anxiety disorder (placebo n = 19; cannabidiol n = 17), MI, ACQ and PDSS by 38 patients with panic disorder with agoraphobia (placebo n = 18; cannabidiol n = 20). Confidence intervals based on robust standard errors. Interactions time2 x drug and session2 x drug were not significant and are not displayed in the table.

* $p < 0.05$.

Supplemental Table 15 Predictors of primary and secondary outcomes in the multilevel analyses on assessments from baseline to second follow-up (T0-T4) in the completers sample, excluding those patients who participated in intensive treatment programs during the follow-up period (between T2 to T4). Placebo $n = 27$; cannabidiol $n = 30$.

	FQ		BAI		BDI-II		BSQ		SPAI-18	
	β	95% CI	*β*	95% CI	*β*	95% CI	*β*	95% CI	*β*	95% CI
Time level										
Time	NA	NA	-0.68*	(-1.18; -0.18)	-0.46*	(-0.70; -0.23)	-0.067*	(-0.10; -0.034)	NA	NA
Time^2	NA	NA	0.011	(-0.00060; 0.022)	0.0082*	(0.0023; 0.014)	0.0011*	(0.00042; 0.0017)	NA	NA
Session	-3.83*	(-5.31; -2.35)	NA	NA	NA	NA	NA	NA	-1.44*	(-2.12; -0.76)
Session^2	0.089*	(0.042; 0.14)	NA	NA	NA	NA	NA	NA	NA	NA
Patient level										
Intercept	60.80*	(52.16; 69.43)	22.60*	(18.46; 26.75)	21.36*	(17.22; 25.50)	2.54*	(2.13; 2.96)	56.12*	(48.30; 63.94)
Antidepressant (no; yes)	NA	NA	NA	NA	-4.38*	(-8.37; -0.39)	NA	NA	NA	NA
Drug (placebo; cannabidiol)	-13.92	(-29.30; 1.45)	4.73	(-2.12; 11.59)	0.85	(-4.81; 6.51)	-0.22	(-0.58; 0.15)	-7.87	(-20.41; 4.66)
Diagnosis (PD + AGO; SOC)	-17.24*	(-29.48; -5.01)	-2.21	(-10.92; 6.49)	4.03	(-5.01; 13.07)	-0.32	(-0.83; 0.18)	13.50*	(3.35; 23.65)
Drug x diagnosis	16.97	(-4.59; 38.52)	-7.09	(-18.66; 4.48)	-1.26	(-12.17; 9.65)	0.062	(-0.56; 0.69)	5.12	(-11.88; 22.13)
Time interaction variables										
Time x drug	NA	NA	-0.19	(-0.75; 0.37)	0.029	(-0.20; 0.26)	-0.22	(-0.014; 0.057)	NA	NA
Time x diagnosis	NA	NA	0.16	(-0.55; 0.86)	0.039	(-0.36; 0.44)	0.024	(-0.011; 0.060)	NA	NA
Time x drug x diagnosis	NA	NA	0.44	(-0.45; 1.34)	-0.2	(-0.73; 0.33)	-0.012	(-0.064; 0.040)	NA	NA

Supplemental Table 15 Predictors of primary and secondary outcomes in the multilevel analyses on assessments from baseline to second follow-up (T0-T4) in the completers sample, excluding those patients who participated in intensive treatment programs during the follow-up period (between T2 to T4). Placebo $n = 27$; cannabidiol $n = 30$.

Session x drug	1.07	(-0.76; 2.90)	NA	NA	NA	NA	NA	NA	0.79*	(0.022; 1.57)
Session x diagnosis	1.75	(-0.12; 3.61)	NA	NA	NA	NA	NA	NA	0.24	(-0.50; 0.98)
Session x drug x diagnosis	-0.6	(-3.12; 1.92)	NA	NA	NA	NA	NA	NA	-0.096	(-1.01; 0.82)

Note: FQ=Fear Questionnaire; BAI=Beck Anxiety Inventory; BDI-II=Beck Depression Inventory-II; BSQ=Bodily Sensations Questionnaire; SPAI-18=Social Phobia and Anxiety Inventory-18; CI=confidence interval; PD + AGO=panic disorder with agoraphobia; SOC=social anxiety disorder; NA=not applicable (non-significant predictor, not included in the final model). Confidence intervals based on robust standard errors. Interactions time2 x drug, time2 x diagnosis, time2 x drug x diagnosis, session2 x drug, session2 x diagnosis, and session2 x drug x diagnosis were not significant and are not displayed in the table.

* $p < 0.05$.

Supplemental Table 16 Predictors of secondary disorder-specific outcomes in the multilevel analyses on assessments from baseline to second follow-up (T0-T4) in the completers sample, excluding those patients who participated in intensive treatment programs during the follow-up period (between T2 to T4).

	LSAS		MI "alone"		MI "accompanied"		ACQ		PDSS	
	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI
Time level										
Time	NA	NA	-0.072*	(-0.11; -0.037)	-0.058*	(-0.097; -0.018)	-0.040*	(-0.066; -0.013)	-0.47*	(-0.68; -0.25)
Time^2	NA	NA	0.0010*	(0.00031; 0.0017)	0.00098*	(0.00014; 0.0018)	0.00047*	(0.000040; 0.00089)	0.0060*	(0.0015; 0.011)
Session	-3.20*	(-4.82; -1.59)	NA	NA	NA	NA	NA	NA	NA	NA
Session^2	0.080*	(0.025; 0.13)	NA	NA	NA	NA	NA	NA	NA	NA

Supplemental Table 16 Predictors of secondary disorder-specific outcomes in the multilevel analyses on assessments from baseline to second follow-up (T0-T4) in the completers sample, excluding those patients who participated in intensive treatment programs during the follow-up period (between T2 to T4).

Patient level										
Intercept	72.73*	(56.46; 88.99)	3.17*	(2.75; 3.58)	2.59*	(2.11; 3.07)	2.26*	(1.93; 2.58)	15.17*	(12.72; 17.62)
Antidepressant (no; yes)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Drug (placebo; cannabidiol)	7.71	(-12.98; 28.41)	-0.34	(-0.93; 0.25)	-0.19	(-0.80; 0.42)	-0.26	(-0.57; 0.062)	0.31	(-2.51; 3.13)
Time interaction variables										
Time x drug	NA	NA	0.024	(-0.016; 0.064)	0.015	(-0.029; 0.059)	0.013*	(0.0020; 0.023)	-0.1	(-0.35; 0.14)
Session x drug	1.02*	(0.095; 1.94)	NA	NA	NA	NA	NA	NA	NA	NA

Note: LSAS=Liebowitz Social Anxiety Scale; MI=Mobility Inventory; ACQ=Agoraphobic Cognitions Questionnaire; PDSS=Panic Disorder Severity Scale; CI=confidence interval; PD + AGO=panic disorder with agoraphobia; SOC=social anxiety disorder; NA=not applicable (non-significant predictor, not included in the final model). LSAS was filled in by 26 patients with social anxiety disorder (placebo n = 13; cannabidiol n = 13), MI, ACQ and PDSS by 31 patients with panic disorder with agoraphobia (placebo n = 14; cannabidiol n = 17). Confidence intervals based on robust standard errors. Interactions time2 x drug and Session2 x drug were not significant and are not displayed in the table.

* $p < 0.05$.

Supplemental Table 17 Predictors of FQ in the per protocol multilevel analysis on assessments from baseline to second follow-up (T0-T4) in patients with medium to high therapist protocol adherence, who (to our knowledge) adhered to the medication regimen, and completed the eight treatment sessions according to the protocol (placebo $n = 25$; cannabidiol $n = 27$).

	FQ	
	β	95% CI
Time level		
Time	-2.09*	(-2.87; -1.32)
Time^2	0.033*	(0.016; 0.050)
Patient level		
Intercept	53.81*	(43.64; 63.97)
Drug (placebo; cannabidiol)	-7.45	(-23.96; 9.06)
Diagnosis (PD + AGO; SOC)	-10.01	(-24.73; 4.71)
Drug x diagnosis	9.1	(-14.90; 33.09)
Time interaction variables		
Time x drug	0.65	(-0.49; 1.78)
Time x diagnosis	0.9	(-0.18; 1.98)
Time x drug x diagnosis	-1.09	(-3.10; 0.92)

Note: FQ=Fear Questionnaire; CI=confidence interval; PD +AGO=panic disorder with agoraphobia; SOC=social anxiety disorder. Confidence intervals based on robust standard errors. Interactions time2 x drug, time2 x diagnosis, and time2 x drug x diagnosis were not significant and are not displayed in the table.

* $p < 0.05$.

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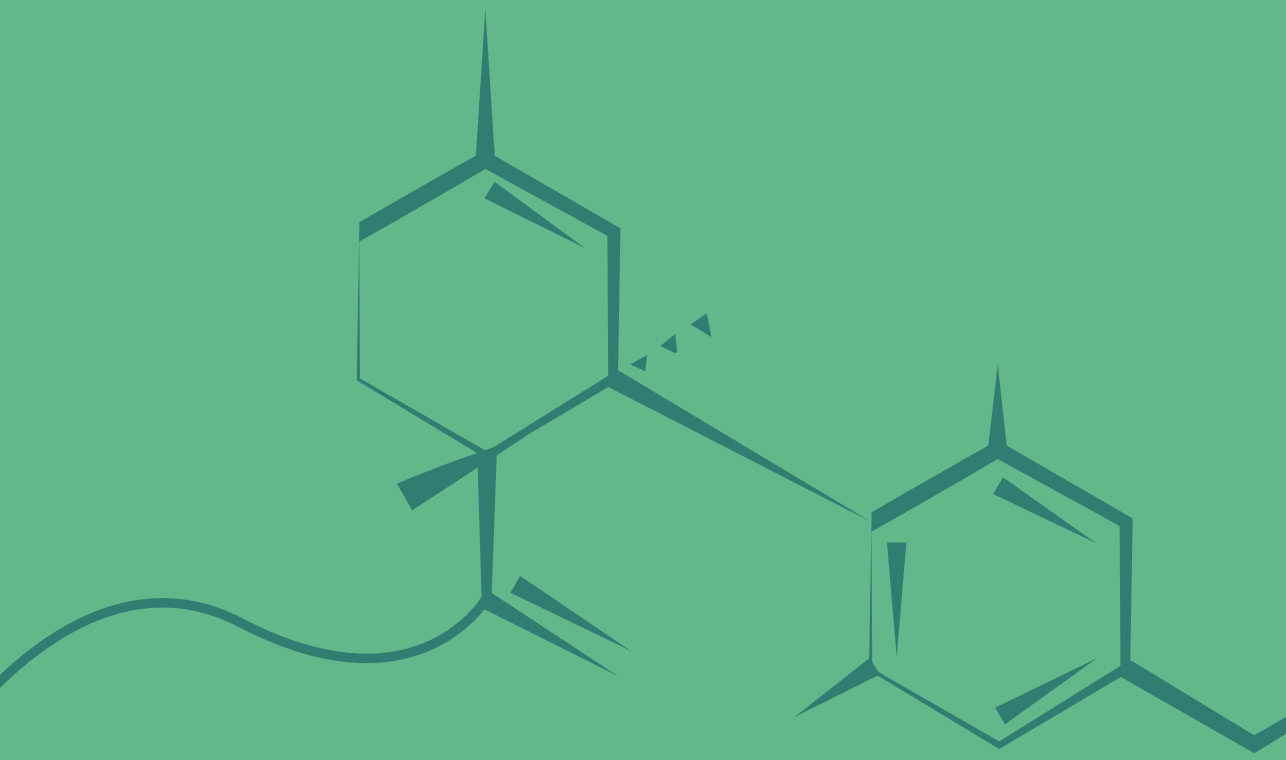

Chapter 6

Effects of cannabidiol on fear conditioning in anxiety disorders: Decreased threat expectation during retention, but no enhanced fear re-extinction

Caroline M.B. Kwee
Febe E. van der Flier
Puck Duits
Anton J.L.M. van Balkom
Danielle C. Cath
Johanna M.P. Baas

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JB (PI), DC, AvB, NB and LG obtained funding for this study. All authors contributed to the design of the study. FvdF and CK coordinated the recruitment of patients and the data collection. CK did the statistical analyses. CK wrote the first draft of the manuscript. All authors (FvdF, JB, DC, PD, AvB, CK) read, contributed to and approved the final manuscript.

Abstract

Rationale

Preclinical research suggests that pharmacologically elevating cannabinoid levels may attenuate fear memory expression and enhance fear extinction.

Objectives

We studied the effects of cannabidiol (CBD) on fear memory expression and fear re-extinction in 69 patients with panic disorder with agoraphobia or with social anxiety disorder. Moderation by sex, diagnosis and serotonergic antidepressant (AD) use was explored.

Methods

A cued fear conditioning paradigm was applied before the first treatment session with 300 mg CBD/placebo augmented exposure therapy. Study medication was administered orally preceding 8 weekly sessions. Fear acquisition and suboptimal extinction took place prior to the first medication ingestion (T0). After the first medication ingestion (T1) we investigated effects on fear memory expression at retention and fear re-extinction. Subjective fear, shock expectancy, skin conductance and startle responses to conditioned (CS+) and safety stimulus (CS-) were measured.

Results

Across the sample, CBD reduced shock expectancy at retention under low and ambiguous threat of shock, but fear re-extinction at T1 was unaffected by CBD. However, in AD users, re-extinction of subjective fear was impaired in the CBD condition compared to placebo. In female AD users, CBD interfered with safety learning measured with fear potentiated startle.

Conclusions

The current findings provide no evidence for enhanced fear re-extinction by CBD. However, CBD acutely decreased threat expectation at retention, without affecting other indices of fear. More studies are needed to elucidate possible interactions with AD use and sex, as well as potential effects of CBD on threat expectancies.

6.1 Introduction

Recurrent excessive fear is a core symptom of anxiety disorders. Several underlying pathological mechanisms have been proposed, including inefficiencies in fear extinction and excessive fear generalization in patients with anxiety disorders compared to healthy individuals (Duits et al., 2015). Exposure therapy is hypothesized to effectuate fear extinction by inhibiting associations between aversive stimuli and essentially neutral conditioned stimuli (Craske et al., 2012). Exposure therapy has shown to be effective in reducing anxiety symptoms (Cuijpers et al., 2016; Van Dis et al., 2020) up to 12 months follow-up (Van Dis et al., 2020), although around one third of patients is left with residual symptoms (Gloster et al., 2013).

The neuromodulatory endogenous cannabinoid system (ECS) is implied as a promising new target for the pharmacological treatment of anxiety disorders (Haller et al., 2002; Marsicano et al., 2002; Myers and Davis, 2007; Sah, 2002). Cannabinoid type 1 (CB1) receptors are the most abundant receptors of the ECS (Pertwee, 1997). By their activation neurotransmission is inhibited throughout the adult human brain, and mostly in the amygdala, hippocampus and associated regions including the prefrontal cortex, where the receptor is densely expressed (Glass et al., 1997). These brain areas are part of the neuronal circuits underlying fear acquisition, expression and extinction (Myers and Davis, 2007; Tovote et al., 2015).

Fear acquisition, expression and extinction refer to experimental phases and to an individual's learning about danger and safety as a result of these phases, either during an experimental fear conditioning task or in daily life situations. During fear acquisition, fear is conditioned to a stimulus that is repeatedly paired with an aversive outcome. During fear extinction, the conditioned stimulus is repeatedly presented without aversive outcomes. A second stimulus that is never paired with aversive outcomes (the 'safety stimulus') and more extinction phases can be included (re-extinction phases), as well as a retention phase during which fear or extinction (memory) expression can be measured.

Endocannabinoids may play a role in fear extinction (Gunduz-Cinar et al., 2013; 2016; Marsicano et al., 2002) through activation of CB1 receptors in the basolateral amygdala, a nucleus that regulates outputs of the central amygdala (McDonald & Mascagni, 2001; Lafenêtre et al., 2007; Tovote et al., 2015). As a proof of concept for the role of the ECS in fear extinction in humans, in healthy individuals polymorphisms in major cannabinoid genes have been associated with poor extinction (e.g., Dincheva et al., 2015; Heitland et al., 2012; Mayo et al., 2018) and more recently, also in patients with posttraumatic stress disorder (PTSD; Ney et al., 2021). Although findings from genetic associations are indirect evidence, they are suggestive of the clinical potential of CB1 receptor activation as an extinction-enhancing strategy.

Recent research suggests that another potential of CB1 receptor signaling might be reduction of fear memory retrieval. Aversive fear memories are at the core of several anxiety disorders (de Quervain et al., 2017) and render patients vulnerable to relapse after exposure therapy (Vervliet et al, 2013). Pharmacological blockade and activation of dorsal hippocampal cannabinoid receptors in rats showed that the decrease in contextual fear memory expression by the stress hormone corticosterone is mediated by the ECS (Atsak et al., 2012). Preliminary evidence suggests that these findings may translate to clinical populations (Hill et al., 2013). This work showed in patients with PTSD that circulating levels of the endocannabinoid anandamide (AEA), a partial cannabinoid receptor agonist (Pertwee, 1999), were negatively related to the degree of intrusive symptoms in patients with PTSD.

Repeated administration of the exogenous cannabinoid cannabidiol (CBD) can increase endogenous AEA levels in humans (Leweke et al., 2012). CBD is a phytocannabinoid without hedonic properties (Katsidoni et al., 2012; Parker et al., 2004) and with a favorable safety profile, although effects and side effects of chronic administration are still understudied (Bergamaschi et al., 2011; Iffland & Grotenhermen, 2017; Kwee et al., 2022a). A recent meta-analysis demonstrated beneficial effects of systemic (mostly single) CBD administration on fear extinction and fear memory expression in rodents and experimentally induced anxiety in humans, although the quality of the evidence was low (Kwee et al., 2023).

Few studies investigated the effect of CBD treatment on fear extinction and fear memory expression in humans. One study investigated the effect of 300 mg orally administered CBD in patients with PTSD on symptoms associated with recalling a traumatic memory (Bolsoni et al., 2022). Compared to placebo (n=16), CBD (n=17) attenuated an increase in cognitive impairment associated with the memory recall. However, CBD had no effect on anxiety, alertness, discomfort or physiological responses associated with recalling a traumatic memory. In a second study in healthy persons who were fear conditioned to a colored box (conditioned stimulus, CS+) 32 mg of inhaled CBD (n=16), compared to placebo (n=16), had no acute effects on fear extinction (Das et al., 2013). Only when administered after successful extinction training, CBD decreased overall shock expectancy (upon context and CS presentations) during a reinstatement phase 24 h later, and enhanced learning of new CS-US contingencies (Das et al., 2013). These findings may be interpreted as enhancement of extinction consolidation by CBD, although effects were limited to the subjective outcomes. A third study did not apply CBD but the fatty acid amide hydrolase (FAAH) inhibitor PF-04457845, that, similar to CBD, acts on cannabinoid receptors indirectly. In this study in healthy individuals, the drug attenuated fear memory expression measured with fear potentiated startle. In the preceding fear extinction phase the PF-04457845 group (n=16) was not different from placebo (n=29; Mayo et al.,

2020).

To date no studies have been published that examined the effect of CBD on fear retention and extinction in patients with anxiety disorders. We recently carried out a randomized controlled clinical trial in treatment refractory patients with social anxiety disorder and with panic disorder with agoraphobia, based on the idea that CBD administration during exposure therapy could augment the efficacy of exposure therapy (Kwee et al, 2022b). To this end, we administered either CBD (300 mg; n=39) or placebo (n=41) orally preceding 8 weekly exposure sessions. On clinical outcome measures, CBD and placebo conditions did not differ (Kwee et al., 2022b). However, to more directly investigate an effect of CBD on fear learning we also included a de novo fear conditioning task, that was executed by 69 patients in this trial. The employed fear conditioning task was adapted from earlier work that showed enhanced fear responses and an overrepresentation of poor extinction trajectories in patients compared to healthy individuals (Duits et al., 2017; 2021). Retention and re-extinction phases executed at the time of first CBD intake were investigated to test potential beneficial effects of acute CBD intake on fear learning, as outlined in the study protocol (van der Flier et al., 2019)

The aim of the present study was two-fold: We investigated whether CBD would attenuate fear memory expression (research question 1) and whether CBD would enhance fear re-extinction (research question 2). For both research questions we explored interaction effects between CBD and potential moderators: diagnosis (panic disorder with agoraphobia; social anxiety disorder), use of serotonergic antidepressant (AD) medication and sex.

In our clinical trial serotonergic AD use at a stable regimen was permitted, given that many treatment refractory patients are receiving this first-line pharmacological treatment (Baldwin et al., 2014). We included in our clinical trial patients with panic disorder with agoraphobia and social anxiety disorder because for both diagnoses, an exposure-based treatment is clinically indicated. Investigation of drug-drug interactions has been advocated, especially in the context of undesired effects of CBD (Bergamaschi et al., 2011; Chesney et al., 2020). We investigated whether the effects of CBD on fear learning could be diagnosis-specific, considering the examples in the literature of ECS manipulations being effective only under aversive conditions (Campolongo et al., 2013; de Oliveira Alvares et al. 2010). How aversive our experimental task is to participants likely depends, at least in part, on an individual's anxiety disorder diagnosis (Cornwell et al., 2006). For instance, social aspects of the experimental situation that are phobic for social anxiety disorder patients may be non-threatening to patients with panic disorder with agoraphobia.

Lastly, we investigated potential differences in CBD effects between the sexes. It is well known that psychotropic drug effects can differ between sexes (Gandhi et al., 2004; Zucker &

Prendergast, 2020). Despite initial studies that demonstrated an absence of consistent sex differences in the effect of CBD on anxiety-like outcomes in rodents (Kasten et al., 2019; Franzen et al., 2023; Stern et al., 2015), this area is still grossly understudied (Kwee et al., 2023).

6.2 Methods

6.2.1 Participants

Participants (n=69) were adult patients with a primary diagnosis of social anxiety disorder or panic disorder with agoraphobia who participated in a fear conditioning task in the context of a multicenter randomized controlled trial on the added effect of CBD augmentation in exposure therapy. Serotonergic antidepressants were allowed, provided that no changes in dosing regimen occurred up to 4 weeks before study entry and during the study. Regular daytime use of benzodiazepines and use of recreational drugs were not allowed (see Table S1 for full in- and exclusion criteria). This study has been approved by the Medical Ethics Committee of the University Medical Centre Utrecht and the study protocol (van der Flier et al., 2019) and results regarding clinical outcomes have been published (Kwee et al., 2022b).

6.2.2 Procedure

6.2.2.1 Randomization and overall study design

The randomization (CBD or placebo with a 1:1 allocation ratio) was conducted by an independent data manager using block randomization, stratifying for study center and primary diagnosis. 300 mg CBD or placebo capsules (identical in appearance) were ingested approximately 2 h prior to the treatment sessions, based on high CBD plasma levels measured from 2 h onwards after oral ingestion (Englund et al., 2013). In order to ensure dosage in the effective and safe range we employed dosages of 300 mg, in line with previous work (Zuardi et al., 1993, 2017). The treatment consisted of 8 weekly 90 min sessions of CBD/placebo augmented standardized exposure therapy (more detailed information in van der Flier et al., 2019).

The fear conditioning task took place at two occasions, see Figure 1. The baseline measurement (T0) was taken approximately 1-2 weeks before the start of treatment, which coincided with the second measurement (T1). At T0, acquisition and a brief extinction training took place, and CBD effects were assessed on retention and re-extinction at T1, 80 minutes after ingestion of the first study medication. Multiple day paradigms often take place on consecutive days (e.g., Klumpers et al., 2012; Mayo et al., 2020), which was not feasible in the context of the clinical

trial. However, conditioned responses can return easily, even after extinction has taken place (Vervliet et al., 2013), which was demonstrated in healthy individuals up to six weeks after fear conditioning (Leen et al., 2021). We thus expected return of fear after ingestion of the study medication, which allowed for assessment of acute effects of CBD versus placebo on fear memory expression and fear re-extinction.

In addition, the fear conditioning task was applied at post treatment (T2). Experimental blocks were the same as at T0 (see 6.2.2.2), except for the addition of a second extinction block (in the same session) that was instructed. The posttreatment assessment was included to investigate whether effects of cannabidiol augmentation on treatment success would translate to improved extinction learning at post treatment (van der Flier et al., 2019). Since we did not find evidence for the impact of CBD on treatment success (Kwee et al., 2022b), no such effects at posttreatment were expected. For completeness the procedure at posttreatment and results are reported in the supplement (6.S.1.2, 6.S.2.1, 6.S.2.2, 6.S.4, 6.S.5, Appendix A).

6.2.2.2 Experimental blocks at baseline (T0)

At T0, the task started with startle habituation and conditioned (CS+) and safety stimulus (CS-) familiarization blocks. These were followed by two successive acquisition blocks, one uninstructed and one instructed. Before the first acquisition block participants were informed that shocks (the unconditioned stimulus, see section 6.2.3.1) might be administered to allow for the spontaneous development of contingency awareness. After the first acquisition block participants were asked to indicate when the shock had been administered. Several options were given orally by the researcher or research assistant: 1) after the startle probe, 2) after both pictures, 3) after one picture, 4) after the fixation cross, 5) no systematic administration has taken place, or 6) don't know. When option 3 was chosen, the participant was asked which of the two pictures predicted the onset of the shock. The criterion for correct report of contingencies was met when a participant chose option 3 and identified the CS+. After the participant's answer was recorded, information about the correct CS-US contingency was provided both orally and written. The task at T0 ended with one uninstructed extinction block, that was aimed at some, but not complete fear extinction to take place.

6.2.2.3 Experimental blocks after first study medication ingestion (T1)

The task at T1 started with two blocks of retention testing to measure the effect of CBD on fear memory expression under increasing levels of threat imminence, following Grillon et al. (1998) who found increased sensitivity to contextual threat measured with fear-potentiated startle in

patients with posttraumatic stress disorder (PTSD). During these two retention blocks, CSs were presented without reinforcement. Participants were informed, orally and in writing, that no shocks would be administered in these blocks. An increase in threat imminence in block two was established by attaching the shock electrodes. Then, two more blocks of uninstructed re-extinction followed.

After each experimental block, VAS scales were presented to collect subjective ratings of fear and expectancy of shock experienced with each CS (outcome measures are discussed in more detail in section 6.2.4).

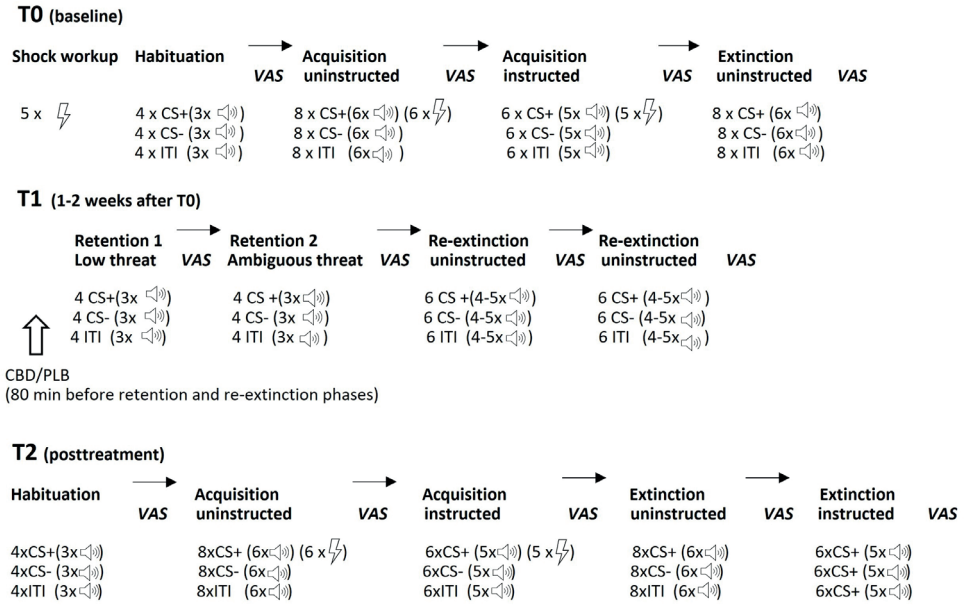

Figure 1. The fear conditioning task.

Note: T0 = Task at baseline, prior to any treatment; T1 = Task after first study medication ingestion; T2 = Task at posttreatment; CS+ = conditioned stimulus; CS- = safety stimulus; ITI = inter-trial-interval; VAS = visual analogue scale; CBD = cannabidiol, PLB = placebo.

6.2.3 The fear conditioning task

6.2.3.1 Stimuli and apparatus

The fear conditioning task was adapted from Duits et al. (2017) and was programmed in Presentation software.

Following Klumpers et al. (2010) and Duits et al. (2017), pictures of neutral faces ((http://pics.stir.ac.uk)) served as CS+ and CS-. A 625-ms electric shock loop, composed of 2-ms pulses delivered at 20 Hz, served as unconditioned stimulus (US). Shocks were administered by a Digitimer DS7A generator, through two disk electrodes attached over the medial nerve on the inner wrist of the subject's non-dominant hand.

6.2.3.2 US titration

A shock workup, previously carried out by Duits et al. (2017), Heitland et al. (2012) and Klumpers et al. (2010), was employed in the present study in order to standardize subjective pain intensity. The shock intensity that received a rating of 4 on a five-point scale, which corresponded to "quite annoying" was used in the fear conditioning task.

6.2.3.3 Task design

During acquisition blocks, CS+ presentations were followed by US presentations according to a partial reinforcement schedule (during uninstructed acquisition 75% of the CS trials (6 out of 8) were reinforced, during instructed acquisition: 83% of the CS trials (5 out of 6)). The CS- was never paired with the US. During extinction, retention and re-extinction blocks all CSs were unreinforced. Stimulus presentations lasted 8 s, and the total duration of each trial (including a black screen with a white fixation cross in the center of 6-8 seconds) was 14-16 s. The CS+, CS- and inter-trial interval (ITI) were presented in fixed order within the conditioning procedure. Figure 2 displays a schematic overview of a trial.

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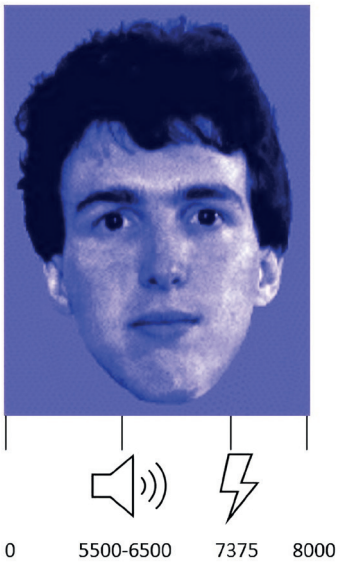

Figure 2. Schematic overview of a CS+ trial reinforced by electric shock

Total duration of one trial was 8000 ms. Auditory startle probes followed 5.5, 6.0 or 6.5 s after stimulus onset in approximately 75% of trials; from 7375 ms onwards an electric shock loop was delivered.

For details and considerations regarding the fear conditioning task, see 6.S.1.2.

6.2.4 Outcome measures

Considering earlier cannabinoid effects in healthy humans on fear reinstatement as measured with shock expectancy (Das et al., 2013), and on fear expression during extinction training measured with skin conductance responses (Klumpers et al., 2012), we included both subjective and physiological measures in the current study. Measures of subjective fear and shock expectancy were used, as well as skin conductance as an index of autonomic arousal and fear potentiated startle as an index of amygdala-based defensive state (Lonsdorf et al., 2017).

6.2.4.1 Physiological measures and processing

Biopac Systems MP150 (Goleta, CA) with AcqKnowledge software, Coulbourn S71-22 skin conductance coupler, and S75-01 bioamplifier apparatus (Alletown, PA) were used to record electromyographic (EMG) and electrodermal (EDA) activity. Data were preprocessed in Brain Vision Analyzer 2.1.

6.2.4.1.1 Fear potentiated startle Auditory startle probes (up to 95 dB, 50-ms white-noise bursts) were administered throughout the conditioning procedure through Sennheiser headphones. The probes followed 5.5, 6.0 or 6.5 s after CS onset or during inter-trial intervals (ITI) in approximately 75% of the stimuli presentations (see Figure 1). EMG activity associated with the eyeblink component of the startle response was recorded with two miniature Ag/AgCl surface electrodes (4 mm diameter) over the left musculus orbicularis oculi and one ground electrode (8 mm diameter), placed on the forehead. The electrodes were filled with electrolyte gel and attached with double-sided adhesive rings.

Following guidelines of Blumenthal et al. (2005) and in-house procedures (Klumpers et al., 2010) a 28 Hz high pass filter and a 50 Hz notch filter to reduce power line interference was applied to the data. The signal was rectified and smoothed using a 14 Hz low-pass filter. Peak amplitudes were determined within a scoring window 25-100 ms post startle probe presentation. Artefact rejection was applied in Matlab R2017a using in-house procedures.

6.2.4.1.2 Skin conductance response Skin conductance response (SCR), the phasic change in EDA in response to the CSs, was recorded with two Ag/AgCl electrodes (8 mm diameter) filled with isotonic electrode gel and placed on the palm of a participant's non-dominant hand.

A 5 Hz low-pass filter was applied to the data according to in-house procedures. SCR was defined as the difference between the highest peak (between .9 and 4 s after stimulus onset) and mean skin conductance level during baseline (between 2 and 0 s before stimulus onset). The time window for peak scoring ensured that recorded responses were elicited by the CS and were unaffected by startle probe presentation. Baseline time window was adopted from Duits et al. (2017). The scored peaks were checked by one of the researchers (AT or CK) in Brain Vision Analyzer 2.1 and coded as response, zero-response, or artefact (SCR starting prior to stimulus onset or too much noise in the signal to identify SCR).

More information about physiological processing is provided in 6.S.1.3.

6.2.4.2 Subjective measures

After each experimental block subjective fear while viewing the CS+ and CS- (response anchors 'not at all fearful/nervous' and 'very fearful/nervous') and shock expectancy following CS+ and CS- presentation (response anchors 'very unlikely' and 'very likely') were measured on visual analogue scales (VASs). Responses were logged on a line that was digitally recoded to a 0-100 scale (numbers were not visible to participants). Participants also rated on VASs their perceived

level of focus, valence of the startle probes and of electrical shocks. In addition, they rated how certain they were of their answers.

After the first study medication ingestion, acute side effects of the study medication were measured at time points 0, 60 and 120 min after ingestion. Patients rated subjective mood, anxiety, alertness, feeling calm, feeling high, apathetic, paranoid, hungry, dizzy on VASs with anchors "not at all- very much".

6.2.5 Statistical analyses

For our analyses regarding clinical outcomes we calculated required the sample size for a power of 0.8, with $\alpha = 0.05$, based on a repeated measures design for two groups with two measurements, and an envisioned effect size of 0.6 Cohen's d. This yielded group sizes of 36 patients per treatment arm (Kwee et al., 2022b).

6.2.5.1 Primary research questions

Analyses were conducted in IBM SPSS Statistics version 28.0.1.1 and HLM 7. Following Duits et al. (2021) and Leen et al. (2021) CS+ and CS- responses were analyzed rather than CS+/CS- difference scores, because the CS- does not represent an absolute absence of threat (Lonsdorf et al., 2017), and may be separately affected by e.g. clinical status (Duits et al,. 2021). Notably, Das et al. (2013) demonstrated effects of CBD on reinstatement in both cues (CS+ and CS-) in healthy volunteers, which would not have become visible when focusing on the CS+/CS- contrast. Therefore, we performed separate analyses for CS+ and CS-.

Multilevel models were used with full maximum likelihood estimation and repeated measurements of responses to CS+ and CS- across experimental blocks (level 1) from each individual (level 2). An advantage of the multilevel approach is the modelling of learning curves that are different for each individual, and take into account within-group variability (Bryk & Raudenbush, 1992). The trends in CS+ and CS- responding over the course of the experimental blocks were modelled by first degree (linear) polynomials. For analyses with more than two experimental blocks, higher polynomials were added to assess a non-linear growth patterns.

Research question 1 (effect of single CBD administration on fear memory expression) was examined in analysis models with Blocks [retention 1 (low threat) and retention 2 (ambiguous threat)], the main and interaction effects of Drug condition and moderator variables (Diagnosis, Sex, and AD medication). Research question 2 (effect of single CBD administration on fear re-extinction) was examined with T0 uninstructed extinction versus T1 uninstructed re-extinction 2, main and interaction effects of Drug condition and moderator variables. In all analyses, cov-

ariates were included to indicate which experimenter conducted the experiment. The reference category was the study experimenter who had seen the most participants (*n*=28) at T0. Three indicator variables (using values 0 and 1) were used for the three study experimenters who had seen the second most participants (together *n*=21) at T0. The remaining study experimenters were grouped under the fourth indicator variable.

For each research question parallel analyses per moderator were conducted to overcome issues with overparameterization (Bates at al., 2018, preprint). Orthogonality checks for moderator variables showed independence across and within drug conditions, ps $\geq$ .22, which provides an indication for an absence of confounding between these moderators. For effects of CBD that did not interact with moderators, the standard criterion of $p < .05$ was applied, and for interactions with moderators a Bonferroni correction (α=.05 divided by three for parallel analyses). Moderator effects in initial models that included moderator X Drug condition interactions are presented in the Supplemental material, Appendix A. The final models are presented in the main text, from which moderators and/or covariates that did not significantly predict outcome were discarded to prevent unnecessarily complex models.

For each research question separate analyses were conducted for all outcome measures (SCR, startle, subjective fear and shock expectancy). For the outcome of fear-potentiated startle, responses during CS presentations were corrected for ITI responses per experimental block. Supplemental analyses on startle *z*-scores (raw score – mean of all startle trials at one measurement occasion within each subject) / standard deviation) were conducted to control for individual differences in variability of startle responding (Bradford et al., 2015). Analyses on raw startle and startle *z*-scores yielded the same results regarding effects of Drug condition, hence, only results on raw startle are reported.

6.2.5.2 Data patterns outside drug effects and subjective side effects

To rule out pre-existing differences between groups assigned to the drug conditions at baseline, we compared sociodemographic and clinical characteristics by means of independent samples t-tests. Multilevel analyses (with the same moderators and covariates described as described in 6.2.5.1) were conducted to investigate differences between drug conditions during T0 fear conditioning (see section 6.3.2 and 6.S.2.3).

Furthermore, we investigated acute side effects of the drug after the first study medication ingestion (see section 6.3.5 and 6.S.3.1), and differences between drug conditions in additional subjective ratings (focus, certainty of subjective ratings and valence ratings of startle probes and of electric shocks) during fear conditioning (see 6.S.5). Percentage correct reporting of the CS-US contingency, that is before instructions were provided about the CS-US contingency,

was compared between drug conditions using the Chi-square test for independence (see section 6.3.2 and 6.S.2.2).

6.3 Results

6.3.1 Baseline characteristics for analyzed sample

The fear conditioning task was administered in two out of the three treatment locations, in total in 69 of the 80 participants randomized to the CBD and placebo condition. Sample size was further reduced due to various reasons.[1]

Table 1. Baseline characteristics of participants did not significantly differ between drug conditions.

Sociodemographics	**Total sample**	**Placebo**	**Cannabidiol**
Age at study entry, mean (SD)	33.8 (9.2)	34.9 (10.2)	32.6 (8.2)
Female sex	21 (41.2)	11 (42.3)	10 (40.0)
Double nationality	1 (3.8)	1 (7.7)	0 (0)
Married or cohabiting	12 (46.2)	7 (53.8)	5 (38.5)
Post-high school education	17 (65.4)	8 (61.5)	9 (69.2)
Currently employed	29 (64.4)	15 (71.4)	14 (58.3)
Clinical variables			
Having received previous treatment	29 (58.0)	16 (61.5)	13 (54.2)
Use of antidepressant medication1	23 (45.1)	10 (38.5)	12 (48.0)
Primary diagnosis social anxiety disorder	24 (47.1)	12 (53.8)	12 (48.0)
Primary diagnosis panic disorder with agoraphobia	27 (52.9)	14 (46.2)	13 (52.0)
Fear Questionnaire (FQ), mean (SD)	48.5 (19.4)	50.6 (15.5)	46.7 (22.5)
Beck Anxiety Inventory (BAI), mean (SD)	24.4 (12.4)	24.9 (11.8)	24.0 (13.1)

Note: Data are n (valid %), unless stated otherwise. 1 Citalopram, escitalopram and sertraline most frequently prescribed.

Baseline characteristics of the participants for which (near) complete data including subjective measures at that time point were available, are displayed for 51 patients at T1 in Table 1.

[1] No administration of the task at T1 in three patients (explicit or implicit refusal or time constraints), data loss due to an administrative error in seven patients, an error in the task in five patients, premature ending of the task due to illness/ task too aversive in three patients, a failure to record physiological measures in two patients, insufficient quality of recording of fear potentiated startle in two patients, insufficient recording of skin conductance in one patient.

At *a*=.05 drug conditions did not significantly differ with respect to these characteristics.

6.3.2 Data patterns at baseline (T0)

Across the entire sample fear acquisition was successful as shown by subjective and physiological outcome measures. Some, but not complete extinction took place across the sample, consistent with the short extinction block at T0. Data are shown in 6.S.2.3.1.

There were no differences between drug conditions (CBD versus placebo) on T0 fear conditioning and extinction, except for subjective fear responses to the CS- within AD users (6.S.2.3.2). AD users in the CBD condition showed stable fear levels during T0, whereas fear in the placebo condition followed a seesaw pattern. At the end of T0 the groups did not differ from one another, $p>.50$. We therefore did not correct for this baseline difference in our analyses for the effects of Drug condition at T1. At T0 CBD and placebo condition did not differ in occurrence of CS-US contingency awareness before instructions about CS-US contingencies were given.

6.3.3 Research question 1: Fear memory expression after a single dose of CBD

Fear memory expression after administration of CBD versus placebo was measured at T1 during conditions of low (retention 1) and ambiguous threat (retention 2, see Table 2 for all statistics). Fear memory expression in terms of shock expectancy to CS+ and CS- showed a main effect of Drug condition across retention blocks (shaded gray in Table 2), see Figure 3. Shock expectancy to the CS+ was significantly lower in the CBD compared to the placebo condition for CS+, β=-20.86, p=.004, and CS-, β=-14.80, p=.03. The size of these effects was large (ES= -1.37 and ES=-1.20, respectively). Further, single CBD administration interacted with Sex and Block (retention 1, retention 2) on CS- subjective fear expression (shaded gray in Table 2, see Fig. S5). Follow-up tests did not indicate significant differences between the groups, $ps \geq .06$. Diagnosis and AD use did not significantly predict fear during T1 retention.

Table 2. Fear outcomes during T1 retention testing, after first CBD/placebo ingestion.

CS+	Subjective fear (n=51)	Shock expectancy (n=51)	Startle (n=52)	SCR (n=53)
	Parameter estimate	Parameter estimate	Parameter estimate	Parameter estimate
Intercept	32.27* (1.02)	55.15* (1.74)	0.04 (0.78)	0.03 (0.15)
Drug condition	-1.74 (-0.03)	-40.39* (-0.64)	-0.03 (-0.13)	0.12 (0.28)
Sex	-	-	-0.07* (-0.35)	-
Experimenter effects	Ind. 2: -33.86* (-0.21)	-	-	-
Block	-0.23 (-0.004)	-13.26 (-0.21)	-0.02 (-0.18)	0.05 (0.12)
x Drug condition	2.95 (0.02)	13.02 (0.10)	0.02 (0.10)	-0.07 (-0.08)
x Sex	-	-	0.04* (0.21)	-
CS-	Subjective fear (n=51)	Shock expectancy (n=51)	Startle (n=52)	SCR (n=53)
	Parameter estimate	Parameter estimate	Parameter estimate	Parameter estimate
Intercept	29.20* (1.11)	44.38* (1.53)	0.03 (0.66)	0.03 (0.16)
Drug condition	-2.20 (-0.04)	-33.22* (-0.57)	-0.02 (-0.28)	0.19 (0.48)
Sex	11.80 (0.22)	-	-	-
Drug condition x Sex	-27.4 (-0.42)	-	-	-
Block	-0.47 (-0.009)	-11.00 (-0.19)	-0.008 (-0.10)	0.04 ^(0.11)
x Drug condition	-1.07 (-0.01)	12.28 (0.11)	0.01 (0.09)	-0.12 (-0.15)
x Sex	-3.72 (-0.04)	-	-	-
x Drug condition x Sex	20.95* (0.16)	-	-	-

Note: Ind.: indicator variable, SCR: skin conductance response. Levels of the within-subjects variable Block were retention 1 and retention 2. Standardized coefficients between brackets. The dashes in the table denote that this variable did not significantly explain variance in the model for this analysis and was not included in the final model; Diagnosis and AD use did not significantly predict fear during T1 retention on any fear outcome.
* $p<1/6$ (=.05/3) for interactions of Drug condition (CBD/placebo) with moderators; $p<.05$ for other effects

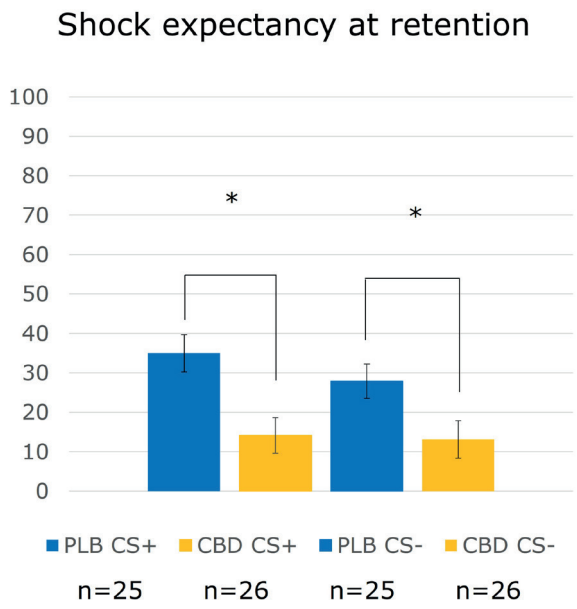

Figure 3. Shock expectancy to the CS+ and CS- during retention testing, split by drug condition.

Note: PLB: placebo; CBD: cannabidiol.

6.3.4 Research question 2: Effects of CBD on fear re-extinction

The analyses on the effect of CBD on fear re-extinction focused on the amount of extinction that was achieved at the end of re-extinction at T1 (after drug treatment), compared to the end of extinction at T0 (before drug treatment). Across the sample, the effects of a single CBD administration during T0 extinction-T1 re-extinction were not significant. Diagnosis did not significantly predict fear during T0 extinction-and T1 fear re-extinction. However, Drug condition interacted significantly with Sex and AD use (shaded gray in Table 3). These involved interaction effects of acute CBD administration with AD use on subjective fear (see Figure 4), and of CBD administration with AD use and Sex on fear-potentiated startle (see Figure 5). These findings are further elaborated in the following paragraphs.

Table 3. Fear outcomes for T0 extinction -T1 fear re-extinction (after first CBD/placebo ingestion).

CS+	Subjective fear (n=51)	Shock expectancy (n=51)	Startle (n=52)		SCR (n=53)
	Parameter estimate	Parameter estimate	Parameter estimate		Parameter estimate
Intercept	37.00* (1.30)	80.58* (2.53)	0.03 (0.75)		0.05 (0.31)
Drug condition	24.31 (0.43)	-13.06 (-0.20)	0.004 (0.05)		0.13 (0.40)
AD use	33.93* (0.60)	-47.83* (-0.75)	-		-
Drug condition x AD use	-62.48* (-0.94)	-	-		-
Block	-2.13 (-0.04)	-21.08* (-0.33)	-0.002 (-0.03)		0.03 (0.09)
x Drug condition	-17.88 (-0.16)	3.71 (0.03)	-0.01 (-0.08)		-0.08 (-0.13)
x AD use	-14.5 (-0.13)	23.43* (0.18)	-		-
x Drug condition x AD use	38.75* (0.29)	-	-		-
CS-	Subjective fear (n=51)	Shock expectancy (n=51)	Startle (n=52)		SCR (n=53)
			Model AD use	Model Sex	
	Parameter estimate	Parameter estimate	P. est.	P. est.	Parameter estimate
Intercept	34.11* (1.24)	6.79 (0.24)	0.02 (0.61)	0.03 (0.74)	0.11* (0.82)
Drug condition	-4.27 (-0.08)	4.21 (0.07)	-0.0007 (-0.009)	0.008 (0.10)	0.02 (0.06)
AD use/Sex	-	-	0.02 (0.27)	0.005 (0.06)	-
Drug condition x AD use/Sex	-	-	-0.06 (-0.69)	-0.08 ˆ (-0.84)	-
Block	-1.53 (-0.03)	13.63 ˆ (0.24)	-0.005 (-0.07)	-0.008 (-0.10)	-0.02 (-0.07)
x Drug condition	3.01 (0.03)	-4.39 (-0.04)	-0.008 (-0.05)	-0.01 (-0.08)	-0.007 (-0.01)
x AD use/ Sex	-	-	-0.03 (-0.19)	-0.02 (-0.11)	-
x Drug condition x AD use/Sex	-	-	0.06* (0.31)	0.06* (0.33)	-

Note: SCR: skin conductance response; P. est.: parameter estimate; levels of the within-subjects variable Block were T0 extinction 1 and T1 re-extinction 2. Standardized coefficients between brackets. Dashes in the table denote that this variable did not significantly explain variance in the model for this analysis and was not included in the final model; Diagnosis did not significantly predict fear during T1 fear extinction on any fear outcome.
ˆ 1/6(.05/3) < p < .05 for interactions of Drug condition (CBD/placebo) with moderators * p<1/6 (=.05/3) for interactions of Drug condition (CBD/placebo) with moderators; p<.05 for other effects

There was an interaction Drug condition x AD use on re-extinction of subjective fear to CS+, with no effect in AD nonusers on re-extinction of conditioned fear to the CS+ (CBD vs placebo condition, β=-17.88, p=.06), but in AD users this difference was significant, β=20.88, p=.03. In AD users who received placebo, a trend of a decrease in subjective fear to the CS+ occurred, β=-

16.63, p=0.05, whereas subjective fear to the CS+ did not change in the CBD condition, β=4.25, p>.50, see Figure 4.

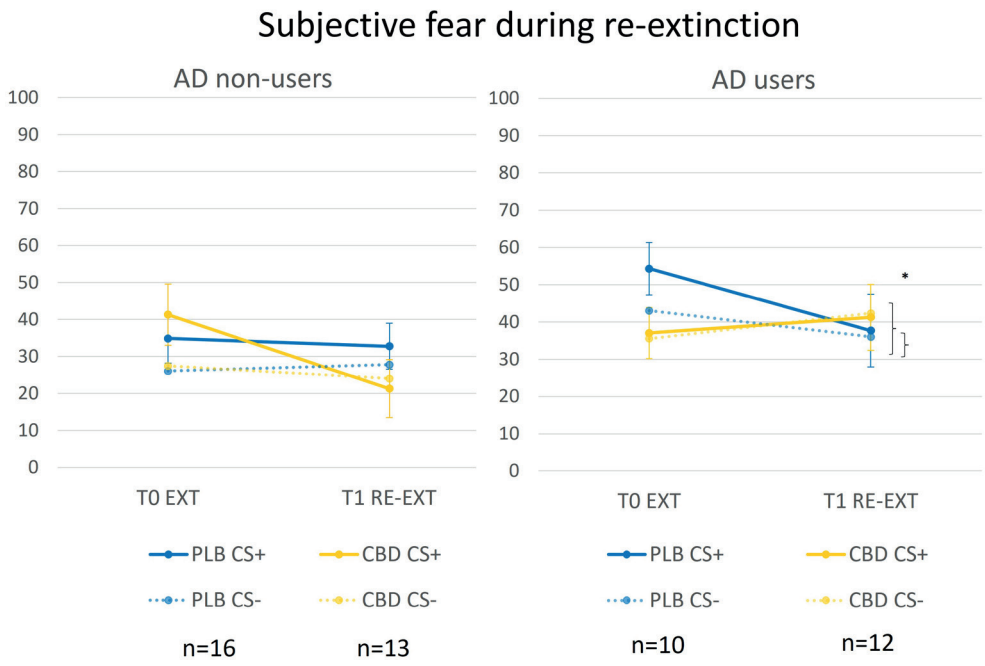

Figure 4. Subjective fear to the CS+ and CS- during extinction at T0 and re-extinction at T1 (after the first study medication ingestion), split by drug condition and AD use.

CBD interfered with safety learning during re-extinction as measured with fear potentiated startle to the CS- in AD users, β=0.05, p=.01, but not in AD nonusers, β=-0.008, p>.50. In addition, CBD interfered with safety learning as measured with fear potentiated startle to the CS- in women, β=0.05, p=.006, but not in men, β=-0.01, p=.23. Because in AD users and in women a similar effect of CBD was observed, we plotted the subgroups jointly in Fig 5 (see 6.S.3.3.2 for elaborate statistics and visualization). Numerically, the interactions of both moderators with CBD seem to result from interference with safety learning by CBD in female AD users, but because of the small groups sizes, no further statistical analysis was conducted.

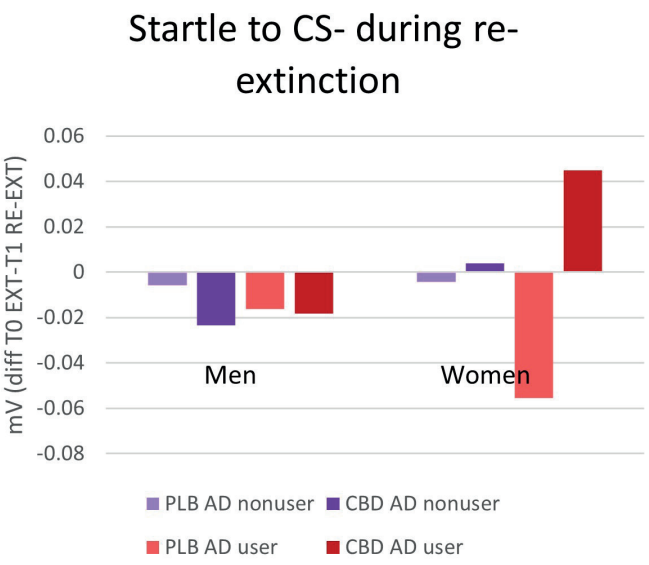

Figure 5. Change in ITI corrected startle responses to the CS- from extinction at T0 (baseline) to re-extinction at T1 (after the first study medication ingestion)

Note: AD: antidepressant; CBD: cannabidiol, PLB: placebo. Positive values indicate an increase from T0 uninstructed extinction to T1 uninstructed re-extinction 2.

Based on these effects of CBD during re-extinction that were seemingly specific to female AD users, post-hoc analyses were conducted in the intent-to-treat sample of the clinical trial the fear condition task was part of. These analyses yielded no significant Drug condition X Sex X AD use interaction effects on primary clinical outcomes, see Table S6.

6.3.5 Subjective drug effects at first ingestion

VASs that measured acute side effects of CBD after the first study medication ingestion at T1 were taken at 0, 60 and 120 minutes. In line with little subjective or side effects of CBD found earlier (Bergamaschi et al., 2011; Chesney et al., 2020), there were no significant differences between the CBD and placebo condition (See 6.S.3.1).

6.4 Discussion

Both in the scientific and popular literature, the exogenous cannabinoid CBD has often been proposed as a drug candidate for the pharmacological treatment of anxiety disorders. In spite

of meagre scientific evidence so far for beneficial effects of this cannabinoid in patients with anxiety disorders (Black et al., 2019; Kwee et al., 2020a; Kwee et al., 2023), CBD is increasingly popular in managing anxiety (Moltke & Hindocha, 2021). To further study its' therapeutic potential we investigated the effects of CBD on fear memory expression and fear re-extinction in a controlled setting, in patients with panic disorder with agoraphobia and social anxiety disorder.

For our first research question we investigated the effect of a single CBD administration on fear memory expression. After study medication ingestion, shock expectancy to the previously conditioned stimulus (CS+) and the non-reinforced cue (CS-) were significantly lower in the CBD condition compared to placebo across the entire sample. Large effect sizes indicated that these differences in shock expectancy were substantial. Further,our findings corroborate previous findings in healthy individuals of lower shock expectancy during a fear reinstatement phase when CBD versus placebo was administered post-extinction (Das et al., 2013). In addition, research with the FAAH inhibitor PF-04457845 showed attenuation of fear memory expression in healthy humans (Mayo et al., 2020). In this study, fear memory expression was measured with change in fear potentiated startle to the CS+ from acquisition to retention. No other fear indices were taken. The result from Mayo et al. (2020) contrasts with our current finding that during retention, physiological measures were unaffected by CBD. Taken together, these findings strongly suggest that treatment with CBD or other FAAH inhibiting compounds holds promise for reduction of aversive fear memory retrieval.

In the current study, CBD was associated with decreased US expectancy ratings on fear expression during retention testing, but did not affect subjective fear and physiological fear responses. In psychopharmacological studies, it is often observed that different indices of fear are differentially affected (e.g., Baas et al., 2002; Heitland et al., 2012; Soeter and Kindt, 2010). Indeed, it has been argued that multiple types of learning involved in fear conditioning lead to systematic dissociations between fear indices (Boddez et al., 2013). US expectancy is considered an index of fear that grasps one component of fear, namely learning about relations between events (Boddez et al., 2013; Chan & Lovibond, 1996). This cognitive process is not specific to fear learning. The current effect of CBD on cognitive threat expectations alone is in line with beneficial effects of (low doses of) CB1 agonists on learning new associations in primarily cognitive tasks in rats (Hill et al., 2006; Pamplona et al., 2006).

For our second research question, we investigated the effect of a single CBD administration on fear re-extinction. Across the sample, fear re-extinction was unaffected by CBD. In contrast to the beneficial effects of CBD on fear memory expression, we observed detrimental effects during further extinction training subsequent to CBD ingestion specifically in female AD users. These contrasting effects of CBD during retention testing and fear re-extinction were not completely

unprecedented. Initial beneficial effects during retention, followed by (potentially) detrimental effects during re-extinction may be in line with the findings of Hill et al. (2006) in rats. They observed that a low dose of a CB1 agonist initially enhanced the learning of new associations to obtain food rewards, but subsequently increased behavior that was only adaptive prior to this learning.

CBD interfered with re-extinction of subjective fear only in AD users, and with safety learning measured with fear potentiated startle only in women and AD users. These potentially detrimental effects did not translate to worse exposure therapy outcome in female AD users who received CBD in the clinical trial the fear condition task was part of (results of these post-hoc analyses are shown in Table S6). Yet, with the current scarcity of human clinical data these preliminary observations warrant attention to such potential interactions with detrimental consequences in further research. These findings may prove to be very relevant, considering that in general, anxiety disorders are more prevalent in women than men (Hallers-Haalboom et al., 2020), and (at least in the USA) women are more likely than men to receive pharmacological treatment for their mental health problems (Terlizzi and Norris, 2021).

Overall, no sex differences have emerged in effects of CBD on unconditioned and conditioned anxiety in rodents from initial studies (Kasten et al., 2019; Franzen et al., 2023; Stern et al., 2015). However, sex-differences have been reported in the desired effects of other psychotropic drugs (Gandhi et al., 2004) and unintended and adverse drug reactions are more common in women across drug classes (Zucker & Prendergast, 2020). Preliminary evidence for such pharmacodynamic effects exist from other pharmaca that, like CBD, indirectly act on cannabinoid receptors. Tabatadze et al. (2015) demonstrated in vitro that the fatty acid amide hydrolase (FAAH) inhibitor URB597 suppressed inhibitory synapses in the hippocampus in female but not in male rats. This could ultimately lead to differential physiological or behavioral output.

Our study focused on the acute effects of CBD on memory expression and re-extinction of previously acquired fear. At baseline we opted for the inclusion of a brief extinction phase immediately after acquisition to answer a different research question (cf. Duits et al., 2021). This may be considered a limitation, because most studies of psychopharmaca to enhance extinction have focused on the first extinction of de novo acquired fear (e.g., Das et al., 2013; Guastella et al., 2007; Klumpers et al., 2012). Research in rats suggests that re-extinction after initial extinction is faster than initial extinction (Quirk, 2002) and that these two types of extinction learning may have distinct susceptibilities to pharmacological intervention (Hart et al., 2009). Therefore, an investigation of drug effects on initial extinction versus re-extinction could yield discordant findings. Re-extinction has been investigated in humans before, and typically a decrease in fear expression over the course of this experimental phase is observed (e.g., Klucken et al., 2016;

Schiller et al., 2010). At face value, exposure therapy in clinical practice is not aimed at recently acquired fears, and hence re-extinction may be a better model for extinction during exposure therapy (Craske et al., 2018), especially in treatment refractory patients.

Finally, no effects of drug condition were expected during the fear conditioning task at baseline (T0), since this preceded the first study medication ingestion. However, in AD users subjective fear to the CS- showed little change during T0 acquisition and extinction in the CBD condition, whereas in the placebo condition it followed a seesaw pattern previously interpreted as 'safety ambiguous' (Leen et al., 2021). However, at the end of T0 the groups did not significantly differ from one another. Thus, we deemed the interpretability of our findings in AD users as not likely to be influenced by the differences between CBD and placebo condition at baseline (T0).

6.4.1 Limitations

Several aspects of this study limit the conclusions that can be drawn. First, data loss due to challenges in implementing an experimental procedure at the participating clinical centers, made our sample size smaller than intended, and also smaller than the original power analysis suggested. Therefore, we had reduced power to investigate potential differences between types of concurrent medication such as antidepressants. Second, potential selection bias (because the task was not applied to the full randomized sample) and relatively small subgroups may have led to the finding of detrimental effects of CBD on safety learning during re-extinction measured with fear potentiated startle in women and in AD users (Figure 5 and 6.S.3.3.2). Because of the small group sizes this finding is very preliminary but its potential clinical significance calls for attention to such interactions in future research.

Third, we investigated drug effects during re-extinction blocks, but did not subsequently investigate potential differences in return of fear at retention some time after the CBD/ placebo administration. This has been investigated in rats, with conflicting findings (Jurkus et al., 2016; Song et al., 2016). This omission in our experimental design precluded determining if CBD administration prior to re-extinction could lead to lasting fear reduction. Fourth, on average, around one fourth of participants' skin conductance responses were excluded from analyses. This might have increased the chance that random noisiness on this outcome obscured any differences between drug conditions. In the study of Das et al. (2013), who administered CBD to healthy participants, skin conductance was also less sensitive than shock expectancy ratings. This may have been due to complete loss of psychophysiological data for some participants and, as a consequence reduced statistical power (Das et al., 2013).

Lastly, our selected 300 mg CBD dose seems to be at the lower end of the most effective

dose range in humans (Kwee et al., 2022a). Therefore, it cannot be completely ruled out that this dose was too low, the more given the large variation in CBD plasma concentrations between individuals (Kwee et al., 2022b). However, the finding of the CBD and placebo condition differing in threat expectancies at retention may argue against this.

6.4.2 Conclusion and recommendations

In conclusion, the current findings provide no evidence for enhancement of fear re-extinction by CBD, which is in line with null findings regarding clinical outcomes in the same patient sample (Kwee et al., 2022b). However, CBD decreased shock expectancy during retention testing, without concurrent effects on direct indices of fear. The exact meaning and significance of this finding with respect to clinical application deserves further investigation.

Finally, findings of the current experimental study provide preliminary evidence that CBD may come with certain disadvantages for women who use antidepressants, although this pattern was not seen in clinical outcomes. The scientific evaluation of drug-drug interactions and of differences in treatment effectiveness between the sexes lags behind the widespread availability and consumption of CBD and other cannabinoid compounds. Covering potential interactions with these factors in future studies that employ CBD in humans may mitigate potential risks and fits with a more personalized approach to pharmacological treatment.

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Conflict of interest

The authors declare that they have no competing interests.

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6.S Supplemental Material

Contents

6.S.1 Supplemental methods

6.S.1.1 In- and exclusion criteria

Supplemental Table 1. Study in- and exclusion criteria.

Inclusion criteria
● Patients between 18 and 65 years with a primary diagnosis of social anxiety disorder or panic disorder with agoraphobia according to DSM-IV criteria.
Exclusion criteria
● Co-morbid psychiatric disorders, i.e. current severe major depressive (Beck Depression Inventory (BDI; Beck et al., 1996) >40)) or bipolar disorder, psychosis, dependence of alcohol and drugs;
● Use of antipsychotic medication;
● Regular daytime use of benzodiazepines;
● Changes in dosing regimen of serotonergic antidepressants <4 weeks prior to study entry;
● Use of recreational drugs <2 months preceding study entry (alcohol and tobacco were permitted);
● Pregnancy or breastfeeding.
● Mental deficiency (IQ <80, as determined by the Nederlandse Leestest voor Volwassenen (NLV; Schmand et al., 1992);
● Inadequate proficiency in Dutch, both verbal and written;
● (A history of) epilepsy, brain damage, cardiac, renal or liver abnormalities;
● History of allergies to medication (adverse reactions or rash);
● (A history of) epilepsy, brain damage, cardiac, renal or liver abnormalities;
● In social anxiety disorder only: Autism traits (Autism Spectrum Quotient (AQ) >32, Hoekstra et al., 2008).

6.S.1.2 The fear conditioning task

Participants were seated in a dimly-lit room while conducting the task. Pictures with blue and yellow backgrounds served as CS+ (or CS-) in the T0 and T1 task. Two different pictures of neutral faces, with orange and purple backgrounds, served as CSs in the T2 task. Which of the two pictures was paired with the US was counterbalanced across patients. Furthermore, by pseudo-randomization two versions with a different order of stimuli presentations and delivery of startle probes were constructed. Regardless of task version, the CS+ and CS- were never presented more than twice consecutively. The task versions were counterbalanced across pa-

tients, to exclude the possibility that characteristics of the stimuli and order effects would bias the results.

The first experimental block at T0 was habituation. Task-irrelevant nuisance that is expected during habituation contains individual differences in baseline responses to the (to be) conditioned and safety stimulus (CS+ and CS-, respectively). Furthermore, habituation of startle responses with repeated presentation of startle eliciting stimuli (see outcome measures) may obscure subsequent learning (Blumenthal, 2005). Before habituation blocks, to signal absence of threat, participants were instructed orally and in writing that no shocks (the unconditioned stimulus (US)) would be administered.

The US was presented within a partial reinforcement schedule to prevent rapid-ceiling level extinction (Lonsdorf et al., 2017). Instructions during acquisition blocks about US reinforcement were implemented to amplify fear learning (Duits et al., 2017; Klumpers et al, 2010) and therefore establish a firm baseline for subsequent fear extinction. As instructions during extinction can enhance extinction learning (Duits et al., 2017; Sevenster et al., 2012), they were not implemented at baseline (T0), to leave room for potential drug effects during re-extinction at T1.

The T2 measurement was 1-2 weeks after the end of the CBD/placebo augmented exposure therapy. At T2, the fear conditioning task was administered with other neutral faces than at T0 and T1, to minimize carry-over of previous learning effects (Torrents-Rodas et al., 2014). Experimental blocks were the same as at T0, except for the addition of a second extinction block (in the same session) that was instructed. Before the first extinction block at T2, participants were informed that they would proceed to the next block. Before the second extinction block they were informed that shocks would no longer be presented.

6.S.1.3 Physiological measures and processing

Baseline artefacts were defined as excessive EMG activity in the window -30 ms before probe up to 20 ms after startle probe presentation (i.e., standard deviation of within-individual activity > the average + 2 standard deviations of activity of all baseline SD's). Latency artefacts were defined as peaks occurring < 45 ms after startle probe presentation or > 99 ms after startle probe presentation. Baseline and latency artefacts were excluded; zero-responses (less than 55% increase in activity from baseline) were included in the calculation of mean startle response per experimental block. Subjects had an average of 7.10% (SD=5.01) missing trials and artefacts.

First and second interval peaks in SCR were not distinguished because of the arbitrariness of boundaries and the risk of underestimating or even overlooking valid responses due to restrictive intervals (Pineles, Orr, & Orr, 2009). Following the recommendations of Lonsdorf et

at al. (2017) SCRs to the US were visually inspected for patients that had zero-responses to the CSs (SCRs falling below a minimum amplitude criterion of 0.01 μS) across all blocks of the fear conditioning task. One patient failed to respond to the US and was excluded from further analysis because the zero-responses were attributed to experimental artefacts (rather than representing a true absence of responding). For the remaining patients magnitude, i.e. the average of responses, including zero-responses (Dawson, et al., 2007), were calculated for each experimental block. In these subjects, an average of 25.16% responses (SD=13.87) were coded as artefacts.

6.S.2 Supplemental results – off-drug group differences in characteristics, contingency learning, baseline fear conditioning

6.S.2.1 Characteristics of sample available at posttreatment (T2)

Data of six patients could not be used for analyses due to data loss due to an administrative error, the task was only administered at one measurement occasion in six patients, an error in the task occurred in five patients, the task was ended prematurely due to illness in one patient, physiological measures were not recorded in two patients, quality of recording of fear potentiated startle was insufficient in two patients, recording of skin conductance was insufficient in one patient. Due to attrition, sample size was further reduced to *n*=42, see Supplemental Table 2.

Supplemental Table 2. Characteristics of participants with subjective measures available at T2 did not significantly differ between drug conditions.

Sociodemographics	Total sample	Placebo	Cannabidiol
Age at study entry, mean (SD)	33.4 (9.1)	34.3 (10.0)	32.6 (8.4)
Female sex	18 (42.9)	9 (45.0)	9 (40.9)
Double nationality	0 (0)	0 (0)	0 (0)
Married or cohabiting	7 (36.8)	4 (44.4)	3 (30.0)
Post-high school education	13 (68.4)	6 (66.7)	7 (70.0)
Currently employed	24 (57.1)	12 (60.0)	12 (54.5)
Clinical variables			
Having received previous treatment	24 (57.1)	12 (60.0)	12 (54.5)
Use of antidepressant medication1	23 (45.1)	10 (38.5)	13 (52.0)
Primary diagnosis social anxiety disorder	20 (47.6)	9 (45.0)	11 (50.0)
Primary diagnosis panic disorder with agoraphobia	22 (52.4)	11 (55.0)	11 (50.0)
Fear Questionnaire (FQ), mean (SD)	48.2 (20.0)	51.2 (15.7)	45.6 (23.2)
Beck Anxiety Inventory (BAI), mean (SD)	24.1 (12.5)	25.3 (12.8)	23.3 (12.6)

Note: Data are n (valid %), unless stated otherwise. 1 Citalopram, escitalopram and sertraline most frequently prescribed.

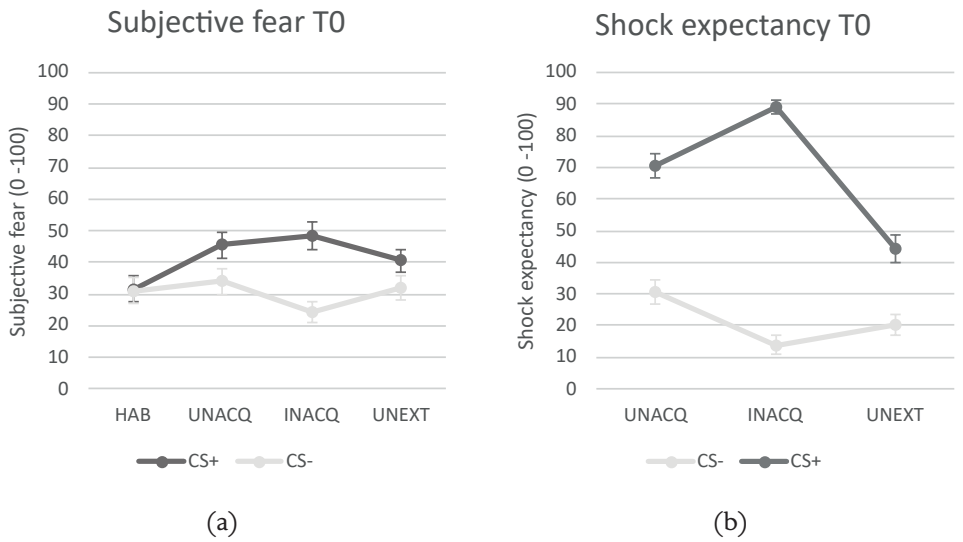
6.S.2.2 Contingency learning at baseline (T0) and at posttreatment (T2)

At T0 and T2, drug conditions did not differ with respect to occurrence of CS plus-US contingency awareness as recorded by the experimenter before instructions about CS-US contingencies were given , $\chi 2=0.58$, $p=.45$ and $\chi 2=0.90$, $p=.64$. A total of 19 (37%) participants were aware of CS-US contingencies after T0 uninstructed acquisition, and a total of 31 (80%) participants were aware of CS-US contingencies after T2 uninstructed acquisition.

6.S.2.3 Fear conditioning at baseline (T0), prior to administration of study medication

The T0 fear conditioning task was administered prior to administration of the study medication. For completeness and to check whether fear acquisition and extinction occurred as expected, we report in 6.S.2.3.1 on the patterns of responding across groups. Differences between drug groups prior to administration of study medication are reported in 6.S.2.3.2.

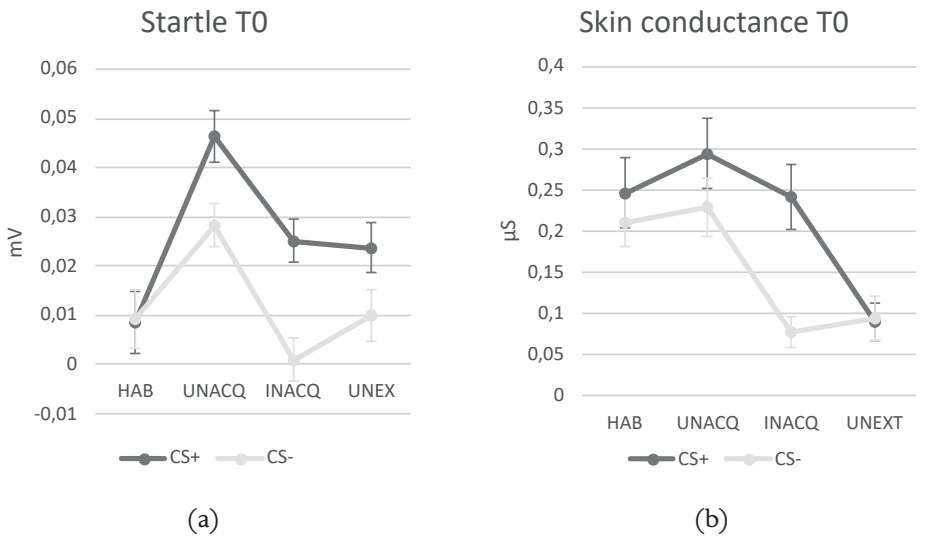
6.S.2.3.1 Patterns of fear conditioning at baseline (T0), prior to administration of study medication Fear to the CS+ showed a linear and quadratic trend, β=30.49, p=.001, β =-5.50, p=.002, respectively. Fear to the CS- showed a linear, quadratic and cubic trend, β=79.00, p=.02, β=-37.25, p<.007, β=5.12, p=.003, see Supplemental Figure 1a. Shock expectancy upon CS+ and upon CS- presentation showed linear trends, β=113.28, p<.001, β=-51.47, p<.0001, and quadratic trends, β=-31.60, p<.001, β= 11.57, p=.001, see Supplemental Figure 1b.



Supplemental Figure 1. Ratings across experimental blocks at T0 (pre-treatment); HAB (habituation); UNACQ (uninstructed acquisition), INACQ (instructed acquisition), UNEXT (uninstructed extinction) for a) subjective fear; b) expectancy of electric shock upon CS+ and CS- presentation. n=51

Fear potentiated startle to the CS+ and CS- showed linear, quadratic and cubic trends, indicated by effects of block (β=0.27 and β=0.24 respectively, ps <.001), block2 (β=-0.11 and β=-0.10 respectively, ps <.001) and block3 (β=0.01 and β=0.01 respectively, ps <.001), see Supplemental Figure 2a.

SCR to CS+ showed a linear-quadratic trend, β= 0.20, p=.005 for block and β=-0.05, p<.0001 for block2, respectively, while SCR to CS- showed linear, quadratic and cubic trends over the course of T0, indicated by effects of block (β= 0.90, p=.005), block2 (β=-0.43, p=.002), and block3 (β=0.06, p=.002), see Supplemental Figure 2b.



(a) (b)

Supplemental Figure 2. Physiological responding across experimental blocks at T0 (pre-treatment); HAB (habituation); UNACQ (uninstructed acquisition), INACQ (instructed acquisition), UNEXT (uninstructed extinction): a) startle responses to the CS+ and to the CS-, corrected for ITI responses, *n*=52; b) skin conductance response upon CS+ and upon CS- presentation, *n*=53.

6.S.2.3.2 **Differences between drug conditions in fear conditioning at baseline (T0), prior to administration of study medication** The check for pre-existing differences between drug groups at baseline, prior to administration of study medication (T0) revealed group effects for the course of subjective fear to the CS-, when comparing users and nonusers of antidepressant (AD) medication, see Supplemental Table 3 and Supplemental Figure 3. Post-hoc tests indicated that subjective fear to the CS- followed a different trajectory in users of AD medication who were in the CBD condition, compared to those in the placebo condition, β= 104.87, p=.02. In the CBD condition, subjective fear to the CS- was not responsive to fear conditioning, instructions about the CS-US contingency, or fear extinction, whereas in the placebo condition, subjective fear to the CS- followed a seesaw pattern.

The trajectories of nonusers of AD medication did not differ between drug conditions, β= -44.32, p>.50. No differences between drug conditions were observed at baseline (T0) fear conditioning on other outcomes than subjective fear to the CS-.

Supplemental Table 3. Differences between drug conditions during fear conditioning at baseline (T0).

CS+	Subjective fear (n=51)	Shock expectancy (n=51) Model AD use	Startle (n=52)	SCR (n=53)
	Parameter estimate	Parameter estimate	Parameter estimate	Parameter estimate
Intercept	-12.96	-8.88	-0.21*	-0.07
Drug condition	14.2	-15.65	0.11	-0.13
AD use	-	12.69	-	-
Experimenter effects	-	-	Ind. 1: -0.02* Ind. 2: -0.01* Ind. 4: -0.02*	Ind. 1: -0.28*
Block	64.7	-27.25*	0.34*	0.66
x Drug condition	-28.93	-5.53	-0.14	0.05
x AD use	-	-3.79*	-	-
Block2	-21	104.17*	-0.13*	-0.27
x Drug condition	13.26	18.59	0.05	0.009
Block3	2.07	- -	0.02*	0.03
x Drug condition	-1.78	-	-0.01	-0.003
x Experimenter effects	-	-	-	Ind. 1: 0.003*
CS-	Subjective fear (n=51) Model AD use	Shock expectancy (n=51)	Startle (n=52) Model diagnosis	SCR (n=53)
	Parameter estimate	Parameter estimate	Parameter estimate	Parameter estimate
Intercept	-0.1	62.31*	-0.22*	-0.56
Drug condition	-54.79	16.97	0.04	0.63

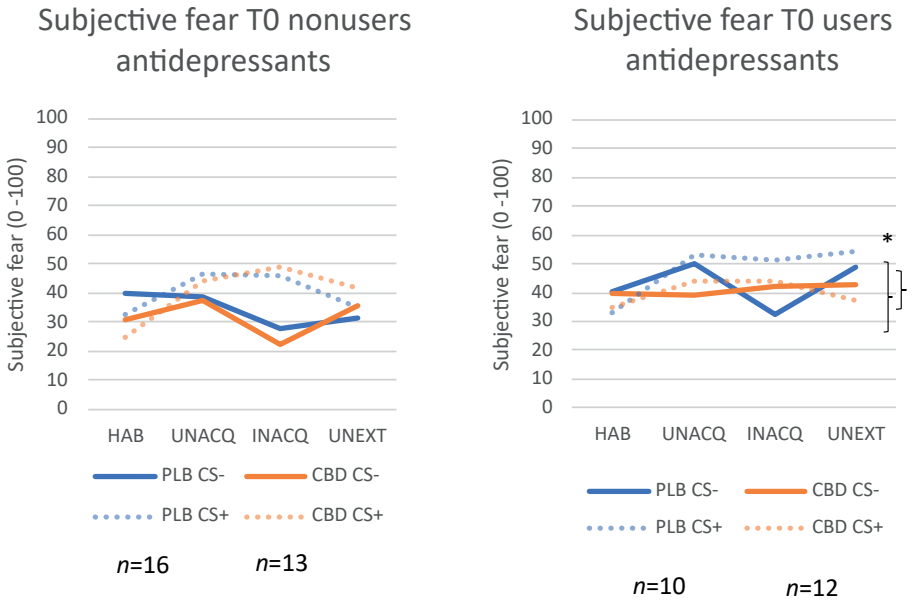
Supplemental Table 3. Differences between drug conditions during fear conditioning at baseline (T0).

AD use/diagnosis	-67.08	-	0.13	-
Drug condition x AD use	166.95	-	-	-
Experimenter effects	Ind. 3: -20.28* Ind. 4: 19.18*	-	Ind. 1: -0.02* Ind. 4: -0.01*	Ind. 1: -0.13* Ind. 4: -0.09*
Block	57.13	8.38	0.38*	1.36*
x Drug condition	73.25	6.5	-0.06	-1
x AD use/diagnosis	108.83	-	-0.21^	-
x Drug condition x AD use	-259.11^	-	-	-
Block2	-28.66	-39.12*	-0.17*	-0.62*
x Drug condition	-31.84	-25.2	0.03	0.42
x AD use/diagnosis	-47.44	-	0.10*	-
x Drug condition x AD use	116.82^	-	-	-
Block3	3.97	-	0.02*	0.08*
x Drug condition	4.31		-0.004	-0.05
x AD use/diagnosis	6.38		-0.01*	-
x Drug condition x AD use	-15.79*		-	-

Note: Ind.: indicator variable; SCR: skin conductance response. Levels of the time variable Block were habituation, uninstructed and instructed acquisition, uninstructed extinction. Block2 and Block3: Second- and third order polynomials for experimental block. The dashes in the table indicate that variables did not significantly explain variance and were not included in the final model; Sex was not a significant predictor of fear during T0 on any fear outcome. The interaction Drug condition x Antidepressant use for subjective fear (gray shaded in Table S3) is illustrated in Figure S3.

ˆ 1/6(.05/3) < p < .05 for interactions of Drug condition with moderators

* p < 1/6 (=.05/3) for interactions of Drug condition with moderators; p<.05 for other effects



(a) (b)

Supplemental Figure 3. Subjective fear to the CS+ and CS- across experimental blocks at T0 (HAB (habituation); UNACQ (uninstructed acquisition), INACQ (instructed acquisition), UNEXT (uninstructed extinction)) split by drug condition and use of AD medication.

Note: PLB: placebo; CBD: cannabidiol. Model estimates of model with indicator variable 1 for experimenter. Indicator variable for experimenter (1-4) affected level of subjective fear (a main effect); we did not examine potential interactions with drug condition.

* p<.05

6.S.3 Supplemental results – acute effects of CBD at (T1)–

6.S.3.1 Subjective drug effects at T1

After the first study drug ingestion, after 0, 60 and 120 min, patients rated subjective mood, anxiety, alertness, feeling calm, feeling high, apathic, paranoid, hungry, dizzy. Model based estimates across groups are listed in Supplemental Table 4. CBD had no acute side effects (see Supplemental Table 5). There were significant main effects of AD use on mood, which are illustrated in Supplemental Figure 4. Moderators Sex and Diagnosis did not predict any subjective drug effect.

Supplemental Table 4. Model based estimates of subjective drug effects 0, 60 and 120 min after first CBD or placebo ingestion across groups.

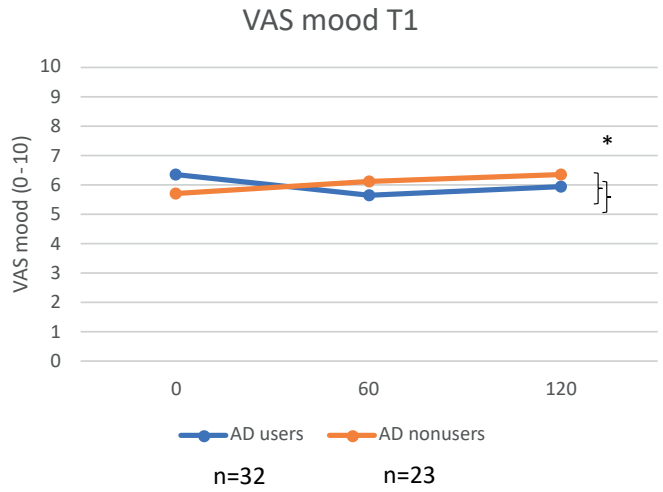
	Mood	Alert	Calm	High	Anxious	Apathic	Paranoid	Hungry	Dizzy
Mean (SD)	6.37 (1.87)	5.57 (1.54)	5.22 (1.67)	0.6 (1.13)	T 0: 4.12 (2.32) T 60: 3.00 (2.18) T 120: 2.67 (2.07)	1.84 (2.28)	0.59 (1.12)	2.34 (1.83)	1.24 (1.59)

Note: Time and Time^2 significantly predicted drug effects only for VAS anxiety, β=-2.31, p=.004 and β=0.40, p=.049, respectively.

Supplemental Table 5. Subjective drug effects 0, 60 and 120 min after first CBD or placebo ingestion.

	Mood	Alert	Calm	High	Anxious	Apathic	Paranoid	Hungry	Dizzy
	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.
Intercept	5.63*	5.85*	3.63*	-1.47	6.08*	3.54*	0.86	3.21*	0.37
Drug condition	-0.2	0.86	-0.74	0.23	-0.58	-1.47	0.51	-0.07	0.74
AD use	3.09*	-	-	-	-	-	-	-	-
Experimenter ind. 1	-0.48	-0.07	-1.22	3.87*	-0.43	-1.35*	-	-	-1.33*
Experimenter ind. 2	-1.16	-1.01*	-0.45	1.14	0.06	-1.18	-	-	-0.74
Experimenter ind. 3	2.95*	0.61	2.25	-1.61	-2.65*	2.98	-	-	-1.36*
Experimenter ind. 4	-0.06	-0.16	-0.74	2.48	-1.37*	-0.66	-	-	-0.01
Time	0.46	-0.01	1.39	1.92*	-2.62*	-1.28	-0.25	-0.79	1.54*
x Drug condition	0.81	-0.25	1.52	-0.2	0.61	1.03	-0.45	-1.09	-1.28
x AD use	-3.12*	-	-	-	-	-	-	-	-
x Experimenter effects	-	-	Ind. 3: -2.32*	Ind. 1: -3.88*	-	-	-	-	-
Time2	-0.04	-0.06	-0.22	-0.4	0.55	0.3	0.07	0.25	-0.34
x Drug condition	-0.18	0.03	-0.39	0.02	-0.3	-0.2	0.03	0.27	0.29
x AD use	0.64*	-	-	-	-	-	-	-	-
x Experimenter effects	-	-	-	Ind. 1: 0.92*	-	-	-	-	-

Note: P. est.: parameter estimate; Ind.: indicator variable, AD: antidepressant. Time2: Second order polynomial for experimental block. The dashes in the table indicate that variables did not significantly explain variance and were not included in the final models. n=55; moderators Sex and Diagnosis did not predict any subjective drug effect.
* p<.05



Supplemental Figure 4. VAS mood at T1 (0, 60 and 120 min after first medication ingestion) in antidepressant (AD) users and nonusers.

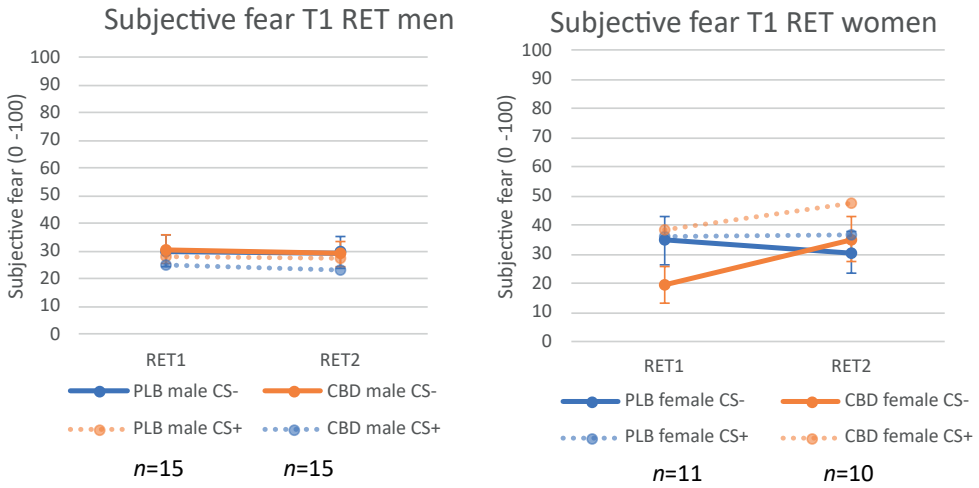
Note: VAS: visual analogue scale. Model estimates of model with indicator variable 1 for experimenter.
* p<.05

6.S.3.2 Fear expression under ambiguous and imminent threat of shock – T1 retention 1 and retention 2

6.S.3.2.1 Patterns of fear expression at T1 retention 1 and retention 2 The main effect of CBD on fear expression is illustrated in the main paper. In 6.S.3.2.2. we show the Sex X Drug condition interaction (dark grey shaded in Table 3 in the main paper). In this section, patterns of fear expression across groups are reported.

Across groups, fear to the CS+ (M= 31.57, SD= 26.11) and CS- (M=29.99, SD=25.10) and shock expectancy upon CS+ (M= 24.74, SD=26.28) and CS- presentation (M=20.63, SD=24.18) did not change from T1 retention 1 to retention 2, β=1.22, p=.57 and β=1.59, p=.44, β=-7.08, p=.14, β=-4.98, p=.25, respectively. Similarly, across groups, fear potentiated startle to the CS+ (M=0.01, SD=0.03) and CS- (M=0.01, SD=0.03) and SCR to the CS+ (M=0.12, SD=0.24) and CS- (M=0.10, SD=0.16) did not change from T1 retention 1 to retention 2, β= 0.009, p=.35, β= -0.001, p=.90, β=0.02, p=.37, β=-0.02, p=.62, respectively.

6.S.3.2.2 Acute effects of CBD on fear expression at T1 retention 1 and retention 2



Supplemental Figure 5. Subjective fear to the CS+ and CS- split by drug condition and sex during RET1 (T1 retention) and RET1 (T1 retention 2), after first study medication ingestion.

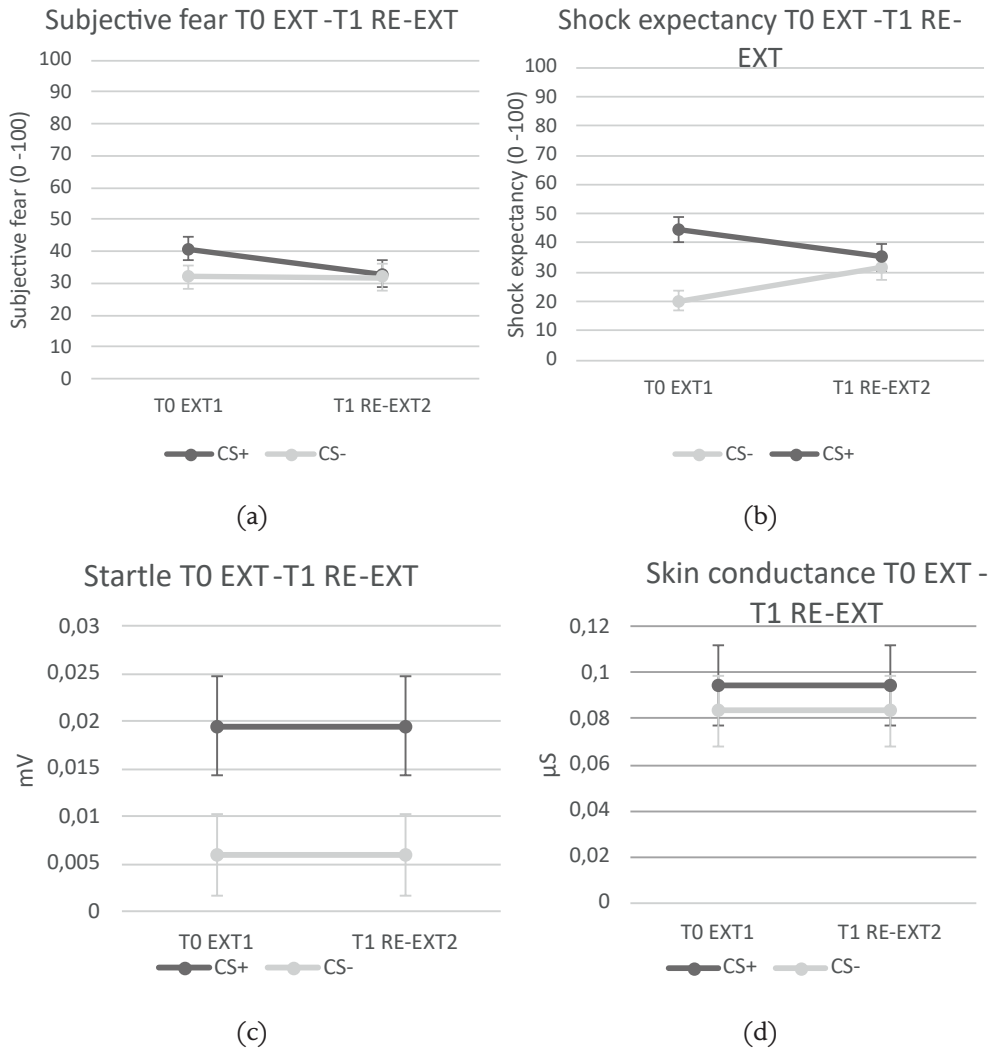
Note: PLB: placebo; CBD: cannabidiol

6.S.3.3 Fear re-extinction – T0 uninstructed extinction to T1 uninstructed re-extinction 2

There were no main effects of CBD on the T0 extinction -T1 re-extinction comparison. Interactions with Sex and AD use are discussed in the main paper and 6.S.3.3.2. For completeness, the patterns of responding across groups are reported in 6.S.3.3.1.

6.S.3.3.1 Patterns of fear extinction from T0 uninstructed extinction to T1 uninstructed extinction 2 Indicative of fear re-extinction, fear to the CS+ decreased from T0 uninstructed extinction (M= 40.82, SD=27.03) to T1 uninstructed re-extinction 2 (M=32.86, SD=29.20), β=-7.96, p=.03, see Figure 6a. Shock expectancy upon CS+ presentation (M=39.84, SD=25.76) and fear to the CS- (M= 31.95, SD=25.28) did not change from T0 uninstructed extinction to T1 uninstructed re-extinction 2, β=-9.25, p=.07 and β=-0.03, p=.99, respectively. Shock expectancy upon CS- presentation increased from T0 uninstructed extinction (M= 20.33, SD= 23.46) to T1 uninstructed re-extinction 2 (M= 31.77, SD= 31.23), β=11.43, p=.01, see Supplemental Figure 6b. Fear potentiated startle and SCR to the CS+ (M= 0.02, SD= 0.04 and M= 0.09, SD=0.13)

and to the CS- (M=0.006, SD=0.03 and M= 0.08, SD=0.11) did not change from T0 uninstructed extinction to T1 uninstructed re-extinction 2, β=-0.008 , p=.19 , β=-0.01, p=.71, and β=-0.008, p=.18 , β=-0.02, p=.29, respectively, see Supplemental Figure 6c and 6d.

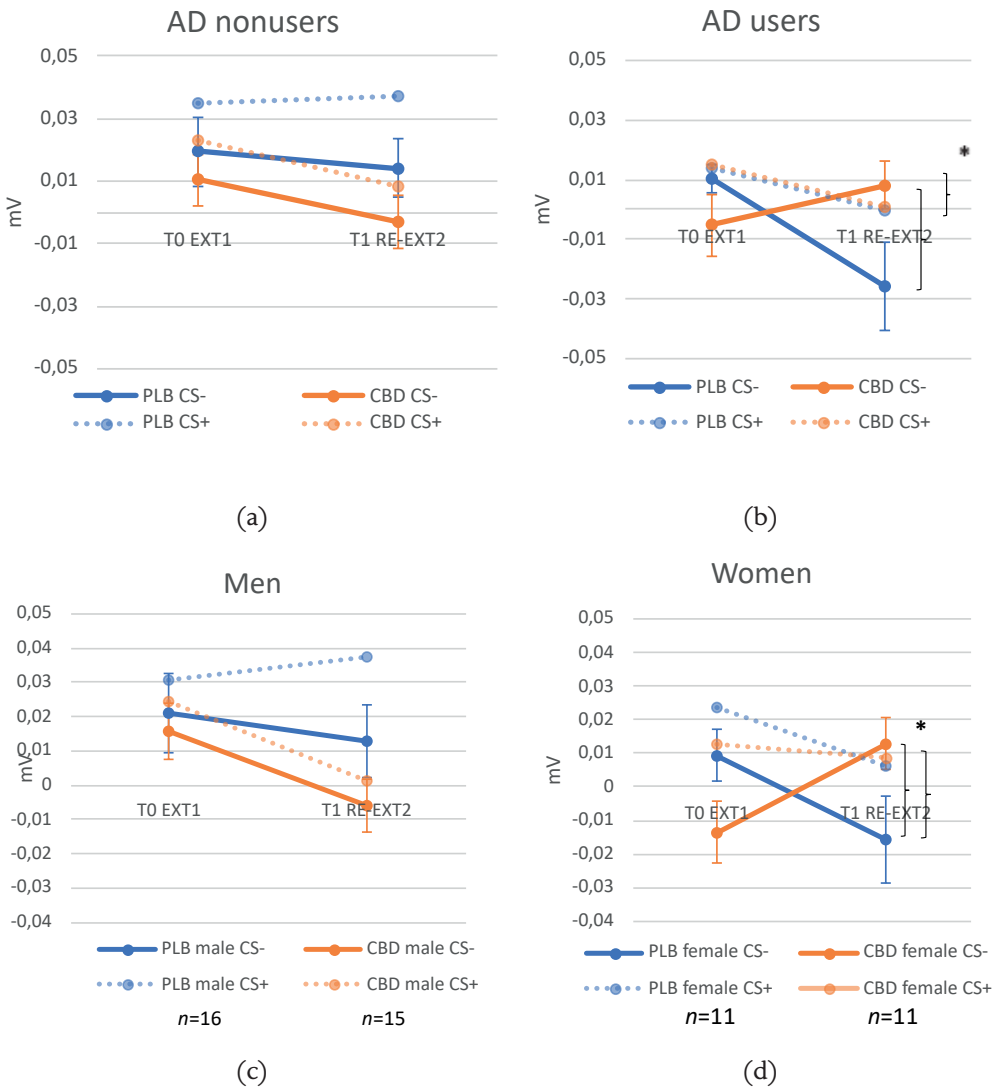


Supplemental Figure 6. Patterns of a) subjective fear, b) shock expectancy, ITI-corrected startle (c) and skin conductance (d) to CS+ and CS- during fear re-extinction from T0 EXT 1 (T0 uninstructed extinction) to T1 RE-EXT2 (T1 uninstructed re-extinction 2). n=51

6.S.3.3.2 Acute effects of CBD on fear re-extinction from T0 uninstructed extinction to T1 uninstructed re-extinction 2 In AD nonusers there was no difference between drug conditions in change in fear potentiated startle to CS- from T0 extinction to T1 re-extinction 2, β=-0.008, p>.50, see Supplemental Figure 7a. Similarly, in men, no differences were observed, β=-0.01, p=.23, see Supplemental Figure 7c.

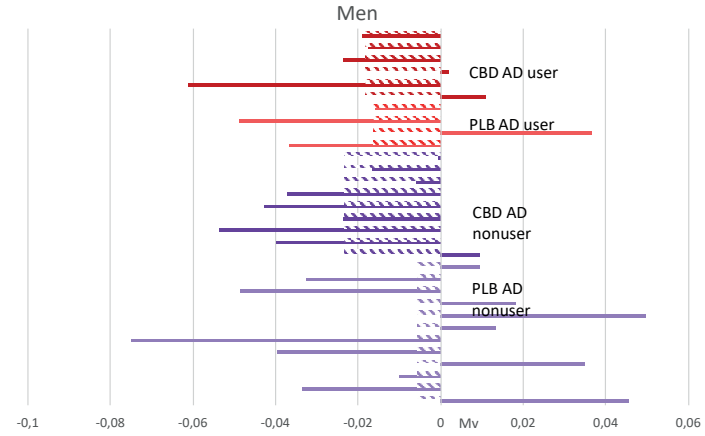
In users of AD medication CBD and placebo condition differed, β=0.05, p=.01. In the placebo condition fear potentiated startle to the CS- decreased from T0 extinction to T1 re-extinction 2, β=-0.04, p=.02. In the CBD condition, no further safety learning occurred after drug ingestion, β=0.01, p=.29, see Supplemental Figure 7b. In women, too, CBD and placebo condition differed, β=0.05, p=.006. In the placebo condition no further safety learning occurred after extinction at T0, β=-0.03, p=.09, while in the CBD condition, startle responses to the CS- increased after extinction at T0, β=0.03, p=.02, see Supplemental Figure 7d.

Considering the detrimental effects of CBD in women and in AD users during fear re-extinction shown by our statistical analyses, we explored whether these effects could be specific to individuals with a combination of these characteristics. In Supplemental figure 8 is plotted change from T0 uninstructed extinction to T1 uninstructed re-extinction 2 per individual.

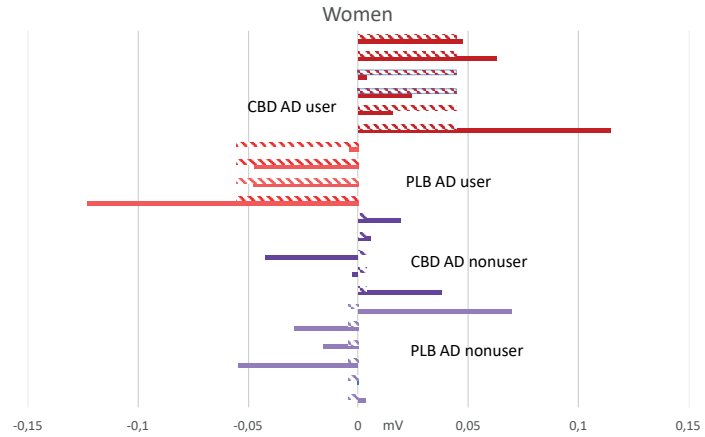


Supplemental Figure 7. ITI corrected startle responses to CS+ and CS- during T0 EXT 1 (T0 uninstructed extinction) to T1 RE-EXT2 (T1 uninstructed re-extinction 2; after the first study medication ingestion) split by drug condition and AD use (a, b) and drug condition and sex (c,d).

Note: PLB: placebo, CBD: cannabidiol.



(a)



(b)

CBD + AD user
Placebo + AD user
CBD + AD nonuser
Placebo + AD nonuser

Supplemental Figure 8. Change in ITI-corrected startle responses from T0 uninstructed extinction to T1 uninstructed re-extinction 2 split by drug condition and use of antidepressant medication for a) men and b)women.

Note: AD: antidepressant; CBD: cannabidiol. Positive values indicate an increase in startle responses to the CS- from T0 extinction to T1 re-extinction. Diagonal-striped bars represent subgroup averages.

6.S.3.3.3 Post-hoc analyses on clinical outcomes based on acute effects of CBD during fear re-extinction

Supplemental Table 6. Post-hoc analyses examining Drug condition X Sex X AD use interactions effects on primary clinical outcomes in the intent-to-treat sample.

n=78	Fear Questionnaire (FQ) Parameter estimate	Beck Anxiety Inventory (BAI) Parameter estimate
Intercept	50.28*	20.15*
Drug condition	4.45	2.9
AD use	-1.54	-0.47
Sex	1.35	0.21
Drug condition X AD use	-21.4	-4.77
Drug condition X Sex	-4.37	6.82
AD use X Sex	2.09	8.19
Drug condition X AD use X Sex	13.29	-9.41
Time		
Intercept	-1.70*	-0.59*
X Drug condition	-0.73	0.21
X AD use	0.44	0.22
X Sex	-0.3	-0.16
X Drug condition X AD use	1.05	-0.44
X Drug condition X Sex	1.22	-0.45

Supplemental Table 6. Post-hoc analyses examining Drug condition X Sex X AD use interactions effects on primary clinical outcomes in the intent-to-treat sample.

n=78	Fear Questionnaire (FQ) Parameter estimate	Beck Anxiety Inventory (BAI) Parameter estimate
X AD use X Sex X Drug condition X AD use X Sex	-0.38 -0.23	-0.64 1.45
Time2 Intercept X Drug condition X AD use X Sex X Drug condition X AD use X Drug condition X Sex X AD use X Sex X Drug condition X AD use X Sex	0.03* 0.02 -0.02 -0.0002 -0.02 -0.03 0.02 -0.004	0.01* -0.005 -0.009* 0.002 0.01 0.01 0.02 -0.03

Note: AD: antidepressant. Assessment points were T0 (0 weeks), T1 (5 weeks), T2 (9 weeks), T3 (21 weeks), and T4 (34 weeks). These planned assessment times were not identical to actual assessment times (the latter were modelled in the analyses). Time2: Second order polynomial for time. * $p<.05$

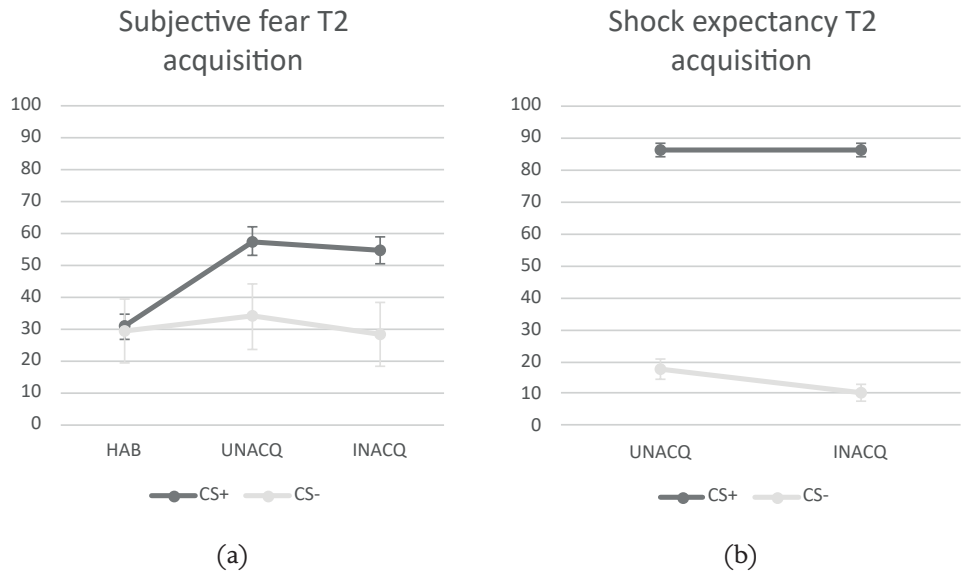
6.S.4 Supplemental results – delayed effect of CBD after repeated administration during posttreatment (T2) fear extinction-

The effect of repeated CBD administration on fear extinction was investigated with T2 Blocks instructed acquisition, uninstructed extinction and instructed extinction, main effects of Drug condition and moderator variables and all interactions modelled. These results are discussed in 6.S.4.3. 6.S.4.1 describes the effect of CBD on T2 fear acquisition and 6.S.4.2 patterns of responding across groups, regardless of drug effects.

6.S.4.1 Patterns of fear acquisition after repeated administration of study medication from T2 habituation, uninstructed to instructed acquisition

The effect of CBD on T2 fear acquisition was not one of our research questions. As a check whether fear acquisition took place prior to T2 extinction (for which CBD-placebo differences are described in the main paper and in 6.S.4.3), we here report patterns of fear acquisition across groups.

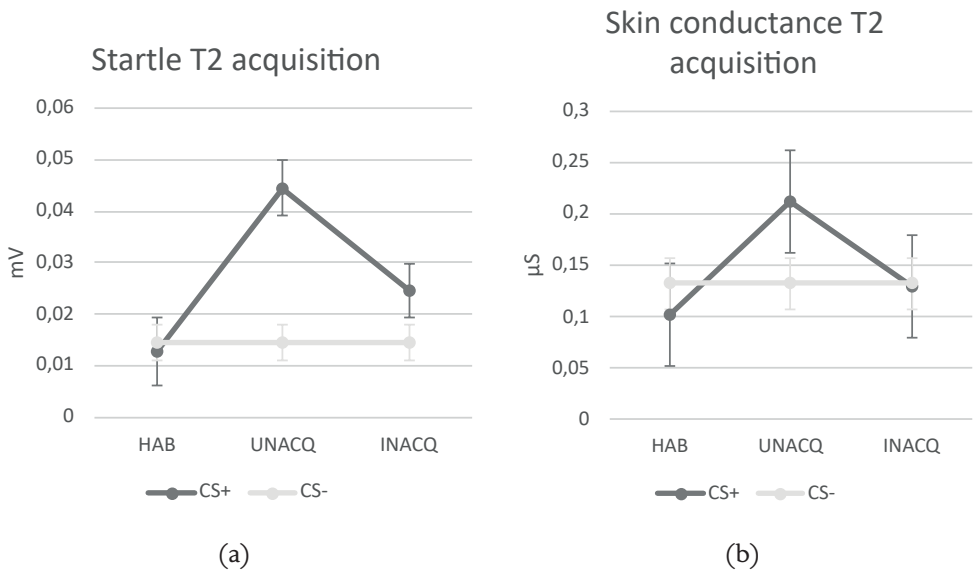
During T2 acquisition, fear responses to the CS+ and to the CS- had linear-quadratic patterns, block: β=70.64, p<.001 and β=18.82, p=.07, block2: β=-14.69, p<.001 and β=-4.82, p=.04, respectively, see Supplemental Figure 9a. Shock expectancy to the CS+ (M= 86.04, SD=13.07) did not change from uninstructed to instructed acquisition, β=4.17, p=.15. Shock expectancy to the CS- decreased from uninstructed to instructed acquisition, β=-7.33, p=.004, see Supplemental Figure 9b.



Supplemental Figure 9. Ratings during acquisition at T2 (posttreatment) blocks HAB (habituation), UNACQ (uninstructed acquisition), INACQ (instructed acquisition) for a) subjective fear to the CS+ and CS-; b) expectancy of electric shock upon CS+ and CS- presentation. *n*=42

During T2 acquisition, startle responses to the CS+ had a linear-quadratic pattern, block: β=0.11, p<.001 and block2: β=-.03, p<.001. Startle responses to the CS- (M=0.01, SD=0.03) did not change across experimental blocks, block: β=0.02, p=.26 block2: β=-0.007, p=.12, see Supplemental Figure 10a. Skin conductance responses to the CS+ had a linear-quadratic pattern, block: β=0.40, p=.009 block2: β=-0.10, p=.009, whereas skin conductance responses to the CS- did not change across experimental blocks habituation, uninstructed and instructed acquisition (M=0.13, SD=.03t), block: β=0.13, p=.28, block2: β=-0.04, p=.20, see Supplemental Figure 10b.

As expected, fear acquisition took place on all outcome measures at posttreatment (T2), however, compared to baseline (T0), skin conductance responses were relatively low (compare Supplemental Figure 10b with Supplemental Figure 2b). However, there was still room for fear extinction, given that skin conductance responses upon CS+ decreased during T2 extinction (see 6.S.4.2).



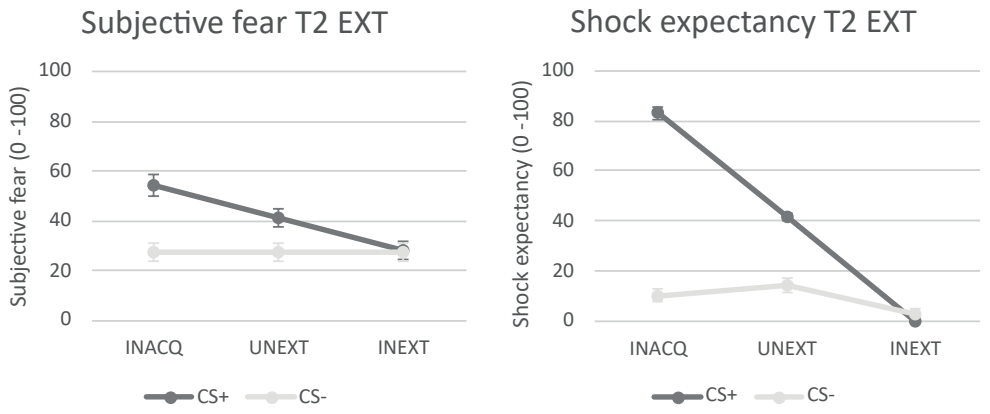
(a) (b)

Supplemental Figure 10. Physiological responses during acquisition at T2 (posttreatment) blocks HAB (habituation), UNACQ (uninstructed acquisition), INACQ (instructed acquisition: a) ITI-corrected startle responses to the CS+ and CS-; b) skin conductance responses upon CS+ and CS- presentation. *n*=47

6.S.4.2 Patterns of fear extinction after repeated administration of study medication from T2 instructed acquisition, uninstructed to instructed extinction

There were no main effects of CBD during fear extinction at posttreatment (T2 instructed acquisition, uninstructed to instructed extinction). The Drug condition X Diagnosis interaction for subjective fear is discussed in 6.S.4.3. For completeness, the patterns of responding across groups are reported in this section.

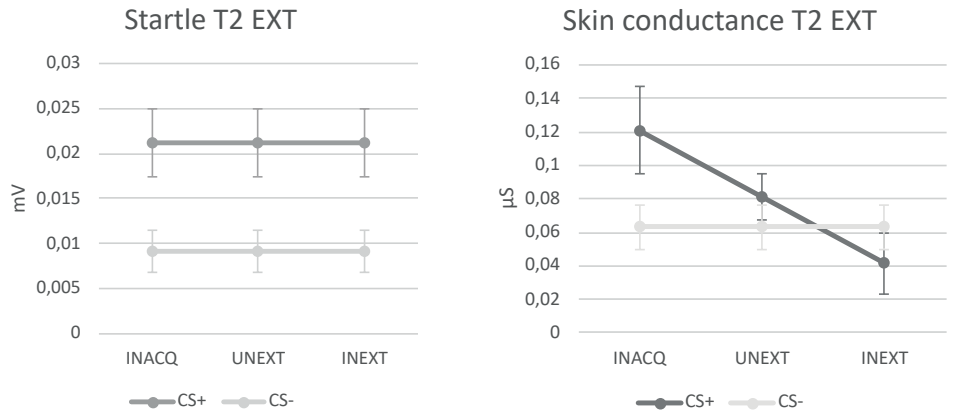
During T2 extinction, subjective fear to the CS+ decreased, β=-13.00, p< .0001, whereas subjective fear to CS- did not change, β=-1.48, p=.26, see Supplemental Figure 11a. Expectancy of shock upon CS+ presentation and upon CS- presentation decreased, β=-41.58, p<.0001 and β=43.46, p=.01, see Figure 11b. In addition, expectancy of shock upon CS- presentation had a linear-quadratic pattern, β=-7.85, p=.006.



(a) (b)

Supplemental Figure 11. Subjective ratings across experimental blocks at T2 (post-treatment) extinction: INACQ (instructed acquisition), UNEXT (uninstructed extinction) and INEXT (instructed extinction) for a) subjective fear to the CS+ and CS-; b) expectancy of electric shock upon CS+ and CS- presentation. *n*=42

Across groups, startle responses to CS+ (M=0.02, SD=0.03) and startle responses to CS- (M= 0.009, SD=0.02) did not change during T2 extinction, for block and block2 β=-0.006, p=.87 and β= -0.06, p=.31, for block2 β=0.0004, p=.94 and β=0.008, p=.29, respectively, see Supplemental Figure 12a. Across groups, SCR to CS+ decreased during T2 extinction, β=-0.04, p=.03, whereas SCR to CS- did not change during T2 extinction, β= -0.02, p=.17. see Supplemental Figure 12b.



(a) (b)

Supplemental Figure 12. ITI-corrected startle responses a) and skin conductance b) to CS+ and CS- across experimental blocks at T2 (post-treatment) extinction: INACQ (instructed acquisition), UNEXT (uninstructed extinction) and INEXT (instructed extinction. *n*=47

6.S.4.3 Delayed effect of CBD after repeated administration on fear extinction from T2 instructed acquisition, uninstructed to instructed extinction

The analyses focused on the amount of extinction that was achieved at extinction at T2 (posttreatment), compared to fear during fear acquisition at T2. Across the sample there were no significant effects of repeated CBD administration on this comparison.

For subjective fear to the CS- there was one significant interaction that survived Bonferroni correction. This was a complex interaction that included Drug condition, Diagnosis and Block2 (quadratic term of Block), which is shaded gray in Supplemental Table 7 (see also Figure S13). Post hoc tests yielded no significant differences between CBD and placebo condition within diagnoses in change in subjective fear to the CS- across the different blocks (from instructed acquisition to uninstructed extinction, p=.31 and p=.17, from uninstructed extinction to instructed extinction, p=.07 and p=.052, and from instructed acquisition to instructed extinction, p=.31 and p=.17). Sex and AD use did not significantly predict fear during T2 fear extinction.

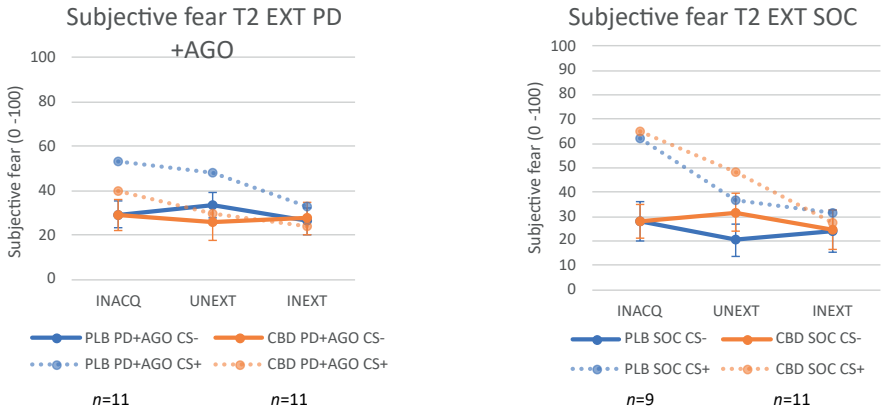
Supplemental Table 7. Fear outcomes during T2 fear extinction.

CS+	Subjective fear β (n=42)	Shock expectancy β (n=42)	Startle β (n=47)	SCR β (n=47)
	Parameter estimate	Parameter estimate	Parameter estimate	Parameter estimate
Intercept	121.35 (4.25)	257.60* (6.53)	0.02 (0.43)	0.43 (2.69)
Drug condition	-30.44 (-0.54)	61.58 (0.80)	0.04 (0.52)	0.5 (1.61)
Block	-26.6 (-0.76)	-108.60* (-2.26)	0.02 (0.32)	-0.15 (-0.79)
x Drug condition	13.96 (0.20)	-41.83 (-0.44)	-0.04 (-0.43)	-0.23 (-0.61)
Block2	1.75 (0.40)	11.50 (1.44)	-0.003 (-0.55)	0.02 (0.63)
x Drug condition	-1.84 (-0.22)	6.34 (0.40)	0.007 (0.60)	0.03 (0.57)
CS-	Subjective fear β (n=42)	Shock expectancy β (n=42)	Startle β (n=47)	SCR β (n=47)
	Parameter estimate	Parameter estimate	Parameter estimate	Parameter estimate
Intercept	-50.64 (-2.14)	-42.85 (-2.65)	-0.07 (-1.67)	-0.6 (-4.30)
Drug condition	117.23 (2.53)	-4.65 (-0.15)	0.35 (4.50)	0.31 (1.15)
Diagnosis	172.41* (3.72)	-	-	1.34* (4.88)
Drug condition x Diagnosis	-287.41ˆ (-12.16)	-	-	-
Block	43.55* (1.51)	42.93 (2.18)	0.04 (0.78)	0.35 (2.05)
x Drug condition	-63.41 (-1.12)	1.03 (0.03)	-0.19 (-2.03)	-0.15 (-0.45)
x Diagnosis	-92.27* (-1.63)	-	-	-0.67* (-2.01)
Drug condition x Diagnosis	153.95ˆ (2.40)	-	-	-
Block2	-5.64* (1.57)	-7.78 (-1.21)	-0.005 (-0.77)	-0.04 (-2.05)
x Drug condition	8.05 (1.14)	-0.13 (-0.02)	0.03 (2.15)	0.02 (0.41)
x Diagnosis	11.47* (1.63)	-	-	0.08* (1.93)
Drug condition x Diagnosis	-19.33* (-2.42)	-	-	-

Note: SCR: skin conductance response. Levels of the within-subjects variable Block were T2 instructed acquisition, T2 uninstructed extinction and T2 instructed extinction. Block2: Second-order polynomial for experimental block. Standardized coefficients between brackets. The dashes in the table denote that this variable did not significantly explain variance in the model for this analysis and was not included in the final model; Sex and antidepressant use (and diagnosis for CS+) did not significantly predict fear during T2 fear extinction on any fear outcome.

ˆ $1/6(.05/3) < p < .05$ for interactions of Drug condition (CBD/placebo) with moderators

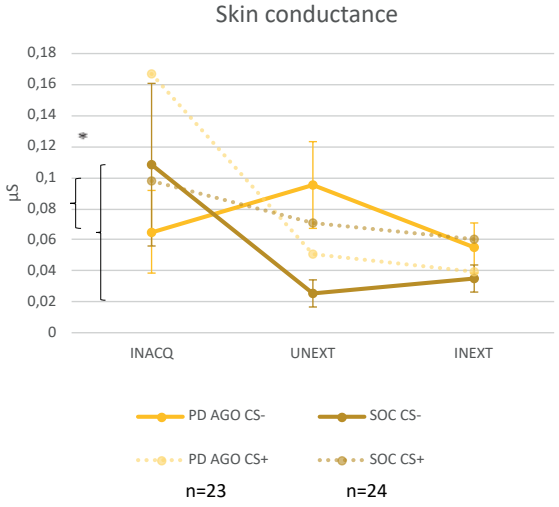
* $p<1/6$ (=.05/3) for interactions of Drug condition (CBD/placebo) with moderators; $p<.05$ for other effects



(a) (b)

Supplemental Figure 13. Subjective fear to CS+ and CS- during extinction at T2 (INACQ: instructed acquisition; UNEXT: uninstructed extinction; INEXT: instructed extinction) split by drug condition and diagnosis.

Note: PLB: placebo; CBD: cannabidiol; PD+AGO: panic disorder with agoraphobia; SOC: social anxiety disorder.



Supplemental Figure 14. Skin conductance responses to CS+ and CS- during extinction at T2 (INACQ: instructed acquisition; EXT1: uninstructed extinction; EXT2: instructed extinction) split by diagnosis.

Note: PLB: placebo; CBD: cannabidiol; PD+AGO: panic disorder with agoraphobia; SOC: social anxiety disorder.

In contrast to the analyses at T0 and T1, we observed effects of diagnosis on fear extinction

at posttreatment (T2), regardless of drug condition. In social anxiety disorder patients the rate of extinction from instructed acquisition to uninstructed extinction was higher compared to panic disorder patients, see Figure S14.

6.S.5 Results on additional subjective ratings at T0, T1 and T2

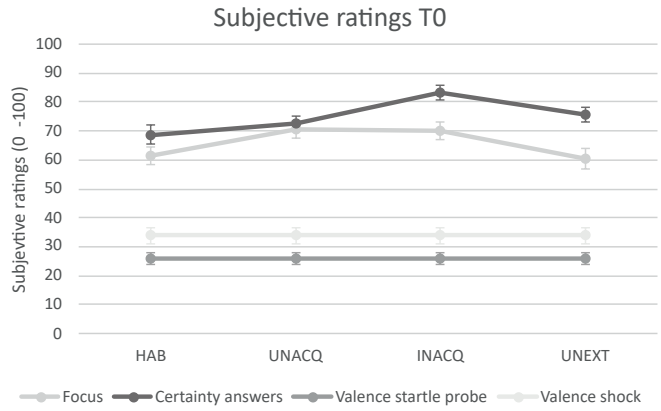
In this section are discussed results of supplementary analyses on focus, certainty of answers and valence ratings of startle probes and of electric shocks at T0, T1, and T2.

6.S.5.1 Patterns of additional subjective ratings at T0, T1 and T2

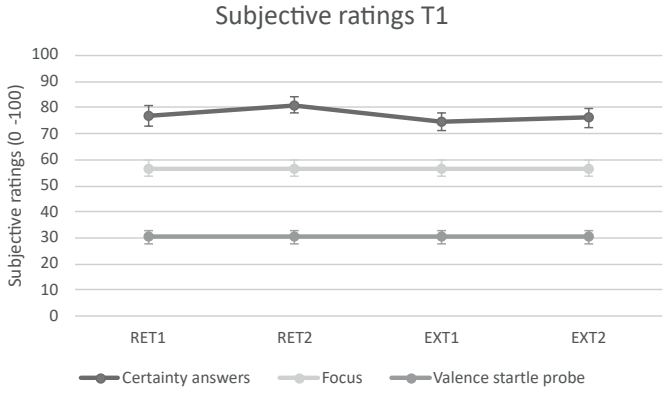
At T0, certainty ratings followed a linear, quadratic and cubic trend, β=-52.78, p=.05 for block, β= 28.60, p=.01 for block2, β=-4.19, p=.006 for block3, see Supplemental Figure 15. Focus ratings followed a linear-quadratic pattern, β= 23.06, p<.0001 for block, β=-4.68, p<.0001 for block2. Valence ratings of startle probes (M=25.87, SD=14.05) and of electric shocks (M=33.82, SD=18.70) did not change across experimental blocks, β=0.98, p=.45 and β= 0.63, p=.87 for block, respectively.

At T1, certainty ratings followed a linear, quadratic and cubic trend, β= 54.59, p=.004 for block, β= -24.19, p=.004 for block2, β= 3.14, p=.006 for block3, see Supplemental Figure 16. Focus ratings (M=56.78, SD=20.33) and valence ratings of the startle probe (M=30.32, SD= 16.97) did not change during the T1 test, β=-1.23, p=.81 for block and β=-0.43, p=.66 for block2, β=-1.20, p=.82 for block and β=0.25, p=.79 for block2, respectively.

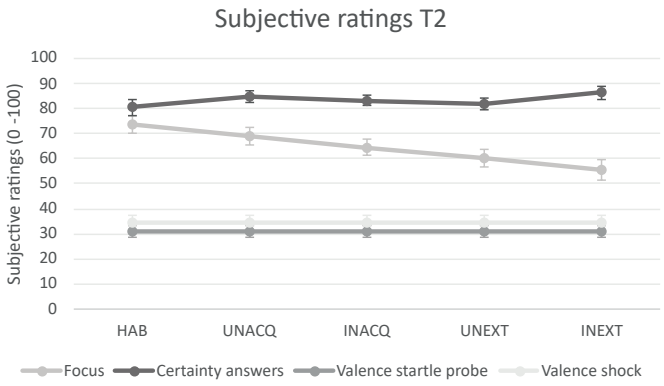
At T2, certainty ratings followed a linear, quadratic and cubic trend, β=23.96, p=.08 for block, β=-8.84, p=.07 for block2, β=0.98, p=.05 for block 3, see Supplemental Figure 17. Focus ratings decreased across experimental blocks at T2, β=-4.43, p<.0001 for block. Valence ratings of startle probes (M= 30.87, SD=15.02) and of electric shocks (M=34.30, SD=21.25) did not change across experimental blocks, β=-2.79, p=.31 for block and β=0.67, p=.09 for block2, β=-0.60, p=.84 for block, respectively.



Supplemental Figure 15. Subjective ratings across experimental blocks at T0 (baseline): HAB (habituation); UNACQ (uninstructed acquisition); INACQ (instructed acquisition); UNEXT (uninstructed extinction). *n*=51.



Supplemental Figure 16. Subjective ratings across experimental blocks at T1 (after first study medication ingestion): RET1: retention 1; RET2: retention 2; EXT1: re-extinction 1; EXT2: re-extinction 2. *n*=51



Supplemental Figure 17. A) Certainty ratings and b) focus ratings across experimental blocks at T2 (post-treatment): HAB: habituation; UNACQ: uninstructed acquisition; INACQ: instructed acquisition; UNEXT: uninstructed extinction; INEXT: instructed extinction. *n*=42

6.S.5.2 Differences between drug conditions in additional subjective ratings at T0, T1 and T2

Focus and certainty of subjective ratings at T0,T1 and T2, and valence ratings of electric shocks at T0 and T2 did not differ between drug conditions and did not interact with sex, use of AD medication, or diagnosis, see Supplemental Table 8 for the final models. Valence ratings of startle probes did not differ between drug conditions at T0 and T2, however, at T1 drug condition interacted with sex for valence ratings of startle probes, which is illustrated in Supplemental Figure 18. Men in the CBD condition (M=31.82) rated startle probes as less unpleasant during re-extinction 2 compared to placebo (M=19.70), although this difference was trend significant, p=.06. No differences between drug conditions were observed during preceding experimental blocks, $ps \geq .14$. Change in valence ratings from retention 1 to retention 2 differed between CBD and placebo condition in women, p=.004. No differences between CBD and placebo were observed during each experimental block; p

With only self-report data available and without follow-up tests, the CBD-placebo differences regarding aversiveness of startle probes discussed above are difficult to interpret.

Supplemental Table 8. Effects of drug condition on subjective ratings during fear conditioning.

T0 (n=51)	Focus	Certainty of answers	Valence ratings of startle probes	Valence ratings of electric shocks
	Parameter estimate	Parameter estimate	Parameter estimate	Parameter estimate
Intercept	77.60*	148.41*	56.39*	15.73
Drug condition	2.67	-16.96	-23.74	33.71
Sex	-	-59.32*	-	-
Experimenter ind. 1	-86.94	-75.82	130.48*	-
Experimenter ind. 2	2.42	-82.96*	-45.12	-
Experimenter ind. 3	-13.28	51.55	191.35*	-
Experimenter ind. 4	52.5	134.13*	-189.48*	-
Block	-33.99	-139.02*	-65.91	5.77
x Drug condition	-0.81	25.53	50.59	-10.49
x Sex	-	96.21*	-	-
x Experimenter ind. 1	138.72*	125.18	-115.94	-
x Experimenter ind. 2	-17.08	133.68*	66.05	-
x Experimenter ind. 3	63.46	-18.43	-324.02*	-
x Experimenter ind. 4	-53.3	-193.02*	288.27*	-
Block2	20.92	66.90*	31.6	-
x Drug condition	1.07	-9.42	-23.95	-
x Sex	-	-40.81*	-	-
x Experimenter effects	Ind. 1: -64.62*	Ind. 2: -60.78*	Ind. 3: 158.60* Ind. 4: -120.29*	-
Block3	-3.42	-9.24*	-4.3	-
x Drug condition	-0.27	1.1	3.34	-
x Sex	-	5.16*	-	-
x Experimenter effects	-	Ind. 1: 8.06* Ind. 2: 8.19*	Ind. 3: -22.46* Ind. 4: 15.29*	-

Supplemental Table 8. Effects of drug condition on subjective ratings during fear conditioning.

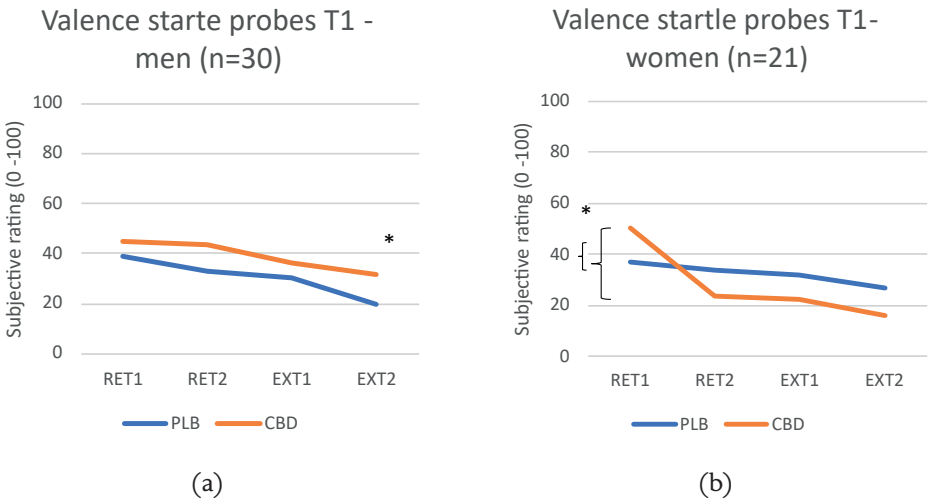
T1 (n=51)	Focus	Certainty of answers	Valence ratings of startle probes	Valence ratings of electric shocks
	Parameter estimate	Parameter estimate	Parameter estimate	Parameter estimate
Intercept	54.17*	42.62*	35.62	-
Drug condition	21.26	0.51	-26.31	-
Sex	-	-	-13.04	-
Drug condition x Sex	-	-	114.73*	-
Experimenter effects	Ind. 3: -98.91*	Ind. 3: 16.44*	Ind. 1: 22.76*	-
Block	15.06	50.99*	-22.76	-
x Drug condition	-21.87	7.34	52.72	-
x Sex	-	-	17.92	-
x Drug condition x Sex	-	-	-162.97*	-
x Experimenter effects	Ind. 3: 63.51*	-	Ind. 1: -7.53*	-
Block2	-9.77	-23.17*	12.53	- -
x Drug condition	12.86	-2.07	-23.67	-
x Sex	-	-	-7.76	-
x Drug condition x Sex	-	-	63.04*	-
x Experimenter effects	Ind. 3: -10.36*	-	-	-
Block3	1.42	3.06*	-1.84	- -
x Drug condition	-1.94	0.16	3.22	-
x Sex	-	-	1.13	-
x Drug condition x Sex	-	-	-7.73*	-

Supplemental Table 8. Effects of drug condition on subjective ratings during fear conditioning.

T2 (n=42)	Focus	Certainty of answers	Valence ratings of startle probes	Valence ratings of electric shocks
	Parameter estimate	Parameter estimate	Parameter estimate	Parameter estimate
Intercept	86.39*	61.26*	41.77*	40.89*
Drug condition	-18.17	5.97	8.71	-2.14
Sex	-39.25*	-	-	-
Experimenter ind. 1	-4.63	-	-21.91	-22.41
Experimenter ind. 2	-8.95	-	-10.35	-18.64
Experimenter ind. 3	-41.78	-	-152.07*	177.24
Experimenter ind. 4	-2.42	-	39.68	-13.82
Block	-10.09	22.36	-22.2	-0.8
x Drug condition	28.71	3.07	1.25	0.39
x Sex	40.18*	-	-	-
x Experimenter effects	Ind. 3: 18.18*	-	Ind. 3: 232.67*	Ind. 3: -69.60*
Block2	2.7	-8.23	9.23*	-
x Drug condition	-10.29	-1.16	-2.71	-
x Sex	-15.13*	-	-	-
x Experimenter effects	-	-	Ind. 3: -88.80*	-
Block3	-0.36	0.91	-1.02*	-
x Drug condition	1.04	0.14	0.44	-
x Sex	1.70*	-	-	-
x Experimenter effects	-	-	Ind. 3: 9.58*	-

Note: Ind.: indicator variable. Levels of the time variable Block were habituation, uninstructed and instructed acquisition, uninstructed extinction (T0), habituation, retention 1 and 2, uninstructed re-extinction 1 and 2 (T1), uninstructed and instructed acquisition, uninstructed and instructed extinction (T2). Block2 and Block3: Second- and third order polynomials for experimental block. The dashes in the table indicate that variables did not significantly explain variance and were not included in the final model; use of antidepressant medication and diagnosis did not significantly predict subjective ratings during fear conditioning. Drug condition X sex interactions (gray shaded) for Valence ratings of startle probes at T2 are illustrated in Supplemental Figure 18.

* $p < 1/6$ (=.05/3) for interactions of CBD with moderators; $p<.05$ for other effects



(a) (b)

Supplemental Figure 18. Valence ratings of startle probes during T1 experimental blocks RET1 (retention 1) RET2 (retention 2); EXT1 (re-extinction 1); EXT2 (re-extinction 2) after first medication ingestion, split by drug condition and sex.

Note: PLB: placebo; CBD: cannabidiol. Model estimates of model with indicator variable 1 for experimenter. Indicator variable for experimenter (1-4) affected level of the ratings (a main effect); we did not examine potential interactions with drug condition.
* $p < .05$.

Appendix A. Moderator effects on fear outcomes during fear conditioning in initial models

Supplemental Table 9. Moderator effects on fear outcomes during T0 fear conditioning at baseline.

CS+	Subjective fear β (n=51)			Shock expectancy β (n=51)			Startle β (n=52)			SCR β (n=53)		
Moderator	Sex	AD use	Diagn	Sex	AD use	Diagn	Sex	AD use	Diagn	Sex	AD use	Diagn
	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.
Sex/AD use/diagn	23.1	-34.03	-50.06	12.29	-19.01	-11.45	0.07	0.15	0.04	0.24	1.02	-0.28
Drug condition x Sex/AD use/diagn	49	60.49	59.53	-15.72	-15.01	50.44	-0.1	-0.05	0.13	-0.01	-1.3	-0.22
Block x Sex/AD use/diagnosis	8.64	56.8	76.73	-22.68	40.67	14.15	-0.09	-0.17	-0.03	-0.06	-1.14	0.6
Block x Drug condition x Sex/AD use/diagn	-96.86	-75.58	-47.25	31.58	16.32	-30.51	0.13	0.04	-0.27	-0.19	1.79	0.24
Block2 x Sex/AD use/diagnosis	-15.44	-25.68	-25.3	8.48	-13.96	-5.41	0.04	0.06	-0.001	-0.08	0.41	-0.3
Block2 x Drug condition x Sex/AD use/diagn	53.09	28.46	12.7	-11.98	-4.75	5.76	-0.06	-0.01	0.13	0.21	-0.74	-0.12

Supplemental Table 9. Moderator effects on fear outcomes during T0 fear conditioning at baseline.

CS-	Subjective fear β (n=51)			Shock expectancy β (n=51)			Startle β (n=52)			SCR β (n=53)		
Moderator	Sex	AD use	Diagn	Sex	AD use	Diagn	Sex	AD use	Diagn	Sex	AD use	Diagn
	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.
Sex/AD use/diagn	85.09	-67.08	17.14	-9.52	11.2	7.01	-0.12	-0.07	0.12	-0.38	0.52	-0.04
Drug condition x Sex/AD use/ diagn	-64.7	166.95	86.25	-14.1	-20.28	-0.98	-0.13	0.03	0.01	0.48	0.1	-0.23
Block x Sex/AD use/diagnosis	-84.3	108.83	-20.5	6.66	-20.37	-26.87	0.21	0.11	-0.22	0.69	-0.46	0.25
Block x Drug condition x Sex/AD use/ diagn	59.24	-259.11^	-110.45	36.21	35.62	20.75	0.14	-0.06	0.02	-0.79	-0.26	0.16
Block2 x Sex/AD use/diagnosis	29.93	-47.44	3.15	1.54	3.84	8.26	-0.09	-0.05	0.1	-0.34	0.12	-0.15
Block2 x Drug condition x Sex/AD use/ diagn	-15.93	116.82^	52.52	-13.17	-8.4	-6.75	-0.05	0.04	-0.02	0.36	0.15	-0.01

Supplemental Table 9. Moderator effects on fear outcomes during T0 fear conditioning at baseline.

CS-	Subjective fear β (n=51)			Shock expectancy β (n=51)			Startle β (n=52)			SCR β (n=53)		
Block3 x Sex/AD use/diagnosis	-3.54	6.38	0.17	-	-	-	0.01	0.007	-0.01	0.05	-0.009	0.02
Block3 x Drug condition x Sex/AD use/ diagn	1.6	-15.79*	-7.36	-	-	-	0.006	-0.007	0.003	-0.05	-0.02	-0.006

Note: P.est.: parameter estimate; AD: antidepressant; Diagn: diagnosis; SCR: skin conductance response. Interactions of drug conditions with Block3 for CS+ were not significant and are not displayed in the table. Significant effects of drug condition that survived Bonferroni correction are shaded gray.

ˆ $1/6(.05/3) < p < .05$ for interactions of CBD with moderators

* $p < 1/6 (=.05/3)$ for interactions of CBD with moderators; $p<.05$ for other effects

Supplemental Table 10. Moderator effects on fear memory expression during T1 fear conditioning (after the first study medication ingestion).

CS+	Subjective fear			Shock expectancy			Startle			SCR		
	β (n=51)			β (n=51)			β (n=52)			β (n=53)		
Moderator	Sex	Antidep	Diagn	Sex	Antidep	Diagn	Sex	Antidep	Diagn	Sex	Antidep	Diagn
	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.
Sex/antidep/ diagn	6.66	8.48	21.01	-5.94	-22.66	3.58	-0.08*	-0.09*	-0.03	-0.13	-0.21*	-0.15
Drug condition x Sex/antidep/diagn	-6.92	-0.73	-10.47	-0.99	-35.5	4.41	0.01	0.09	0.09	0.1	0.18	0.32
Block x Sex/antidep/ diagn	1.35	6.39	-10.71	12.15	-11.28	-5.49	0.04	0.06*	0.009	0.05	0.13	0.03
Block x Drug condition x Sex/antidep/diagn	9.95	-0.88	14.3	-4.75	18.53	0.4	0.005	-0.07	-0.06	-0.09	-0.15	-0.1

Supplemental Table 10. Moderator effects on fear memory expression during T1 fear conditioning (after the first study medication ingestion).

CS-	Subjective fear β (n=51)			Shock expectancy β (n=51)			Startle β (n=52)			SCR β (n=53)		
Moderator	Sex Antidep Diagn			Sex Antidep Diagn			Sex Antidep Diagn			Sex Antidep Diagn		
	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.
Sex/antidep/diagn	11.8	20.81	10.01	11.94	18.71	-11.24	-0.03	-0.06	-0.01	-0.1	-0.07	-0.14*
Drug condition x Sex/antidep/diagn	-27.4	-15.86	7.92	-6.54	-14.69	39.14	-0.03	0.06	0.06	-0.23	-0.08	-0.02
Block x Sex/antidep/diagn	-3.72	-0.75	-6.12	-1.26	-10.56	3.71	0.01	0.03	0.01	0.03	0.02	0.06
Block x Drug condition x Sex/antidep/ diagn	20.95*	4.87	6.87	2.13	8.1	-19.8	0.02	-0.04	-0.05	0.14	0.04	-0.02

Note: P. est.: parameter estimate; AD: antidepressant; Diagn: diagnosis; SCR: skin conductance response. Significant effects of drug condition that survived Bonferroni correction are shaded gray.

* $p < 1/6$ (=.05/3) for interactions of CBD with moderators; $p<.05$ for other effects

Supplemental Table 11. Moderator effects on fear outcomes during T1 fear extinction (after first study medication ingestion).

CS+	Subjective fear β (n=51)			Shock expectancy β (n=51)			Startle β (n=52)			SCR β (n=53)		
Moderator	Sex	AD use	Diagn	Sex	AD use	Diagn	Sex	AD use	Diagn	Sex	AD use	Diagn
	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.
Sex/AD use/diagn	17,43	33.93*	-25,33	22,96	-30,55	-25,19	0,02	-0,004	0,009	-0,06	0,07	0,15
Drug condition x Sex/AD use/diagn	-18,09	-62.48*	39,24	-52,89	-33,34	6,16	-0,05	-0,004	-0,01	0,17	-0,07	-0,27
Block x Sex/AD use/diagnosis	-7,94	-14,5	10,91	-2,36	7,94	7,48	-0,02	-0,02	-0,02	0,004	-0,1	-0,13
Block x Drug condition x Sex/AD use/ diagn	23,37	38.75*	-8,61	24,06	29,79	4,61	0,04	0,02	0,009	-0,09	0,07	0,21
CS-	Subjective fear β (n=51)			Shock expectancy β (n=51)			Startle β (n=52)			SCR β (n=53)		
Moderator	Sex	AD use	Diagn	Sex	AD use	Diagn	Sex	AD use	Diagn	Sex	AD use	Diagn
	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.
Sex/AD use/ diagn	2,15	25,71	2,56	39.81*	-17,78	8,68	0,005	0,022	-0,04	0,005	0,03	0,11
Drug condition x Sex/AD use/diagn	18,11	-27,97	2,55	-56.81^	9,12	-9	-0.08^	-0,06	0,03	0,1	-0,01	-0,24
Block x Sex/AD use/diagnosis	-2,21	-8,77	-3,79	-15,49	2,46	-7,92	-0,02	-0,03	0,02	-0,05	-0,05	-0,09
Block x Drug condition x Sex/AD use/ diagn	4,57	19,07	11,52	32,75	4,61	9,54	0.06*	0.06*	-0,02	-0,03	0,05	0,12

Note: P.est.: parameter estimate; AD: antidepressant; Diagn: diagnosis; SCR: skin conductance response. Significant effects of drug condition that survived Bonferroni correction are shaded gray.

ˆ 1/6(.05/3) < p < .05 for interactions of CBD with moderators

* p < 1/6 (=.05/3) for interactions of CBD with moderators; p<.05 for other effects

Supplemental Table 12. Moderator effects on T2 fear extinction.

CS+	Subjective fear β (n=42)			Shock expectancy β (n=42)			Startle β (n=47)			SCR β (n=47)		
Moderator	Sex	AD use	Diagn	Sex	AD use	Diagn	Sex	AD use	Diagn	Sex	AD use	Diagn
	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.
Sex/AD use/diagn	226,43	49,87	244,41	-22,1	210.02*	109,21	-0,11	-0,18	-0,31	0,26	-0,6	-1.3*
Drug condition x Sex/AD use/diagn	-149,4	-125,39	-247,14	1,11	-246,35	-128,85	-0,08	-0,02	0,18	0,37	0,17	0,75
Block x Sex/AD use/diagnosis	-122,2	-30,73	-122,65	10,89	-160.32*	-71,33	0,04	0,1	0,15	-0,27	-0,32	0.66*
Block x Drug condition x Sex/AD use/diagn	93,8	68,89	143,92	4,13	178,87	92,47	0,07	0,01	-0,09	-0,18	-0,19	-0,47
Block2 x Sex/AD use/diagnosis	16.11*	4,39	14,7	-0,6	27.17*	10,51	-0,003	-0,01	-0,02	0,02	-0,04	-0.08*
Block2 x Drug condition x Sex/AD use/diagn	-12,57	-8,5	-18,7	-1,57	-29,39	-14,46	-0,01	-0,002	0,01	0,02	0,03	0,07

Supplemental Table 12. Moderator effects on T2 fear extinction.

CS-	Subjective fear β (n=42)			Shock expectancy β (n=42)			Startle β (n=47)			SCR β (n=47)		
Moderator	Sex	AD use	Diagn	Sex	AD use	Diagn	Sex	AD use	Diagn	Sex	AD use	Diagn
	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.	P. est.
Sex/AD use/diagn	-100,72	209.38*	172.41*	13,46	105,99	25,99	0,11	-0,17	-0,11	-1,06	-0,63	0,96
Drug condition x Sex/AD use/diagn	27,15	-209,68	-287.41ˆ	-48,91	-161,91	-102,1	-0,51	0,11	0,41	-0,4	-0,49	0,77
Block x Sex/AD use/diagnosis	56,12	-99.34*	-92.27*	-3,5	-82,29	-21,08	-0,06	0,09	0,05	0,49	0,32	-0,51
Block x Drug condition x Sex/AD use/ diagn	-10,01	96,91	153.95ˆ	35,73	128.95ˆ	65,71	0,27	-0,05	-0,23	0,21	0,16	-0,33
Block2 x Sex/AD use/diagnosis	-6,41	12.08*	11.47*	0,6	14,34	3,22	0,01	-0,01	-0,007	-0,06	-0,04	0,06
Block2 x Drug condition x Sex/AD use/diagn	0,9	-11,02	-19.33*	-6,21	-22.42ˆ	-9,59	-0,03	0,004	0,03	-0,03	-0,01	0,04

Note: P. est.: parameter estimate; AD: antidepressant; Diagn: diagnosis; SCR: skin conductance response. Significant effects of drug condition that survived Bonferroni correction are shaded gray.

ˆ 1/6(.05/3) < p < .05 for interactions of CBD with moderators

* p < 1/6 (=.05/3) for interactions of CBD with moderators; p<.05 for other effects

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Part III

Predicting exposure treatment success

Chapter 7

Predicting exposure therapy response by latent fear learning trajectories in two independent patient samples with anxiety disorders

Caroline M.B. Kwee

Puck Duits

Neeltje M. Batelaan

Danielle C. Cath

Johanna M.P. Baas

Authors' contributions:
JB and DC obtained funding for this study from the Helmholtz Institute and GGZ Drenthe. All authors contributed to the design of the study. PD and CK coordinated the recruitment of patients and the data collection. CK did the statistical analyses and wrote the manuscript. All authors (JB, DC, PD, NB, CK) read, contributed to and approved the final manuscript.

Abstract

Background and Objectives

A substantial proportion of patients with anxiety disorders shows insufficient response to currently available cognitive-behavioral treatments. Previous research demonstrated deficits in extinction and safety learning in patients compared to healthy individuals. In a previous study (DOI:10.1016/j.janxdis.2021.102361, referred to as study 1, (dys)functional fear learning trajectories were determined by latent class growth analyses (LCGA) prior to the start of exposure based therapy. Evidence whether dysfunctional fear learning trajectories predict poor treatment response was inconclusive, partly as a result of small sample size. Here, we reinvestigate this question including a replication in an independent sample (study 2).

Methods

Fear learning was measured using subjective fear and shock expectancy self-reporting to the conditioned (CS+) and safety stimulus (CS-) across (un)instructed acquisition and uninstructed extinction phases. We re-examined latent fear learning trajectories up to the uninstructed extinction phase in study 1 and classified participants of study 2 according to these trajectories. We investigated prediction of treatment response based on the number of dysfunctional trajectories in both samples separately with Bayesian Analysis of Variance.

Results

The hypothesis of the number of dysfunctional trajectories being unrelated to treatment response received moderate-to-substantial support both in study 1 and study 2 (BF 7.205 and 17.52). The alternative hypothesis of the highest number of dysfunctional trajectories being associated with poor treatment response also received moderate support (BF 9.22 and 4.89). Our findings were to some extent sensitive to the choice of the scale factor for the prior distribution. With a higher scale factor there was also some support in the data for the hypothesis that the number of dysfunctional trajectories would be linearly and negatively associated with treatment response (BF 3.061 and 1.427).

Limitations

The size of both samples was small and limits the power to draw a conclusion.

Conclusions

Our findings are inconclusive. Studies with larger samples are needed to determine whether fear learning determined with LCGA can predict the success of subsequent exposure therapy.

7.1 Introduction

Anxiety disorders are prevalent mental disorders with a high disease burden and societal costs (for a review of epidemiological surveys, see Bandelow & Michaelis, 2015). Exposure therapy has been found to be effective at posttreatment and at long term follow-up (for (reviews of) meta-analyses, see Butler et al., 2006; Hofmann et al., 2012; Hofmann & Smits, 2008; Van Dis et al., 2020). However, around one third of patients do not respond satisfactorily (Taylor et al., 2012) and some even show symptom exacerbation after treatment (Hansen et al., 2002). Given the emotional distress of those who undergo exposure therapy (Olatunji et al., 2009), treatment nonresponse should be minimized.

Predictive factors of treatment (non)response that were mentioned in the literature are pretreatment symptom severity and comorbidity, and an unsupportive or overinvolved interpersonal environment (Taylor et al., 2012). In panic disorder with agoraphobia specifically, positive mental health emerged as a predictor of remission after exposure-based treatment (Teisman et al., 2018) leading to new interventions that are effective in treating anxiety disorders (Haller et al., 2021). Unfortunately, these research findings do not easily translate to prediction and prevention of treatment nonresponse on a case-by-case basis.

Individual differences in fear conditioning may constitute a candidate for predicting treatment response. Fear conditioning is widely used across species as a laboratory model of how fear is acquired and extinguished in exposure therapy. During the acquisition phase of conditioning, fear is acquired by pairing an initially neutral stimulus (conditioned stimulus; CS+) with an aversive outcome (unconditioned stimulus; US). By contrast, over the course of fear extinction the CS+ no longer predicts the US. Learning this new, inhibitory association (CS+ no US) is currently thought to be the prime mechanism of fear extinction and of symptom reduction during exposure therapy (Craske, 2015; Craske et al., 2018).

Aberrant patterns of fear conditioning have been observed in patients with anxiety disorders. Meta-analyses showed that on average, patients responded with stronger fear (on subjective and physiological measures) to the CS+ during fear extinction, compared to healthy subjects (Duits et al., 2015; Lissek et al., 2005). In addition, fear responses to a safety cue (CS-) that was never paired with a US were elevated in patients during fear acquisition and extinction

(Duits et al., 2015). This result is in line with deficits in learning about safety demonstrated in earlier work in highly anxious individuals (Chan and Lovibond, 1996) and claustrophobic patients (Telch et al., 1994).

These comparisons between patient- and control samples do not readily provide information regarding individuals in these groups. A data-driven method introduced by Galatzer-Levy et al. (2017) in this field showed how individual learning trajectories during fear conditioning can be identified, by classification to latent (not directly observable) groupings with similar trajectories. This approach was applied by Duits et al. (2021) in patients with anxiety disorders (n=104) and healthy subjects (n=93). This yielded classification of some, but not all patients into trajectories that may be dysfunctional, suggesting that fear learning deficits may underlie pathology and/or treatment nonresponse in some, but not all patients. This opens up the possibility to predict who may do well in exposure treatment based on the results of a laboratory fear conditioning experiment.

The trajectories, based on subjective fear ratings, that occurred relatively frequent in patients were: poor fear extinction to the CS+ (n = 28 (27%) patients) compared to (n = 8 (9%)) controls, and high fear responses to the CS- during fear acquisition and extinction, which was interpreted as impaired safety learning (n=41 (39%) patients; n= 18 (19%) controls). Clinical status (patient, control) was not related to learning trajectories based on US expectancy ratings (Duits et al., 2021). These trajectories were replicated in an entirely healthy sample (n=300; Leen et al., 2021). In this sample, all potentially dysfunctional trajectories (except for US expectancy upon CS+ presentation) were associated with higher state anxiety compared to the other trajectories.

Moving away from cross-sectional associations, Duits et al. (2021) investigated whether latent fear learning trajectories would also predict future exposure therapy response (n=63). Fear extinction is widely recognized as the central mechanism of symptom improvement during exposure therapy. Therefore, when this learning is impaired, treatment response is expected to be curbed (Craske et al., 2018). However, Duits et al. (2021) found that the only trajectory that significantly predicted poor treatment response was that of high US expectancy ratings to the CS-. Numerically however, trajectories of poor extinction of fear and US expectancy upon seeing the CS+, and of high fear ratings to the CS- also seemed to coincide with poor treatment response: % improvement on the Brief Symptom Inventory M(SD) = 8.7(57.7) vs 39.1(39.0) for normal conditioners on subjective fear upon CS+; 9.3(78.3) vs 36.8(40.5) for normal conditioners on US expectancy upon CS+; 22.9(55.3) vs 49.7(40.5) for normal safety learning on subjective fear for the CS (Duits et al., 2021). Finally, a high number of poor extinction and impaired safety learning trajectories (hereafter referred to as 'dysfunctional trajectories') seemed to be associ-

ated with poor treatment response (Duits et al., 2021). No statistical tests were conducted on these data because of the small group sizes.

The availability of a new patient sample from our recent study that investigated enhancement of treatment response by cannabidiol (CBD; null findings have been published in Kwee et al., 2022) offers a great opportunity to replicate these tentative conclusions. In this independent sample a cued fear conditioning paradigm based on Duits et al. (2021) was applied to 53 patients with panic disorder and agoraphobia and social anxiety disorder. This work (hereafter referred to as 'study 2') differs from the study of Duits et al. (2021; hereafter referred to as 'study 1'), because it was aimed at incomplete extinction to measure subsequent effects of CBD. Therefore, study 2 included only uninstructed extinction, whereas study 1 included an additional instructed extinction phase, before which participants were instructed about US nonoccurrence.

Uninstructed extinction is the most common way to assess extinction in the fear conditioning literature (Lonsdorf et al., 2017). The first step of our current analysis was therefore to reidentify latent fear learning trajectories, omitting the instructed extinction phase that was included by Duits et al. (2021). We then reinvestigated prediction of treatment response in study 1, and attempted to replicate the findings in study 2.

The current analysis allows for testing whether individual differences in the most common assessment of extinction, without verbal instructions, predicts treatment success. Our a priori hypothesis was, in line with the previous findings of Duits et al. (2021), that only patients with the highest number of dysfunctional trajectories would have worse treatment response than other patients. Our alternative hypothesis was that the number of dysfunctional trajectories would be linearly and negatively associated with treatment response.

7.2 Methods

7.2.1 Participants and treatment

7.2.1.1 Study 1

Fear conditioning data from 93 healthy subjects and 101 patients with various anxiety-related disorders (who agreed upon their data being reused) were used for reidentification of latent fear learning trajectories without instructed extinction phase. After participation in the fear conditioning task, patients received exposure-based cognitive behavioral therapy (CBT) encompassing 45-minute sessions at the Altrecht Academic Anxiety Center (n=70) and the Center for Psychological Psychotherapy of the University of Greifswald (n=31), respectively. This study was conducted in a naturalistic setting, hence, the number of treatment sessions until posttreat-

ment assessment (after treatment termination) was highly variable (see Table 1). Results of previous analyses into prediction of treatment response have been published in Duits et al. (2021).

7.2.1.2 Study 2

Subjective fear conditioning data prior to treatment was available for 53 patients with panic disorder and agoraphobia or social anxiety disorder. The treatment consisted of 8 weekly 90-min sessions of standardized exposure therapy, augmented with either 300 mg CBD (n=27) or placebo (n=26), which was orally administered approximately 2 h prior to treatment sessions. The protocol (van der Flier et al., 2019) and null effects of CBD augmentation on clinical outcomes have been published (Kwee et al., 2022). At the posttreatment assessment, all patients received about the same amount of treatment. After the assessment, some patients followed further treatment(outside of the study protocol).

Table 1 displays patient and treatment characteristics for study 1 and 2.

Table 1. Patient and treatment characteristics.

	Study 1	Study 2
Diagnosis, n		
social anxiety disorder	26	25
panic disorder and/or agoraphobia	24	28
obsessive compulsive disorder	17	NA
generalized anxiety disorder	12	NA
post-traumatic stress disorder	10	NA
hypochondriasis	7	NA
specific phobia	5	NA
Symptom severity	BSI	BAI
Pretreatment, mean (SD)	1.24 (0.73)	25.74 (13.17)
Pre-posttreatment, % change (SD)	32.91 (47.27)	17.82 (96.07)
Psychotropic medication, n (%)	54 (53%) mainly AD	22 (42%) Mainly serotonergic AD
Sessions until posttreatment, mean (SD)	23.13 (13.54)	8.23 (2.05)

Note: NA: not applicable; AD: antidepressant; BSI: Brief Symptom Inventory; BAI: Beck Anxiety Inventory

7.2.2 Fear conditioning task

A fear conditioning task was administered before psychotherapy (study 1 and 2) and CBD/placebo administration (study 2) commenced.

7.2.2.1 Stimuli and apparatus

The fear conditioning task was programmed in Presentation software. Pictures of neutral faces served as CS+ and CS-. They were taken from the Psychological Image Collection at Stirling; `http://pics.stir.ac.uk`, following Klumpers et al. (2010) and Duits et al. (2017). A 625-ms electric shock loop, composed of 2-ms pulses delivered at 20 Hz, served as US. Shocks were administered by a Digitimer DS7A generator, through two disk electrodes attached over the medial nerve on the inner wrist of the subject's non-dominant hand. In both studies, physiological responses were recorded, including startle which was evoked with startle noises in 75% of the trials. These data are not the focus of this report, as the analyses by Duits et al. (2021) indicated that no latent trajectories could be discerned in the physiological data.

7.2.2.2 US titration

A shock workup, previously carried out in work by, for example Baas et al. (2009), Heitland et al. (2012) and Klumpers et al. (2010), was employed in order to standardize subjective pain intensity. The workup comprised of five sample shocks and was followed by rating on a five-point scale to indicate how annoying/painful the shocks were being perceived. The shock intensity that received a rating of 4, which corresponded to "quite annoying" was used in the fear conditioning task.

7.2.2.3 Task design

During acquisition phases, CS+ presentations were followed by US presentation according to a partial reinforcement schedule. The CS- was never paired with the US. During extinction phases all CSs were unreinforced (no USs were presented anymore). Stimulus presentations lasted 8 s, and the total duration of each trial (including a black screen with a white fixation cross in the center of 6-8 seconds) was 14-16 s. The CS+, CS- and inter-trial interval (ITI) were presented in fixed order within the conditioning procedure.

7.2.2.4 Procedure

Participants were seated in a dimly-lit room while conducting the fear conditioning task.

The task started with startle habituation and CS familiarization phases. These were followed by two successive acquisition phases, one uninstructed and one instructed. After each phase, visual analogue scales (VASs) were presented to collect subjective ratings of fear and expectancy of shock experienced with each CS. Before the first acquisition phase participants were merely informed that shocks might be administered to allow for the spontaneous development of contingency awareness. Before the second acquisition phase participants were asked to indicate when the shock had been administered (response correctness is reported in Kwee et al., submitted for publication). After the participant's answer was recorded, information about the correct CS-US contingency was provided both orally and written.

The task in study 2 ended with one uninstructed extinction phase. In study 1 this was followed by a second instructed extinction phase that was omitted from analyses for reasons of comparison. Figure 1 provides an overview of the remaining phases.

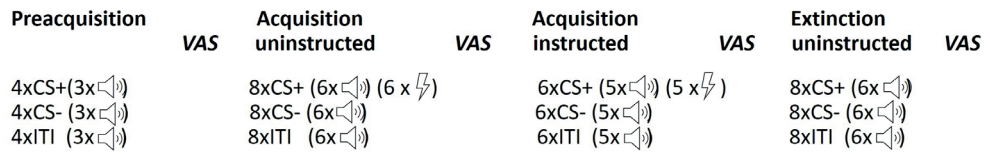

Figure 1. The fear conditioning task at baseline.

Note: CS+ = conditioned stimulus; CS- = safety stimulus; ITI = inter-trial-interval; VAS = visual analogue scale.

7.2.2.5 Outcome measures

Fear learning was measured with subjective fear and US expectancy upon CS+ and CS- presentation that were rated after experimental phases on VASs. Response anchors were 'not at all fearful/nervous' and 'very fearful/nervous' for subjective fear and 'very unlikely' and 'very likely' for US expectancy. Responses were logged on a line that was digitally recoded to a 0-100 scale (numbers were not visible to participants).US expectancy was not measured after preacquisition, given that no shocks were administered yet at that time.

7.2.3 Measures of treatment response

Brief Symptom Inventory

Patients in the study 1 filled out the Brief Symptom Inventory (BSI), a generic 53 item self-report scale of psychological symptoms with good psychometric properties (de Beurs & Zitman, 2006). Respondents rated on a 5-point Likert scale (anchors ranging from "none" to "very much") how much they were bothered by a symptom in the past week including today. The BSI

has shown sufficient responsiveness to change in anxiety symptom severity (van der Mheen et al., 2018).

Beck Anxiety Inventory

The Beck Anxiety Inventory (BAI; Beck et al., 1988) was used as a measure of treatment response in study 2. The BAI consists of 21 items and patients rated on a 4-point Likert scale (anchors ranging from "not at all" to "severely, I could barely stand it") how much they were bothered by a symptom in the past week including today. The BAI is a reliable and well validated measure of anxiety symptoms (Fydrich et al., 1992), and is sensitive to measure change associated with treatment (de Beurs et al., 1997).

No golden standard exists to operationalize treatment response (Taylor et al. 2012). Nevertheless, percentage change between baseline and post-treatment has often been used (e.g., Erzegovesi et al., 2001; Duits et al., 2021; Hofmeijer-Sevink et al., 2017), which is what we calculated in the current work for the BSI (study 1) and BAI (study 2). When symptom severity is underreported initially (e.g., Pierce and Kirkpatrick, 1992) percentage change can take on extreme values. This issue was mitigated by the use of trimmed means (see section 2.4.2).

7.2.4 Statistical analyses

We used latent class growth analyses (LCGA) to discern classes of fear learning trajectories (steps one and three) and Bayesian Analysis of Variance (ANOVA) for comparisons between classes (steps two, four and five):

1. Reidentification of latent fear learning trajectories (measured with subjective fear and shock expectancy to the CS+ and CS-) in study 1, omitting the instructed extinction phase (n=194);

2. Reinvestigation of prediction of treatment response in study 1 (n=62). The following hypotheses were evaluated: the null hypothesis that the number of dysfunctional trajectories is unrelated to treatment response, hypothesis 1 that patients with only the highest number of dysfunctional trajectories would have worse treatment response than other patients; and hypothesis 2 that the number of dysfunctional trajectories would be linearly and negatively associated with treatment response;

3. Classification of patients from study 2 (n=53) to the fear learning trajectories reidentified in step 1;

4. Translation of findings from step two into hypotheses which were tested in study 2 (n=43);

5. Sensitivity analyses were conducted to examine whether substantiated conclusions would change by altering the scale factor for the prior distribution (Hoijtink et al., 2019).

These five steps are described in more detail in section 2.4.1-2.4.5:

7.2.4.1 Step 1: Reidentification of latent fear learning trajectories(study 1)

We first reidentified latent classes with latent class growth analyses (LCGA; Jung & Wickrama, 2008), a type of latent variable mixture modeling (LVMM; Berlin et al., 2014) in Mplus version 8.8 (Muthén and Muthén, 1998-2017). Individuals within these latent classes displayed more pattern similarity compared to individuals between classes (Jung & Wickrama, 2008).

Latent intercept and slope variables were calculated that describe the (change in) level of our subjective outcome measures across the experimental phases (preacquisition, uninstructed acquisition, instructed acquisition, uninstructed extinction). Depending on the number of experimental phases in the model, we included slope variables to describe potential linear (2 or more phases), quadratic (3 or more phases) and cubic trends (4 or more phases). Separate analyses were conducted for the four combinations of stimulus type (CS+ and CS-) and fear measure (subjective fear, US expectancy).

In order to prevent the chance of local maxima and to optimize the reliability of the loglikelihood estimation, the number of random sets on starting values was set at 800 and the number of final optimizations at 200.

Following Duits et al. (2021) and Leen et al. (2021) model selection was based on entropy scores, and apparent drops in Bayesian Information Criterion (BIC) scores between two adjacent models.

We always chose the model with the smallest number of classes considered theoretically meaningful (van de Schoot et al., 2017). Based on the premise that deficits in extinction learning to the CS+ and safety learning to the CS- may underlie resistance to exposure treatment (Duits et al., 2015; 2021; Lissek et al., 2005), we required that the selected models uncovered individual differences regarding these types of learning (as in Duits et al. (2021) and Leen et al. (2021)).

Difficulties with extinction learning may have translational value with respect to treatment nonresponse (Craske, 2015; Craske et al., 2018; Duits et al., 2021), whereas this is not necessarily true for differences in ratings during (pre)acquisition. Therefore, to allow for model selection based on differences in fear extinction learning, we simplified the models by including the instructed acquisition and the uninstructed extinction phase only. This was a post hoc step, taken only after seeing the latent classes for models including (pre)acquisition phases (see Supplemental results), that resulted in more concise models than tested by Duits et al., 2021 and Leen

et al., 2021, and the CS- fear models tested in the current work.

7.2.4.2 Step 2: Prediction of treatment outcome by latent fear learning trajectories (study 1)

Our second step was to investigate whether the number of dysfunctional trajectories would predict treatment response in study 1.

We employed Bayesian ANOVA using R package bain. This yields Bayes factors that quantify the support in the data for competing hypotheses (Gu, 2016; Gu et al., 2018; Hoijtink et al., 2019), relative to the unconstrained hypothesis (no restrictions between means). In order to prevent results from being unduly affected by outliers, we calculated robust Bayes factors using 20% trimmed means as replacements for the standard arithmetic means (Bosman et al., 2018) with R package WRS2 (Mair et al., 2017). This approach is suitable for small sample sizes (Wilcox, 2017, pp. 90-93).

7.2.4.3 Step 3: Classification of individuals to latent fear learning trajectories (study 2)

Sample size of study 2 was small (n=53), and therefore accuracy of class assignment might suffer when conducting LCGA's (Depaoli, 2013). We therefore imposed the latent trajectories identified with our LCGA's in study 1 (characterized by the growth curve parameters that are displayed in Table 7) upon the replication sample (study 2). Individuals of study 2 were classified to these trajectories based on the similarity of their fear learning trajectories to each latent class. We used entropy scores to determine quality of classification.

7.2.4.4 Step 4: Prediction of treatment outcome by latent fear learning trajectories (study 2)

Our fourth step was to investigate whether the number of dysfunctional trajectories would predict treatment response in study 2, which was evaluated with Bayesian ANOVA, using robust Bayes factors to compare the support in the data for competing hypotheses. Because there were some differences between the two conditioning tasks, the most important one being that instructed fear acquisition and extinction followed uninstructed phases in study 1 but not in study 2, we decided to analyze the samples separately and use study 1 as a discovery sample and study 2 as a replication sample instead of evaluating the joint evidence.

7.2.4.5 Sensitivity analyses

We conducted sensitivity analyses to see if our findings were robust with respect to the choice for the scale factor for the prior distribution. To compute Bayes factors, each hypothesis has to be translated in a so-called prior distribution. With the exception of the scale of the prior distribution all the information necessary for its specification comes from the hypothesis. The default scale used in bain is chosen such that the resulting Bayes factor somewhat favors the classical null-hypothesis to protect the user against finding effects that do not exist. However, this is an arbitrary choice. The effects of this choice can be determined by doing a so-called sensitivity analysis, that is, running bain with the default scale factor (fraction = 1) and larger scale factors (fraction = 2, 3). The latter render a Bayes factor with decreasing preference for the classical null-hypothesis. If the outcomes of these analyses are comparable, the outcomes are not sensitive to the choice of the scale factor. The interested reader is referred to Hoijtink et al. (2019) for further elaborations.

7.3 Results

7.3.1 Step 1: Re-identification of latent fear learning trajectories (study 1)

CS+ fear trajectories

We first compared BIC scores between adjacent (1-2, 2-3, 3-4 class) models. The most substantial drop in BIC was seen moving from a 1 to a 2 class model, see Table 2. In addition, the highest entropy was associated with the 2 class model. However, for the two classes with low (class 1) and high fear (class 2) during both acquisition as well as extinction, no differentiation could be made in rate of extinction learning. The addition of an extra class led to a poorer entropy score, however, in this 3 class model trajectories of low fear (n=61), poor and successful extinction (both n=66) could be distinguished (see Figure 2), which corroborates with previous research (Duits et al., 2021; Leen et al., 2021). Therefore, we proceeded our analysis with the 3 class model.

Table 2. Fit indices for one-to four class Latent Growth Models based on fearfulness ratings to the CS+, only including experimental phases instructed acquisition and uninstructed extinction.

Classes	BIC	Entropy	n per class based on most likely class membership
1	3786.48	-	193
2	3623.47	0.904	71/122
3	**3601.02**	**0.807**	**66/66/61**
4	3563.13	0.876	61/33/66/33

Note: The chosen model is highlighted in bold.

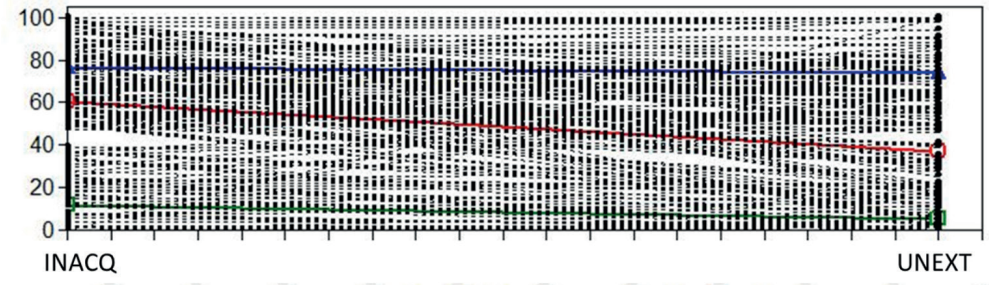

Figure 2. Estimated means and observed individual trajectories for subjective fear to the CS+ for the 3 class model with only the instructed acquisition and the uninstructed extinction phase.

Note: INACQ: Instructed acquisition; UNEXT: uninstructed extinction.

CS+ US expectancy trajectories

For CS+ US expectancy, evaluation of latent class growth models yielded no preferred model, because no distinction between functional and dysfunctional trajectories (poor extinction) could be made, neither in models with all experimental phase (see Supplemental results) nor in models that included only the instructed acquisition and the uninstructed extinction phase, which always included one class with <5% of individuals (see Table 3). We therefore did not include CS+ US expectancy for our analyses of prediction of treatment outcome.

Table 3. Fit indices for one-to four class Latent Growth Models based on US expectancy ratings upon CS+ presentation, only including experimental phases instructed acquisition and uninstructed extinction.

Classes	BIC	Entropy	n per class based on most likely class membership
1	3505.945	-	193
2	3418.326	0.996	189/4
3	3364.338	0.972	4/16/173
4	3355.535	0.896	44/2/13/132

Note: None of the models were selected for subsequent analyses.

CS- fear trajectories

For CS- fear, all experimental phases (from preacquisition until uninstructed extinction) were included in the LCGA's, given that differences in fearfulness between latent trajectories were observed in previous studies during the entire fear conditioning task (Duits et al., 2021; Leen et al., 2021). Evaluation of the latent class growth models yielded the 2 class model as the preferred model. The most substantial drop in BIC was seen moving from a 1 to a 2 class model, see Table 4. However, entropy showed a linear increase with number of classes, which complicated model selection. The 3 class model included a trajectory of initially high fear (during preacquisition), followed by a moderate level of fear during the remaining experimental phases. As in previous studies (Duits et al., 2021; Leen et al., 2021) we selected the most parsimonious 2 class model with trajectories characterized by low (n=138) and high fear (n=56) to the CS- (see Figure 3).

Table 4. Fit indices for one-to four class Latent Growth Models based on Fear to the CS-, including experimental phases preacquisition, uninstructed acquisition, instructed acquisition and uninstructed extinction.

Classes	BIC	Entropy	n per class based on most likely class membership
1	7155.45	-	194
2	**6949.61**	**0.901**	**56/138**
3	6863.77	0.929	23/48/123
4	6808.41	0.958	13/29/118/34

Note: The chosen model is highlighted in bold.

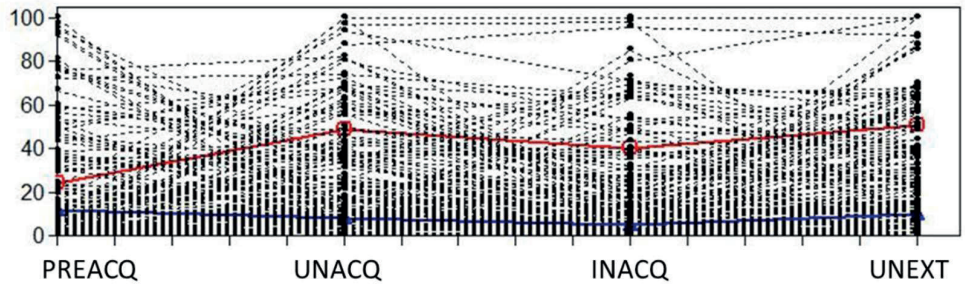

Figure 3. Estimated means and observed individual trajectories for subjective fear to the CS- for the 2 class model with phases preacquisition, uninstructed and instructed acquisition and uninstructed extinction.

Note: PREACQ: preacquisition; UNACQ: uninstructed acquisition; INACQ: instructed acquisition; UNEXT: uninstructed extinction.

CS- US expectancy trajectories

For US expectancy upon CS- presentation, following Duits et al. (2021) and Leen et al. (2021), experimental phases from uninstructed acquisition until uninstructed extinction were included in the LCGA's. Evaluation of latent class growth models yielded the 2 class model as preferred model. The most substantial drop in BIC was seen moving from a 1 to a 2 class model, see Table 5. However, entropy was higher for the 3 class model. The 3 class model included a trajectory of moderate US expectancy across experimental phases. Because of the small size of this class (n=8), and in order to strive for congruence with previous studies (Duits et al., 2021; Leen et al., 2021) we selected the 2 class model with trajectories characterized by low (n=173) and high (n=20) US expectancy (see Figure 4).

Table 5. Fit indices for one-to four class Latent Growth Models based on US expectancy upon CS- presentation, including experimental phases uninstructed acquisition, instructed acquisition and uninstructed extinction.

Classes	BIC	Entropy	n per class based on most likely class membership
1	5333.35	-	193
2	**5191.28**	**0.972**	**173/20**
3	5086.29	0.987	162/8/23
4	5051.74	0.934	33/20/8/132

Note: The chosen model is highlighted in bold.

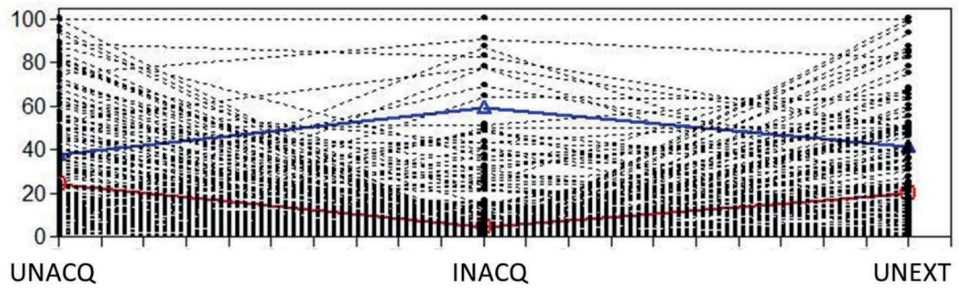

Figure 4. Estimated means and observed individual trajectories for shock expectancy upon CS- presentation for the 2 class model with phases uninstructed and instructed acquisition and uninstructed extinction.

Note: UNACQ: uninstructed acquisition; INACQ: instructed acquisition; UNEXT: uninstructed extinction.

7.3.2 Step 2: Prediction of treatment outcome by latent fear learning trajectories (study 1)

In the group for whom clinical outcome data after treatment was available, we created groups of 0 (n=31) 1 (n=14), or 2 or 3 (n=17) dysfunctional trajectories (patients with 2 and 3 dysfunctional trajectories were grouped together because of the small group size (n=4) of patients with 3 dysfunctional trajectories). Considering that we calculated robust Bayes factors using 20% trimmed means (see section 2.4.2) our results are not driven by 3 statistical outliers (< 75th percentile + 1.5 X interquartile range for each group mean) on % pre-to post treatment improvement on the BSI (see Figure 5).

Robust Bayes factors for the null hypothesis and two informative hypotheses (relative to the unconstrained hypothesis) are listed in Table 6, together with the posterior probabilities. The results show that the most support in the data is for the null hypothesis, followed by hypothesis 1, however, hypothesis 2 cannot be ruled out. In addition, due to the low probability of the unconstrained hypothesis, it is highly probable that either the null hypothesis, hypothesis 1 or hypothesis 2 (and nothing else) is true. This means that there is no other pattern not included by our informed hypotheses that would be even more likely.

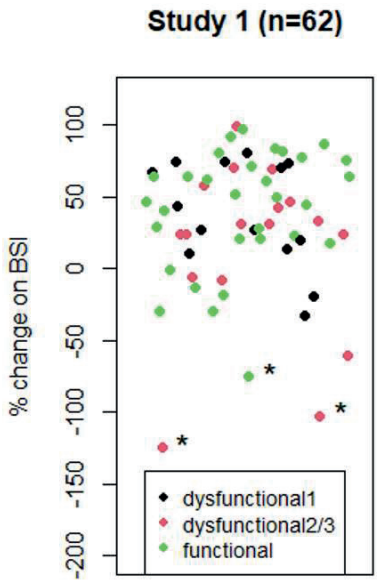

Figure 5. Scatterplot showing percentage pre-to posttreatment change on the BSI for study 1.

Note: * < 25th percentile - 1.5 X interquartile range or > 75th percentile + 1.5 X interquartile range for each group mean. Arbitrary values on the x axis.

Table 6. Robust Bayes factors and posterior probabilities for competing hypotheses about number of dysfunctional learning trajectories in study 1.

Hypothesis	Bayes factor	Posterior probability
Null	7.205	0.352
1	9.22	0.45
2	3.061	0.149
Unconstrained	1	0.049

Note: Hypothesis 1: Patients with the highest number of dysfunctional trajectories have worse treatment response than other patients; Hypothesis 2: The number of dysfunctional trajectories is linearly and negatively associated with treatment response.

7.3.3 Step 3: Classification of individuals to latent fear learning trajectories (study 2)

Classification of individuals of study 2 in the trajectories identified in study 1 yielded similar entropy scores to those of study 1, see Table 7. Percentages of individuals in each latent class were

similar for all outcome measures except CS- fear: a higher percentage of individuals in study 2 was classified to the 'impaired safety learning' trajectory, compared to study 1. This difference, however, was to be expected given the association between clinical status and CS- fear learning (Duits et al., 2021) and taking into consideration that only in study 1 healthy individuals participated.

Table 7. Classification of individuals to latent fear learning trajectories

Outcome measure	Imposed growth curve parameters				Entropy		% per class	
	i	s	q	c	Study 1	Study 2	Study 1	Study 2
CS+ fear			*Class 1*					
	60	-23	NA	NA				
			Class 2					
	13	-6	NA	NA	0.807	0.791	34/32/34	35/33/31
			Class 3					
	77	-3	NA	NA				
CS- fear			*Class 1*					
	24	59	-43	9				
			Class 2		0.901	0.901	71/29	51/49
	11	-1	-4	1				
CS- US expectancy			*Class 1*					
	24	-37	17	NA				
			Class 2		0.972	0.949	90/10	86/14
	38	41	-20	NA				

Note: i: intercept; s: linear trend; q: quadratic trend; c: cubic trend; values rounded to integers. NA: not applicable.

7.3.4 Step 4: Prediction of treatment outcome by latent fear learning trajectories (study 2)

We created groups of 0 (n=20) 1 (n=11), or 2 or 3 (n=12) dysfunctional trajectories (patients with 2 and 3 dysfunctional trajectories were grouped together because of the small group size (n=2) of patients with 3 dysfunctional trajectories). We identified 4 statistical outliers (< or > 75^{th} percentile + 3 X interquartile range for each group mean) on % pre-to post treatment improvement on the BAI (see Figure 6), that were not included in our calculation of robust Bayes factors using 20% trimmed means. Robust Bayes factors and posterior probabilities are listed in Table 8. The results show that the most support in the data is for the null hypothesis, how-

ever, hypothesis 1 cannot be ruled out. In addition, it is highly probable that either the null hypothesis or hypothesis 1 (and nothing else) is true.

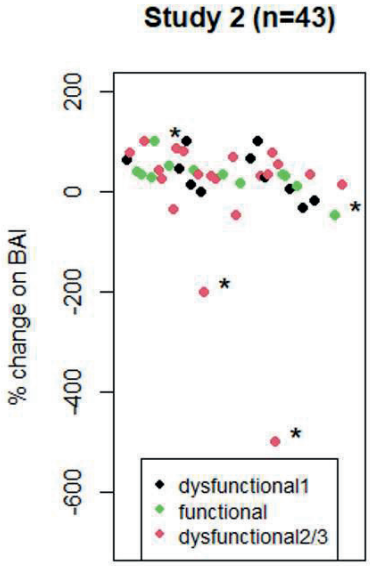

Figure 6. Scatterplot showing percentage pre-to posttreatment change on the BAI for study 2.

Note: * < 25th percentile - 1.5 X interquartile range or > 75th percentile + 1.5 X interquartile range for each group mean. Arbitrary values on the x axis.

Table 8. Robust Bayes factors and posterior probabilities for competing hypotheses in study 2.

Hypothesis	Bayes factor	Posterior probability
Null	17.518	0.705
1	4.894	0.197
2	1.427	0.057
Unconstrained	1	0.04

Note: Hypothesis 1: Patients with the highest number of dysfunctional trajectories having worse treatment response than other patients; Hypothesis 2: The number of dysfunctional trajectories is linearly and negatively associated with treatment response.

7.3.5 Sensitivity analyses

Sensitivity analyses with scale factor = 3 for the prior distribution yielded some-to-moderate support in study 1 and study 2 for all three hypotheses: the null hypothesis, hypothesis 1 (pa-

tients with the highest number of dysfunctional trajectories have worse treatment response than other patients) and hypothesis 2 (the number of dysfunctional trajectories is linearly and negatively associated with treatment response, see Table 9).

Table 9. Robust Bayes factors and posterior probabilities for competing hypotheses in study 1 and 2 from sensitivity analyses with scale factor = 3 for the prior distribution than in our main analyses, which employed scale factor = 1.

	Study 1		Study 2	
Hypothesis	Bayes factor	Posterior Probability	Bayes factor	Posterior Probability
Null	2.402	0.204	5.839	0.526
1	5.323	0.452	2.825	0.255
2	3.061	0.26	1.427	0.129
Unconstrained	1	0.085	1	0.090

Note: Hypothesis 1: Patients with the highest number of dysfunctional trajectories having worse treatment response than other patients; Hypothesis 2: The number of dysfunctional trajectories is linearly and negatively associated with treatment response.

7.4 Discussion

A substantial proportion of patients with anxiety disorders does not respond satisfactorily to currently available therapies. Research suggests that individual differences in experimental fear learning are associated with anxiety pathology (Duits et al., 2015, 2021; Lissek et al., 2005; Leen et al., 2021). To date, evidence for prediction of treatment response by these types of learning is inconclusive (Duits et al., 2021). In this paper we reinvestigated these data omitting instructed extinction (study 1). We included a replication in an independent sample (study 2), aiming to identify robust predictors of treatment (non)response.

We omitted the instructed extinction phase in study 1 given that study 2 was aimed at incomplete extinction to measure subsequent effects of CBD, and included only an uninstructed extinction phase. Reidentification of latent fear learning trajectories without the instructed extinction phase in study 1 yielded largely similar classes as identified in previous studies (Duits et al., 2021; Leen et al., 2021). For US expectancy and fear upon CS- presentation trajectories of normal and impaired safety learning were identified. For CS+ fear a differentiation was made between individuals with low fear during acquisition and extinction, and those with successful and poor extinction. However, this distinction was less readily apparent in the data without the instructed extinction phase compared to when this phase was included (Duits et al., 2021). Thus, for the current research question of prediction of treatment response by latent fear learn-

ing trajectories, the inclusion of an additional extinction phase would have allowed for a better differentiation based on extinction success.

The poor extinction class was larger in our analyses with only uninstructed extinction compared to the analyses including instructed extinction (34% versus 18% of individuals; Duits et al., 2021). In addition, this 'poor extinction' class included 39 of individuals (40%) who did extinguish fear after verbal instructions. This is in line with an unpublished exploratory between-group comparison in the study reported by Leen et al. (2021), in which 40 additional participants were subjected to the same task but without extinction instructions. In this sample of healthy controls, merely 12% of individuals were classified to poor extinction of fear to the CS+ with the instruction of US nonoccurrence during the last part of extinction. A much larger subset of 27% was classified to the poor extinction trajectory without this instruction (Leen et al., unpublished data).

For US expectancy upon CS+ presentation, no differentiation could be made between individuals with successful and poor extinction learning, omitting the instructed extinction phase. Hence, we only investigated prediction of treatment (non)response by fear learning trajectories measured with CS+ and CS- fear, and US expectancy upon CS- presentation. To that end, individuals of study 1 and study 2 were assigned to the functional and dysfunctional fear learning trajectories reidentified in study 1. The similar quality of classification (as indicated by entropy scores) and similar proportions of individuals in latent classes in study 1 and study 2 support our approach of selecting models that conceptually replicate the latent classes that were previously identified (Duits et al., 2021), and then assigning individuals from an independent sample (study 2) to these latent classes.

In both studies, the null hypothesis that the number of dysfunctional trajectories was unrelated to treatment response received most support. In study 1, there was similar support for the null hypothesis (posterior probability of 35%) and the informative hypothesis that the highest number of dysfunctional trajectories was associated with poor treatment response (posterior probability of 45%). In study 2 the null hypothesis received by far the most support (posterior probability of 71%), but again the same informative hypothesis could not be ruled out completely (posterior probability of 20%). Sensitivity analyses pointed out that our findings were to some extent sensitive to the choice of the scale factor for the prior distribution. These sensitivity analyses showed equivalent support in the data for all three hypotheses: the null hypothesis and the two informative hypotheses that highest/ higher number of dysfunctional trajectories was/were associated with poor treatment response.

Prediction of poor treatment response by dysfunctional learning trajectories would argue for explicitly addressing fear learning deficits prior to exposure therapy. The results from the

current analysis are in line with the findings of previous analyses including instructed extinction (Duits et al., 2021). It is as of yet not completely clear whether we can predict exposure therapy success using classification of fear learning trajectories. The potential benefit of including explicit extinction instructions also needs further investigation. Experimental findings indicate that verbal instructions about US nonoccurrence may help to extinguish conditioned fear (Duits et al., 2017). This is not the common practice, neither during experimental fear extinction (Lonsdorf et al., 2017) nor exposure therapy, where maximum violation of negative expectancies is advocated (Craske, 2015). Further research is needed to (re)investigate what the optimal level of threat anticipation is for awareness about safety to set in, and what role instructions may play. After all, robust fear responses (to the CS+ and CS-) despite instructions about safety may be what truly sets apart treatment responders from treatment non-responders.

7.4.1 Limitations

Our research question was investigated in two small sized samples. We expect that additional data will lead to a higher degree of support in favor of one of our hypotheses (Moerbeek, 2021), which will lead to more decisive conclusions about the predictive value of latent fear learning trajectories. However, we refrained in this work from combining the evidence from study 1 and 2, because we expected that the findings would be predominantly driven by the larger sample of study 1. When, in the future, more data becomes available, the Bayesian approach allows for updating the evidence for one of the preferred hypotheses (Kuiper et al., 2012; Moerbeek, 2021).

We also refrained from conducting exploratory analyses into the predictive value of individual trajectories of subjective fear or US expectancy to the CS+ or CS- considered on their own. Because individuals are always characterized by multiple learning trajectories, the limited sample sizes argued for considering these trajectories together. However, larger sample sizes would allow the analysis of the effect of each (dys)functional trajectory to a background of trajectories on the other measures.

We opted for symptom improvement to operationalize treatment (non)response. While subjectively experienced symptoms can be considered a reasonable indicator of pathological anxiety, impaired functioning in important life domains is also a defining feature of many patients (Hendriks et al., 2014; Substance Abuse and Mental Health Services Administration, 2016). Further, impairments in fear extinction and/or safety learning are most likely to become apparent when there is a learning opportunity, for example during treatment sessions. Posttreatment assessment of symptoms is a rather distal outcome in this regard (relative to within-session learning), that can be influenced by a multitude of other factors during therapy

(e.g., protocol adherence, a patient's motivation to engage in therapy). A more proximal outcome, such as the ability to complete steps of an exposure hierarchy in a fixed time (Raeder et al., 2020), may more sensitive to detect differences based on fear learning trajectories.

Our requirement that the latent class growth models uncovered individual differences regarding fear extinction and safety learning was based on our current understanding of anxiety pathology (e.g., Craske et al., 2018; Duits et al., 2015, 2021; Lissek et al., 2005). This prerequisite allowed us to investigate the predictive value of individual differences in these learning trajectories on treatment outcome. We focused on fear responses and threat expectancy during instructed acquisition and uninstructed extinction, instead of studying fear responses during the entire task. As a recent paper makes a case for perturbed anticipatory responding in anxiety disorders during the entire fear conditioning task (Abend et al., 2020), our work is limited in that we did not investigate the predictive value of individual differences in general threat anticipation.

7.4.2 Conclusion

We investigated in two independent samples whether individual differences in fear conditioning would predict exposure therapy response. Our findings are inconclusive. They suggest that number of dysfunctional fear learning trajectories are either unrelated to treatment response, that patients with a higher number of dysfunctional trajectories are more likely to have poor treatment response, or that only patients with the highest number of dysfunctional trajectories are more likely to have poor treatment response.

In anxiety patients, converging evidence points to deficits in learning about safety and absence of threat (Chan and Lovibond, 1996; Duits et al., 2015; Lissek et al., 2005; Telch et al., 1994), which may underly symptom persistence and treatment non-response (Craske, 2015; Craske et al., 2018). An empirical demonstration would lay a strong foundation for explicitly addressing these learning deficits during exposure therapy. Studies with larger samples are needed to determine whether fear learning determined with LCGA can predict the success of subsequent exposure therapy.

Conflict of interest

The authors have no conflicts of interest to declare.

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7.S Supplemental results

Contents

The final model selection of analysis step 1, to reidentify latent fear learning trajectories, is described in section 3.1. The models fitted for CS+ fear trajectories and CS+ US expectancy trajectories that included (pre)acquisition phases are discussed in the following.

7.S.1 CS+ fear trajectories

For CS+ fear, evaluation of latent class growth models including all experimental phases did not yield one preferred model. The most substantial drop in BIC was seen moving from a 1 to a 2 class model, see Supplemental Table 1. However, entropy showed a linear increase with number of classes. Notably, congruency with previous studies (Duits et al., 2021; Leen et al., 2021) was limited for all models tested for CS+ fear. In the 2-class model classes mostly differed in level of fear during acquisition and extinction, and in 3- and 4 class models also during preacquisition. In contrast, in Duits et al. (2021) and Leen et al. (2021) classes differed based on their responding to the CS+ only during extinction.

Supplemental Table 1. Fit indices for one-to four class Latent Growth Models based on fearfulness ratings to the CS+, including all experimental phases under study (preacquisition, uninstructed and instructed acquisition, and uninstructed extinction).

Classes	BIC	Entropy	n per class based on most likely class membership
1	7482	-	194
2	7246.34	0.878	71/123
3	7165.9	0.9	102/28/64
4	7124.31	0.923	8/64/25/97

Note: None of the models were selected for subsequent analyses.

7.S.2 CS+ US expectancy trajectories

The most substantial drop in BIC was seen moving from a 1 to a 2 class model, see Supplemental Table 2. Entropy was highest for the 3 class model. 2- to 4 class models contained one very small

class with <5 % of participants who had relatively low US expectancy upon CS+ presentation across the task. Further, the 2- to 4 class models classes did not differ in fear extinction learning (instructed acquisition vs uninstructed extinction), but in CS+ US expectancy that already was evident during acquisition.

Supplemental Table 2. Fit indices for one-to four class Latent Growth Models based on US expectancy ratings upon CS+ presentation, including experimental phases uninstructed acquisition, instructed acquisition and uninstructed extinction.

Classes	BIC	Entropy	n per class based on most likely class membership
1	5333.35	-	193
2	5191.28	0.972	189/4
3	5086.29	0.987	163/8/22
4	5051.74	0.934	16/4/154/19

Note: None of the models were selected for subsequent analyses.

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Chapter 8

General summary and discussion

The main aim of this thesis was to study the added benefit of cannabidiol (CBD) in the treatment of anxiety disorders. In this final chapter, I first present a summary of the main findings. I then give consideration to methodological strengths and limitations of the studies in this thesis. Thereafter, I discuss our findings in the context of the broader literature. I conclude this chapter with patient and clinician relevant conclusions and suggestions for further research.

8.1 Summary of main findings

8.1.1 Chapters 2 and 3: systematic literature reviews on cannabidiol and anxiety

The primary aim of the quantitative review in **Chapter 2** was to systematically review and meta-analyze the research published so far on anxiety-reducing effects of compounds that enhance the synaptic concentrations of the endocannabinoid anandamide (AEA). Our synthesis of 120 studies consisted of predominantly preclinical research conducted in animals (114 studies). Investigated compounds were the phytocannabinoid CBD (61 studies), fatty acid amide hydrolase (FAAH) inhibitors URB597 (39 studies) and PF-3845 (6 studies), and the AEA reuptake inhibitor AM404 (14 studies). The effects of these compounds on conditioned and unconditioned anxiety outcomes in animals were meta-analyzed. The pooled effects derived from individual studies showed that CBD, AM404 and PF-3845 but not URB597 reduced anxiety in tests of unconditioned anxiety and CBD, URB597 and AM404 reduced anxiety in tests of conditioned anxiety in animals. CBD was the only compound for which sufficient studies in humans were available to meta-analyze. CBD reduced experimentally induced anxiety in humans. Few studies reported on toxicity (n=17), but the profile of functional or behavioral side-effects confirmed earlier work that indicated no to very mild toxicity of CBD.

Among the moderators that we examined, publication year, the presence/ absence of a pre-existing anxiety condition and type of anxiety test were found to significantly moderate the effects of CBD on unconditioned anxiety. More recent publications were associated with smaller effects of CBD. Compared to approach avoidance tests, tests of repetitive-compulsive behavior were associated with larger effects of CBD. Larger effects were also observed in animals with a pre-existing anxiety condition (i.e. animals with increased anxiogenic behavior induced by stressor exposure). For URB597 treatment, the social interaction test was associated with smaller effects than approach-avoidance tests. A discussion of these moderator effects in the context of the broader literature is provided in section 8.3 of this chapter.

This generally positive meta-analytic evidence for anxiety reducing effects of these AEA en-

hancing drugs was put in perspective by the low quality of evidence. Information to assess risk of bias was underreported in the large majority of included studies, further there were strong indications of publication bias and high heterogeneity in study methods and effect sizes between studies. Importantly, studies with directly patient relevant outcomes in humans were too few in number to be included in our meta-analysis. Furthermore, as mentioned above, we found that more recent studies with CBD reported smaller effects compared to older publications. Such an effect of time is not unprecedented, and has been explained by the generally more rigorous methodology in later studies (Schooler, 2011). Also, null findings may be published more readily after other null findings have started to appear in the literature. This suggests that a large part of the CBD literature overestimates the true anxiety-reducing effects of this cannabinoid.

The aim of the semi-quantitative review in **Chapter 3** was to determine the therapeutic window of dosing to reach anxiety-reducing effects of CBD in humans based on preclinical and clinical studies. We combined the data of two systematic literature searches into pharmacokinetic and pharmacodynamic effects regarding anxiety reduction by CBD. We used the IB-de-risk tool, a tool that can be used to summarize data from an Investigator's Brochures of novel drugs in development, or from published preclinical and clinical literature (van Gerven and Cohen, 2018). Using this tool, we integrated the pharmacokinetic (PK), pharmacodynamic (PD) and safety outcomes from the included single administration studies (n=87) and aimed to estimate an active and safe dose range in humans. Variable CBD plasma concentration-effect relations across species (rat, human, mice) and the absence of a consistent linear effect of CBD on anxiety reduction precluded translation to a straightforward dosing recommendation in humans. However, some evidence for an inverted U-shaped dose-response curve was suggested when looking within species. This putative inverted U-shape is in line with the currently available data from human studies that reported anxiety reducing effects with oral CBD dosages between 300-600 mg, and less so with lower dosages. Studies with higher dosages in humans were largely lacking. Further research should establish whether higher dosages constitute the right leg of an inverted U-shaped dose-response curve (see 8.5) to inform optimal dosing of CBD in anxiety disorders.

8.1.2 Chapters 4, 5 and 6: clinical research with cannabidiol in patients with anxiety disorders

The effect of exposure therapy (the first-line psychotherapeutic treatment for anxiety disorders) is thought to share an underlying mechanism with fear extinction, and rodent studies suggest that CBD could enhance this type of learning. Therefore, we investigated the efficacy of CBD as an augmentation strategy for exposure therapy in our preregistered (**Chapter 4**) randomized controlled trial (RCT) (**Chapter 5**). The expectation was that this augmentation

step could provide additional benefit for patients who are refractory to currently available treatments. Study medication (300 mg CBD or placebo) was administered orally preceding 8 weekly exposure therapy sessions. We examined whether CBD augmentation would lead to faster, stronger and/or more enduring improvement on clinical outcomes compared to placebo augmented exposure therapy. We also explored enhancement of within-session fear extinction by CBD and measured CBD plasma levels at two time points of treatment. Included were 43 treatment refractory patients with panic disorder with agoraphobia and 37 patients with social anxiety disorder. Our primary outcome measure was the Fear Questionnaire (FQ), which measures level of avoidance, and our most important secondary outcome measure was the Beck Anxiety Inventory (BAI) which measures predominantly physiological and subjective fear responses. Disorder-specific outcome measures were also taken (for panic disorder with agoraphobia and for social anxiety disorder). Blinding was successful and marked CBD plasma levels were observed in the CBD condition, but not the placebo condition. This indicated high adherence to the assigned drug treatment. Adverse events were equally distributed over the CBD and placebo condition. In addition, no serious adverse events occurred.

Our results showed that regardless of assigned drug condition, patients improved over the course of therapy as FQ scores decreased. However, exposure treatment efficacy was not significantly improved in the CBD condition, compared to the placebo condition. Exploratory analyses on between session symptom change and regression analyses on within-session extinction showed that CBD augmentation did not lead to early treatment benefits. There was also no effect of CBD on long-term extinction learning, indicating an absence of more enduring effects of CBD. In conclusion, in this first clinical trial examining CBD as an adjunctive therapy in treatment refractory patients with anxiety disorders, CBD did not lead to faster, stronger and/or more enduring improvement on clinical outcomes.

The aim of the fear conditioning experiment in **Chapter 6** was to experimentally investigate the effect of CBD on attenuation of fear memory expression at retention, and enhancement of fear re-extinction in patients who participated in the RCT. To this end, a differential cued fear conditioning paradigm was administered and subjective and physiological indices of fear were measured after a single dose of 300 mg CBD. Experimental phases assessed under influence of CBD/placebo were retention and re-extinction. The second extinction phase was called re-extinction given the presence of an initial brief extinction phase, prior to CBD/placebo administration. Results showed that CBD acutely decreased threat expectation to the CS+ and CS- under increasing levels of threat imminence at retention, without affecting other indices of fear. There was no evidence for enhanced fear re-extinction by CBD. Exploratory analyses, however, showed potential disadvantageous effects of CBD in two subgroups: woman and users of sero-

tonergic antidepressants (AD users). In female AD users, CBD interfered with safety learning measured with fear potentiated startle to the CS-, and in AD users of any sex, CBD interfered with re-extinction learning measured with subjective fear to the CS+. The study was not set up a-priori to test these subgroups, so these effects must be considered as very preliminary.

8.1.3 Chapter 7: prediction of exposure treatment success

The aim of the study in **Chapter 7** was to predict which patients would remain treatment refractory, by characterizing patients prior to the start of exposure therapy using the results of a fear conditioning task. This endeavor is clinically relevant because it could help elucidate before treatment commencement who needs an augmentation strategy to standard exposure therapy. We hypothesized that the number of potentially dysfunctional latent trajectories of fear extinction and safety learning during fear conditioning would be predictive of treatment response in two independent samples. Our findings were inconclusive: Both the null hypothesis and one of the informative hypotheses received support. The null hypothesis that number of potentially dysfunctional trajectories would be unrelated to treatment response received moderate-to-substantial support in study 1 and study 2. The informative hypothesis of highest number of dysfunctional trajectories being associated with poor treatment response also received moderate support.

8.2 Methodological strengths and limitations

8.2.1 Overall strengths and limitations of this thesis

Our work provides an important contribution to research into CBD in the treatment of anxiety disorders. We synthesized a large body of preclinical literature in order to elucidate the conditions needed for such an application to be effective. In addition, we conducted the first study that investigated CBD augmentation of exposure therapy as a next step treatment for those patients who fail to respond to the available treatments in first line care. Given the size of the group of patients who remain treatment refractory despite recurrent treatment attempts, our research is of great value. We aimed to include treatment refractory patients by recruiting among patients who were referred to specialized treatment centers for anxiety. It should be noted that in order to maximize sample size we also included patients that did not receive previous treatment for their anxiety complaints but who were referred to the second line care because of their complex clinical picture with severe and/or treatment resistant symptomatology.

Because the placebo response is robust in CBD research (Gournay et al., 2023) and in psychiatric research more generally (Bandelow et al., 2015; [Box 1]), assessment of true pharmacological benefits requires inclusion of a control group that does not receive the active drug. The placebo control group receives an inactive medication that is indistinguishable from the medication under study (see Box 1). The inclusion of only placebo-controlled designs in our reviews (**Chapters 2 and 3**) and of a placebo-control group in the RCT (**Chapters 5 and 6**) is therefore a major strength of our work. Further, for the studies in this thesis methodological standards for conducting studies and reporting were followed, for systematic reviews/meta-analyses (PRISMA) and randomized controlled trials (CONSORT). Following these standards provides a protection against common risks for biased outcome reporting and can improve the reproducibility of research findings (Ioannidis et al., 2014).

Chapters 3-6 of this thesis focused on administration of synthetic (relatively pure) CBD, and not on other chemical constituents of cannabis. These other cannabinoids and terpenes, alone or in combination with CBD, may have very different pharmacokinetic and pharmacodynamic profiles. We considered these to be beyond the scope of this thesis.

Box 1 Placebo effects with cannabidiol treatment

When unraveling the therapeutic benefit many users of CBD experience, not only actual pharmacological effects should be taken into consideration. As with any treatment, when a person believes that CBD has anxiety-reducing properties, this belief alone can decrease physiological stress reactivity and anxiety (Spinella et al., 2021). Currently in the Netherlands CBD is often sold in an oil solution, and in absence of robust studies that determined the correct dose for an effect (PK/PD studies, see Chapter 3), the number of drops of CBD is increased until an effect occurs. Such a titration may actually capitalize on this placebo effect ("if only I take the 'right' dose, CBD will do its work"). In research, open-label studies have the drawback that patients and other persons involved in the research are informed about the assigned treatment condition throughout the study. This means that pharmacological and placebo effects cannot be distinguished in this type of studies.

8.2.2 Chapters 2 and 3: Systematic literature reviews on cannabidiol and anxiety

In contrast to the numerous narrative reviews into the potential of CBD for treating anxiety symptoms, **Chapters 2 and 3** employed prespecified and preregistered eligibility criteria and synthesized the literature in a (semi-)quantitative way. This work lays the foundation for mak-

ing important steps forward in the field of cannabinoid research.

In general we found extensive evidence for beneficial effects of AEA enhancing compounds in preclinical tests of anxiety in our meta-analysis. Importantly however, our assessment of the quality of evidence of the reviewed literature revealed strong indications for publication bias. Specifically, for studies with a small sample size, small and non-significant effects, and effects in the opposite direction were missing. Thus, the literature to date likely gives an overly positive picture and the meta-analytic findings are likely to be overestimations of the true anxiety-reducing effects of the investigated compounds. To date no procedures are available to estimate the extent of this bias for multilevel meta-analysis. The extent to which publication bias inflated the anxiety-reducing effects of AEA enhancing compounds demonstrated in our literature review/ meta-analysis across-the-board, is therefore unknown. We would expect more modest overall effects if works that currently remained unpublished would become available.

Our observations regarding publication bias stress the importance of study preregistration in the field of cannabinoid medicine. For animal studies, supporting infrastructure only exists since 2018 (van der Naald et al., 2021). The practice of preregistration can ensure that publication chances are less affected by whether or not findings are statistically significant or in line with prior expectations. Additionally, a Bayesian framework for statistical inference would be a good alternative to the frequentist approach with p-values as a hard cut-off for the meaningfulness of research findings.

In an effort to accelerate the development of tailored clinical applications of CBD, we applied Bayesian regularized meta-regression to research the causes of between-study variability in effect sizes. This novel technique allowed for exploring sources of between-study variability in the context of a limited number of studies.

In the semi-quantitative review of **Chapter 3** we synthesized a large body of preclinical and human literature that would otherwise have been analyzed as separate chunks of data on anxiety reduction by CBD, pharmacokinetic data and safety data. The IB-de-risk tool (van Gerven and Cohen, 2018) was developed to facilitate this integration and provides a valuable instrument for the translation of preclinical data to a dose recommendation in humans (Ferreira et al., 2023). We furthermore aimed to explain the variability in the studies' findings regarding anxiety-reduction and side effects by CBD, by drug concentrations in plasma that were measured or otherwise estimated based on available data. In line with the IB-de-risk approach, the included studies were color-coded based on statistical significance of effects, with p-values serving as a hard cut-off for anxiety reducing effects of CBD. A limitation of the reviewed literature was the sparse pharmacokinetic data available for certain administration routes, that formed the basis of some of our estimations of plasma concentrations. This means that our sorting of

studies by plasma concentrations may have lacked sensitivity to detect an association between drug efficacy and drug concentrations. Our review pointed out this omission in the literature, which should be addressed by the field to be able to inform optimal dosing of CBD in anxiety disorders in the future.

This sensitivity may also have been lowered because variability in anxiety-reduction is likely to (also) be affected by other potential causes than drug concentrations. Our synthesis, that was aimed at gaining an overall perspective, did not account for such causes. One such cause, identified in the quantitative review of **Chapter 2**, was type of anxiety test. Exploratory analyses in **Chapter 2** indicated an association between pharmacodynamic effects of AM404 and drug dose in tests of repetitive compulsive-like behavior. No linear or non-linear associations were found between pharmacodynamic effects of CBD and drug dose in tests of repetitive compulsive-like behavior in our meta-analysis. However, tests of repetitive-compulsive behavior were associated with larger effects of CBD compared to approach avoidance tests (**Chapter 2**), and some indications for an inverted U-shaped dose-response curve were found in **Chapter 3**. Based on this combined evidence, I made a post-hoc visualization of effect sizes indicating pharmacodynamic effects of CBD in tests of repetitive-compulsive behavior in mice and rats. This visualization plots effect sizes quantified by the statistic 'Hedge's' (see Figure 1), and is suggestive of a curvilinear relation between drug dose and effect size. Activation of the transient receptor potential vanilloid subtype 1 (TRPV1) may explain why anxiety-reducing effects disappear at higher CBD concentrations (Campos and Guimarães, 2009); (see further: 8.3.5). Whereas this dose-effect relation for repetitive-compulsive behavior in rodents cannot be directly translated to humans, the pattern shown here strengthens the evidence for an anti-compulsive effect of CBD (see further: 8.3.3).

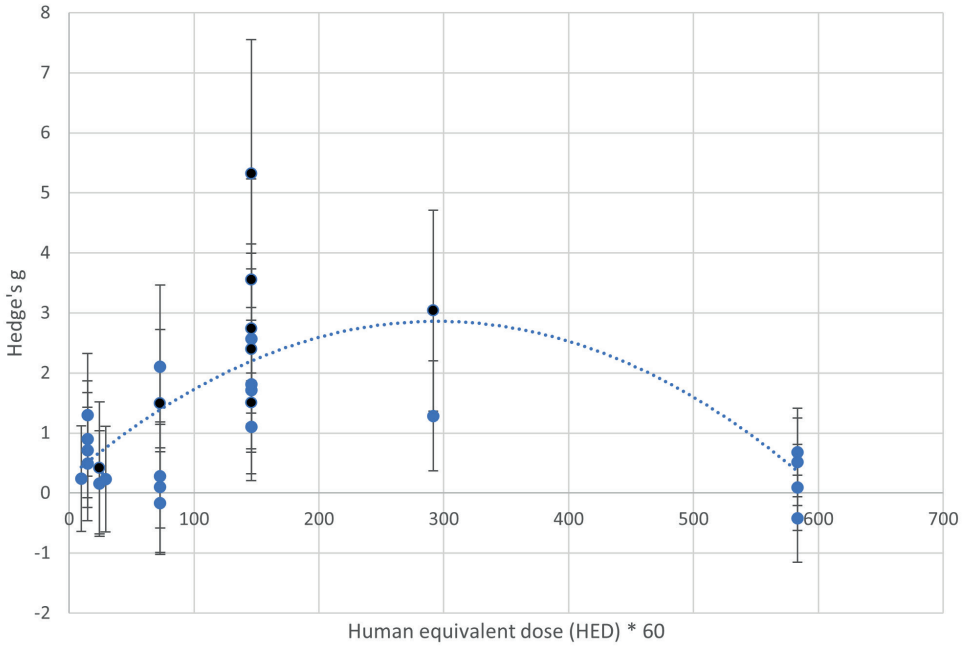

Figure 1. Effect sizes for single CBD administration in tests of repetitive compulsive-like behavior in rodents plotted against human equivalent dose in mg/kg * 60 to arrive at an equivalent human dose.

Note: A second order polynomial trendline is fitted to the data. Black data points refer to effects of Casarotto et al. (2010; see 8.3.3).

8.2.3 Chapters 4, 5 and 6: Clinical research with cannabidiol in patients with anxiety disorders

Pioneering in the field of human CBD research, we based our choice for a 300 mg dosage on the significant anxiolytic effects that had been demonstrated in a stress exposure paradigm, indicating that this dose is high enough to exert significant effects on the fear system (Crippa and Zuardi, unpublished data, personal communication). In addition, the data available to date suggest that anxiety-reducing effects of CBD in humans occur with 300-600 mg doses (see **Chapter 3**). However, considering that the measured plasma concentrations in the CBD condition of our RCT (**Chapter 5**) fall at the lower end of what seems to be the most effective concentration range in humans (**Chapter 3**), the possibility still exists that we might have applied a subtherapeutic dose. This could hypothetically be an explanation for the null effect of CBD on exposure treatment outcome and on fear re-extinction in a fear conditioning task, in patients with

panic disorder with agoraphobia or with social anxiety disorder (as discussed in **Chapters 5 and 6**). However, in the fear conditioning experiment, we did find a difference between CBD and placebo on threat expectancies at retention. In any case, given that a dissolving vehicle improves drug absorption and minimizes variability in plasma levels after oral CBD consumption (Izgelov et al., 2020, **Chapter 3**), plasma levels in our RCT could have fallen well within the most effective concentration range when a dissolving vehicle would have been used. Our application of CBD in powder form at a relatively low dose should therefore be considered a limitation.

Our design in **Chapter 6** allowed for assessing effects of CBD on fear memory expression as well as fear re-extinction. Due to practical reasons we did not include a second retention test after extinction learning. This could have given more insight into longer-term benefits of CBD in patients with anxiety disorders that may result from drug-enhanced memory consolidation after extinction learning. Some evidence for an effect of CBD on extinction consolidation may be found in differences in freezing between CBD and vehicle treated rats that were re-exposed to the context where fear conditioning took place (Song et al., 2016). Such differences were not observed in rats during a memory retention test after cued fear conditioning (Jurkus et al., 2016). In healthy individuals, Das and coworkers (2013) provided evidence for enhancement of extinction consolidation by CBD, indicated by decreased shock expectancy (but no effects on skin conductance) during a fear reinstatement task. Investigation of CBD on extinction memory consolidation would be highly valuable, considering these currently inconclusive findings about the effect of CBD on extinction memory consolidation and the evidence for involvement of CB1 receptors in (fear) memory consolidation under highly stressful conditions (Campolongo et al., 2013; de Oliveira Alvares et al. 2010; Morena et al., 2014) [Box 2].

In our randomized controlled trial (RCT) administration of CBD preceded exposure treatment sessions (**Chapter 5**). This choice for administration was based on a previous study that suggested an association between human CB1 receptor functioning and within-session extinction learning (Heitland et al., 2012). We left the possibility open that CBD in our study would affect consolidation of extinction learning apart from effects on within-session extinction learning, because the drug is not expected to be fully eliminated from the body by the time a treatment session ended (Millar et al., 2018). This allowed us to explore CBD enhancement of consolidation of (mal)adaptive learning during exposure therapy sessions, for which we found no evidence.

Box 2 The endocannabinoid system and memory consolidation under highly stressful conditions

The involvement of the endocannabinoid system (ECS) in stress has been repeatedly demonstrated in rodent research. Under normal circumstances, in the absence of ECS manipulations, CB1 receptor signaling contributes to neuronal, endocrine and behavioral responsivity to acute stress (Hill et al., 2009; Patel et al., 2005; Rademacher et al., 2008) and habituation to stress (Hill et al., 2010; Patel et al., 2005; Rademacher et al., 2008).

Importantly, effects of endocannabinoids have mostly been observed in the regulation of memory function under high rather than low stress conditions in rodents. For example, one study demonstrated CB1-mediated enhancement of memory consolidation after an aversive experience, as measured by active avoidance behavior in rats (Morena et al., 2014). Effects on memory consolidation can be isolated by administering the compound after the aversive experience. Such an experience led to increased AEA levels in the BLA, hippocampus and medial prefrontal cortex, but a less aversive experience had no effect (Morena et al., 2014). Experimentally, effects of AEA enhancing compounds on memory consolidation were demonstrated by administering these compounds after the aversive experience (Morena et al., 2014). Two other studies also showed effects of an ECS manipulation under aversive conditions. In the first study, object recognition was improved in rats who were injected with a CB1 agonist after initial object exploration under high (but not low) arousing conditions (Campolongo et al., 2013). In the second study, freezing behavior was decreased in rats who had a CB1 antagonist infused in the dorsal hippocampus after strong (but not weak) foot shocks (de Oliveira Alvares et al. 2010). The involvement of CB1 receptors in memory consolidation demonstrated in these studies suggests that enhancing CB1 receptor activation may also have a facilitating effect on consolidation of extinction learning.

A strength of our human fear conditioning study (**Chapter 6**) is the use of multiple indices of fear to measure anxiety-reduction, including fear-potentiated startle, skin conductance response (SCR), shock expectancy and subjective fear. Whereas subjective ratings that reflect declarative knowledge about cues that signal danger, and subjective and physiological in response to these cues are strongly associated (Baas et al., 2008; 2013; Hamm and Vaitl, 1996; Soeter and Kindt, 2010), a dissociation in the effects of pharmacological agents on these different output systems has also often been observed (Baas et al., 2002; Graham et al., 2005; Heitland et al., 2012; Soeter and Kindt, 2010). Put differently, an anxiety reducing effect of a drug on one out-

put system, but not on the other, is not uncommon. Thus, to not overlook an anxiety-reducing effect of CBD, our inclusion of multiple fear indices is of great importance.

In our study, CBD only attenuated shock expectancy at retention, other fear indices were unaffected by the drug. In theory, a failure to detect SCR differences between drug conditions may be due to our search window of 0.9-4 s after stimulus onset (following Duits et al., 2017), that might have been too restrictive. Perhaps a broader search window may have been more sensitive. However, our method was sensitive enough to detect differential conditioning at the end of fear acquisition (high SCR to the CS+ and low SCR to the CS-).

We explored effects of CBD in subgroups (of AD users and non-users; men and women; patients with panic disorder with agoraphobia, and patients with social anxiety disorder) in the fear conditioning task (**Chapter 6**). For the clinical outcomes in the RCT (**Chapter 4, 5**) we took a more stringent approach as this was adequately powered for main analyses regarding clinical outcomes, but not for such subgroup analyses. In addition, the sample size of the experimental part of the study was even smaller because not all participants could be included. Hence, even though subgroup analyses are important, the reduced statistical power may have made the study less sensitive to detect smaller sized effects. Reversely, the effects that were detected are at risk of being unrealistically large (Button et al., 2013). The potentially disadvantageous effects of CBD that we observed during fear re-extinction in AD users (subjective fear) and in female AD users (fear-potentiated startle) (**Chapter 6**) therefore require further investigation in adequately powered, independent samples, also with respect to directly clinically relevant outcomes.

8.2.4 Chapter 7: Prediction of exposure treatment success

The investigation of prediction of treatment nonresponse by dysfunctional latent trajectories of fear extinction and safety learning (**Chapter 7**) was aimed at following-up on earlier preliminary findings in a similar sample of anxiety disordered patients (Duits et al, 2021). Our analyses yielded inconclusive findings. The challenges of including patients in experimental studies make that most of these studies, including our own, are underpowered (Forcadell et al., 2017; Lange et al. 2020; Lubin et al., 2023; Raeder et al., 2020; discussed in section 8.3.7). Efforts to make the fear conditioning task easier to administer are likely to lead to larger sample sizes in this type of prospective research (Leen et al., 2021). We took a Bayesian approach to statistical analyses to acquire information on the strength of evidence for our results. This approach yields more informative outcomes (a probability of the hypothesis being true) than a frequentist approach that yields point estimates (p-values) and is therefore also suitable for small samples. When more data becomes available the evidence will become more strongly in favor of one of

the hypotheses that currently are plausible (Kuiper et al., 2012; Moerbeek, 2021). In our study we focused on the predictive value of fear extinction and safety learning, and did not investigate individual differences in general threat anticipation, which would be a worthwhile avenue for future research considering the demonstrated cross-sectional association with anxiety disorders (Abend et al., 2020). Further, our pretreatment fear conditioning task did not include an assessment of fear extinction retention. This could have been a relevant addition as a study in individuals with spider phobia showed an association between lower US expectancy at retention and completing exposure steps in a predetermined time (Raeder et al., 2020). Additionally, this study probably has an increased signal-to-noise ratio compared to our own work due to the more homogenous group of participants and prediction target.

8.3 Findings in the context of the broader literature

8.3.1 An interpretation of mixed effects of cannabidiol on anxiety outcomes

As outlined in **Chapter 1**, despite the availability of evidence-based psychotherapeutic and pharmacotherapeutic treatments for anxiety disorders, many patients are treatment refractory. A promising avenue for these patients are drugs that act synergistically with psychotherapy. Considering the involvement of the endocannabinoid system (ECS) in emotional memory processing under stressful conditions (e.g., Atsak et al., 2012; Campolongo et al., 2013; de Oliveira Alvares et al. 2010; Morena et al., 2014) and in fear extinction specifically (e.g., Marsicano et al., 2002; Pamplona et al., 2006; Suzuki et al., 2004), it made sense to investigate CBD in patients who have difficulties with fear extinction and are plagued with recurrent excessive fear. However, our findings in an experimental fear conditioning task did not show enhancement of fear re-extinction by CBD in patients with social anxiety disorder or panic disorder with agoraphobia (**Chapter 6**). Corroborating these laboratory-based findings, CBD as an adjunctive therapy to exposure treatment did not improve treatment outcome in these same patients (**Chapter 5**).

These results are contrary to our expectations and contrast with the acute anxiety reducing effects of CBD in the 300-600 mg range that have been found in other studies in social anxiety in humans (Bergamaschi et al., 2011; Crippa et al., 2011; Linares et al., 2019; Masataka, 2019; Zuardi et al., 1993; 2017), and in one human study that used an anxiety provocation test (Crippa et al., 2004).

The null effects on fear re-extinction in the fear conditioning experiment in **Chapter 6** contrast with the pivotal role of the CB1 receptor in fear extinction demonstrated in mice (Marsicano et al., 2002) and with our meta-analytic evidence for anxiety-reduction, including

effects on fear-extinction in animal studies (see **Chapter 2**). One explanation for this null finding in humans may be that re-extinction, in contrast to extinction, is not susceptible to CBD, given that these types of fear learning have distinct susceptibilities to pharmacological intervention (Hart et al., 2009). Since we did not include an assessment of CBD's effects on initial extinction, this needs further verification. However, at face value, the choice to study effect on re-extinction aligns better with exposure therapy in clinical practice, which is also not aimed at recently acquired fears. Therefore, re-extinction may be a better model for extinction during exposure therapy (Craske et al., 2018), especially in treatment refractory patients. Whether the difference between initial extinction and later extinction training may explain the null findings in this thesis awaits further research.

Our findings do fall into a pattern seen in more recent randomized placebo-controlled studies into anxiety-reducing effects of CBD in humans. These studies that are too recent to have been included in our (semi)quantitative reviews of Chapter 2 and 3 also yielded mixed findings. In these studies, CBD was investigated as a stand-alone therapy rather than as an augmentation of learning processes during extinction or exposure therapy.

In the first of these more recent studies, the effect of 150-600 mg CBD was investigated in healthy individuals on self-reported fear and panic symptoms and the heart rate increase induced by a biological challenge (inhaling 10% carbon dioxide (CO2)-enriched air, n=84; Leen-Feldner et al., 2022). No effect of CBD on fear reactivity was found, and the research group subsequently hypothesized that CBD may affect cognitive aspects of anxiety (i.e., worry), based on previous findings of a CBD-induced reduction of social evaluation concerns (Bergamaschi et al., 2011). The question whether CBD would reduce worry severity was investigated in individuals with elevated trait worry (n=63; Gournay et al., 2023). Acute effects and effects after 2 weeks of repeated dosing of 50 mg and 300 mg of CBD on worry severity were investigated, but no CBD/placebo differences were found. A third study investigated the effect of a single dose of 300 mg orally administered CBD on symptoms associated with recalling a traumatic memory in patients with posttraumatic stress disorder (PTSD; Bolsoni et al., 2022). Also in this study, the hypothesized effect was not there.

However, other effects that were not specified in the main hypotheses were reported: Greater reductions in physiological arousal symptoms (during the past week) after repeated dosing of 300 mg CBD (Gournay et al., 2023) and attenuation of cognitive impairment associated with traumatic memory recall by a single dose of 300 mg CBD (Bolsoni et al., 2022) are suggestive of beneficial effects of CBD on specific aspects of anxiety. However, the method of measuring arousal and cognitive impairment (i.e., self-report rather than a physiological measurement or behavioral test (Bolsoni et al., 2022; Gournay et al., 2023)) precludes strong

conclusions about the exact nature of these effects. Together with the evidence from this thesis, these studies suggest that CBD may have beneficial effects only on specific aspects of anxiety. In general, the current absence of a straightforward dosing recommendation should be taken into consideration when interpreting these findings. There is a possibility that plasma (and brain) concentrations in these studies fell outside of the window for therapeutic effects of CBD.

An effect on cognitive impairment as found in PTSD patients (Bolsoni et al., 2022) may suggest that CBD affects processes that are not unique to anxiety or fear. This possibility is supported by findings from fear conditioning experiments: CBD reduced threat expectancy at retention in our clinical sample (**Chapter 6**) and during a fear reinstatement phase in healthy individuals (Das et al., 2013), without affecting other indices of fear. Interestingly, emotion and cognition are interrelated processes in patients with anxiety disorders (e.g., Arntz et al., 1995). That is, patients with anxiety disorders seem to rely not only on objective information, but also on their anxious feelings when assessing the amount of danger when facing threat situations (Arntz et al., 1995). This interplay between emotion and cognition is reflected in the concept of 'anxiety sensitivity', which is the tendency to respond fearfully to anxiety symptoms based on expectancies of negative consequences of these symptoms (McNally, 1989; Reiss et al., 1986). Anxiety sensitivity is increased in patients with anxiety disorders compared to healthy individuals (Olatunji & Wolitzky-Taylor, 2009).

8.3.2 Cannabidiol and social anxiety

To date, the type of anxiety that has been investigated most in CBD research in humans entails social anxiety, with generally beneficial effects of CBD (Bergamaschi et al., 2011; Crippa et al., 2011; Linares et al., 2019; Masataka, 2019; Zuardi et al., 1993; 2017). Our meta-analytic results suggest that increasing anandamide (AEA) levels may lead to social withdrawal in rodents, operationalized as less time in active social interaction (**Chapter 2**). For the drug URB597 that shares FAAH inhibition as the mechanism of action with CBD, there was a negative median effect size for the social interaction test (Hedge's G = -2.65), indicating that animals in the URB597 condition spent less time in active social interaction compared to animals in the placebo condition. Although statistically, the effect of CBD in the social interaction test was not different from the effect in approach avoidance tests, numerically, effect sizes were relatively small for this test (median Hedge's G =0.13).

One potential interpretation of these seemingly contradictory results may be found in parallel cannabinoid effects in the dopaminergic (reward) system. This view is based on ideas about how emotional salience attributions during social interaction may be changed by CBD. The mesolimbic system is involved in attributions of salience to incoming stimuli that signal

threat or opportunity for reward (Hudson et al., 2018). CBD injected into the cerebral ventricles of rats induced increases in dopaminergic signaling in the mesolimbic neural circuit (Murillo-Rodríguez et al., 2006), with the nucleus accumbens (Norris, 2016), and the ventral hippocampus (Loureiro et al., 2015; 2016) as potential sites of action. By changing salience attributions, CBD potentially changes motivational needs, such as the need to explore the environment. There are several examples in which CBD changed the animal's original responsiveness to both potential threat and to reward, depending on the level of threat and opportunity for reward in the experimental situation. Stimulating intra-ventral hippocampus CB1 receptors in rats increased responses to stimuli that are normally below the threshold for both anxiety responses (stronger anxiety-like behavior after low intensity foot shocks; Loureiro et al., 2016) and for subthreshold rewarding stimuli (increase place-preference related to low dose morphine injections; Loureiro et al., 2015; 2016). Conversely, this ECS manipulation blunted the emotional response to normally suprathreshold aversive stimuli (i.e.; high intensity foot shocks; Loureiro et al., 2016), and decreased the animals' natural preference for social novelty (Loureiro et al., 2015; 2016). CBD injected into the nucleus accumbens shell blocked freezing behaviors in rats who were fear conditioned to an olfactory cure, whereas significant freezing was observed when no active drug was administered (Norris et al., 2016). These findings indicate that effects of CBD and perhaps also other FAAH inhibitors are sensitive to the test situation and depend on how strongly salient the aversive and/or appetitive experimental manipulations are.

In human cannabinoid research a social stress test is often applied, i.e., Simulated Public Speaking Test (Bergamaschi et al., 2011; Linares et al., 2019; Zuardi et al., 1993; 2017). This test subjects participants to a threatening situation, especially for socially anxious individuals who may fear to be scrutinized. In this context, a reduction in salience of threatening aspects of the situation by CBD may in fact explain the anxiety-reducing effects on the participant. In contrast to the high social threat test in humans, the social interaction test in rodents is a milder social challenge and may not primarily measure anxiety-like behavior. It rather measures social behavior, which for a large part is modulated by social motivation (Davidson and Gabos-Grecu, 2020). Based on the literature regarding differential effects of CBD depending on the intensity of incoming stimuli reviewed in the preceding paragraph, CBD could decrease an animal's motivation for (potentially rewarding) social interactions, which may explain the relatively small effect sizes of CBD and negative effect sizes of the FAAH inhibitor URB57 in the social interaction test (see **Chapter 2**).

What could these preclinical findings mean for effects of CBD in clinical populations? Speculating, it could be that when using CBD patients may feel less motivated to engage in social interactions or be less perceptive to reinforcement by the therapist. For patients with panic

disorder with agoraphobia, it is extremely hard to avoid major fear-evoking situations once in therapy, because the typical phobic situations in panic disorder and agoraphobia are readily encountered (e.g., crossing the street, using public transport, experiencing physical arousal symptoms). On the other hand, patients with social anxiety disorder may avoid fear-evoking situations more easily. This is especially true for those who are currently not in the workforce or otherwise isolated from society as a consequence of the disorder. If, theoretically, CBD would make engagement in social activities even less appealing than it already was to socially anxious individuals, this could hinder participation in exposure exercises and be detrimental for treatment outcome. Indeed, this is in line with a finding in our RCT (Supplemental material of **Chapter 5**). In the completers sample of patients with social anxiety disorder the CBD group had worse exposure treatment response compared to placebo, which was contrary to expectations. Exploratory post-hoc analyses (not reported in this thesis) showed that CBD exerted this effect on social anxiety symptoms only in women.

Cannabinoid research with a focus on other target populations than anxiety disorders can be informative. Disturbances in social behavior are not only observed in anxiety disorders, but also in the context of schizophrenia. In an animal model of schizophrenia cognitive deficits (Seillier et al., 2010) and social withdrawal (Seillier et al., 2010; 2013) were mediated by aberrant endocannabinoid transmission. In this schizophrenia model, increasing AEA levels with a FAAH inhibitor increased time in social interaction (Seillier et al., 2010; 2013). In healthy animals, however, the FAAH inhibitor URB597 (Seillier et al., 2010; 2013; 2018) and an AEA reuptake inhibitor (OMDM-2; Seillier et al., 2018) decreased time in social interaction. It has been suggested that only when AEA levels in the medial prefrontal cortex and amygdala are deficient, pharmacologically elevating them has beneficial effects on social behavior (Seillier et al., 2013). With an eye on translation to (socially) anxious individuals, it would be interesting to also study this in animals with a pre-existing anxiety condition, e.g., persistent anxiety-like behavior after exposure to predator-scent (Shallcross et al., 2019).

8.3.3 Potential application of cannabidiol in obsessive-compulsive disorder

Our meta-analysis showed a larger reduction of unconditioned anxiety by CBD in animals with a pre-existing anxiety condition, in whom anxiogenic behavior was induced by a stressor, than in animals without (**Chapter 2**). This finding seems to imply that CBD indeed may be a reasonable drug candidate for treating pathological anxiety. We also made an important step forward in identifying conditions under which clinically beneficial effects of CBD can be expected. Based on our meta-analytic results (**Chapter 2**), larger effects of CBD on anxiety-like behavior were seen with tests of repetitive compulsive-like behavior when compared to approach avoidance

tests. Tests of repetitive compulsive-like behavior are often employed as a screening tool for the pharmacological treatment of obsessive-compulsive disorder (OCD; de Brouwer et al., 2019). The preclinical literature provides strong evidence for an anti-compulsive effect of CBD in rodents [Box 3].

Box 3 Anti-compulsive effect of cannabidiol in rodents

Most included studies on repetitive-compulsive behavior in our meta-analysis used the marble burying test (MBT), in which a mouse is placed in an arena with marbles and burying material, with the number of marbles buried within a given time as an indicator of repetitive compulsive-like behavior. Because burying is part of the normal behavioral repertoire of rodents, clear criteria should be applied to determine when marble burying constitutes a pathological process. For example, the C57BL/6J mice strain previously showed higher burying scores than other strains, in addition to spontaneous avoidance of the marbles (Nicolas et al., 2006) and may therefore be an anxious behavioral phenotype with translational relevance for human OCD (de Brouwer et al., 2019). In only one of our included studies in **Chapter 2** marble burying was measured in the C57BL/6J strain (Casarotto et al., 2010). This study yielded large effect sizes of CBD on marble burying relative to other studies (see black data points in Figure 1). Further, CBD reduced marble burying with no effect on locomotor activity with the same dose (Casarotto et al., 2010; Nardo et al., 2014). This combined effect is characteristic for most known anxiety reducing drugs (Nicolas et al., 2006). In addition, the dose-effect associations for AM404 and CBD treatment on tests of repetitive compulsive-like behavior (**Chapter 2** and Figure 1 in the current chapter) strengthen the evidence for an anti-compulsive effect of CBD (Schünemann and Santesso, 2010).

In patients with OCD, compulsions are often performed in response to obsessions, with the function to reduce excessive fear and anxiety (Abramowitz and Jacoby, 2015). The focus on compulsions of preclinical models could be a limitation to the translational validity of these models. Research translating these effects to humans is scarce. A first placebo-controlled study in patients with OCD (n=14) showed no acute effect on OCD of smoked cannabis containing primarily tetrahydrocannabinol (THC) or CBD (Kayser et al., 2020). However, this area certainly deserves further investigation.

8.3.4 Potential explanations for AD use- and sex-specific effects of cannabidiol

In the experimental study we included sex and use of antidepressants as moderators and found preliminary evidence that both moderators may influence effects of CBD. Note that the interactions between CBD and AD use and sex are based on very preliminary data, and post-hoc analyses of the data from the randomized controlled trial in the same patient sample (**Chapter 5**) suggest that the findings regarding interactions with AD use in our laboratory task do not translate to clinical outcomes. Here, I discuss two potential explanations for the AD-use and sex-specific effects of CBD in **Chapter 6:** 1. desensitization of 5HT1A receptors in AD users and 2. metabolic drug-drug interactions.

Regarding the first potential explanation for the AD-use specific effects of CBD, it is important to note that CBD does not only act on cannabinoid receptors. Other targets seem to be also pivotal in mediating this cannabinoid's therapeutic effects, including agonism of CBD at serotonin 5HT1A receptors (Russo et al., 2005) which may mediate some of its stress-protective effects (Campos et al., 2012; Rock et al., 2017). What is more, 5HT1A receptor desensitization appears to underly the therapeutic effects of repeated administration of both CBD and selective serotonin reuptake inhibitors (SSRIs; Blier, 2010; de Gregorio et al., 2019). After a single administration however, CBD dose-dependently decreased firing rate of 5HT neurons in the rat dorsal raphe nucleus through 5HT1A and transient receptor potential vanilloid subtype 1 (TRPV1) receptor mediated mechanisms (de Gregorio et al., 2019). Because patients who are on a stable SSRI regimen are expected to have desensitized 5HT1A receptors (Blier, 2010), we could hypothesize that they are less responsive to the effects of a single CBD dose. However, because disadvantageous effects of CBD were found only in AD users, but not in AD nonusers (**Chapter 6**), this line of reasoning does not easily explain observed effects.

The second potential explanation for CBD/AD and CBD/sex interactions may be found at the level of pharmacokinetics of both ADs and CBD. Several medications including traditional ADs are inhibitors of the CYP2C19 enzyme (Brown and Winterstein, 2019), which is an important enzyme in the metabolism of CBD (Beers et al., 2021). Therefore, CBD concentrations may be higher in AD users compared to AD nonusers. This may theoretically explain the potentially disadvantageous effects of CBD in AD users, in contrast to non-users (**Chapter 6**). Given the unclear concentration-effect associations for CBD (elaborated upon in 8.3.5), at this point in time we can only speculate that differential pharmacodynamic effects in AD users versus AD nonusers are explained by differences in available CBD plasma concentrations. Effects of CBD in women but not in men may possibly be explained by the use of oral contraceptives. That is, women using oral contraceptives show reduced activity of the CYP2C19 enzymes, compared

to non-users (Hägg et al., 2001), which would theoretically result in higher CBD concentrations in the former group. Note that no data on oral contraceptives use was collected in our studies, and even though the popularity of the oral contraceptive pill is declining, still a third of adult women in the Netherlands use it daily (CBS, 2014).

It is important to note that CBD is often used under the assumption that it has such a favorable side effects profile, and that if it does not help, it does not hurt either. Even if the indications of potential interactions in our data are preliminary, it is important to further investigate the possibility that CBD may have disadvantageous effects on anxiety symptoms in patients that also use traditional anxiolytic medication. Also a potential moderating effect on anxiety reduction by CBD of the subject's sex and/or use of oral contraceptives certainly deserves attention in future research.

8.3.5 What constitutes an effective cannabidiol dose?

Given the absence of clear concentration-effect associations across and within species (**Chapter 3**), at this point in time it is difficult to establish what would ascertain 'effective' and 'ineffective' CBD doses. Despite some suggestions in the literature for an inverted U-response curve for the anxiolytic effects of CBD, whether this truly exists is disputable. In general, for psychotropic medications linear relations between dose and therapeutic effect have been demonstrated in humans (Bishara et al., 2013; Jakubovski et al., 2016; Linssen et al., 2012), with unwanted effects also becoming larger with increasing doses (Hunault et al., 2014; Kinon et al., 2001). However, for other cannabinoids such as THC (e.g., El-Alfy et al., 2010) an inverted U-shaped dose-response function has been demonstrated, however, Hunault et al. (2014) reported multiple linear dose-response functions for THC, except for 'wanting more of this drug' (inverted U-shape). In theory, for CBD, multiple dose-response (desired and undesired) relations in combination lead to the optimal dose to be somewhat in the middle of the dose range. In this context it is worth mentioning that it is not likely that there is one 'good' dose for everyone. This is likely to depend, among many other individual difference factors, on the presence and type of co-administered medication.

AD users, but not AD non-users who were assigned to placebo showed fear re-extinction on the startle outcome measure (Supplemental material of **Chapter 6**). It is noteworthy because it is in line with meta-analytic evidence that points out that SSRIs may facilitate fear extinction to a cue (Heesbeen et al., 2023). However, no fear re-extinction as measured with startle was observed in AD users who received CBD (**Chapter 6**). Activation of TRPV1 may explain why anxiety-reducing effects disappear at higher CBD concentrations (Campos and Guimarães, 2009). The extinction-facilitating effect of certain SSRIs is thought to be mediated by increases

in AEA levels in the brain (Gunduz-Cinar et al., 2016). Therefore, if CBD theoretically induces additional AEA increases (Leweke et al., 2012), agonistic properties at TRPV1 detrimental to anxiolysis (Bisogno et al., 2001, Ross, 2003) may be achieved even faster. Together this could mean that the addition of CBD may cause an excess and thereby counteract beneficial effects of traditional antidepressants. The importance of hitting just the right dosage was seen with beneficial effects of only a moderate dose of CBD (300 mg) in a Simulated Public Speaking Test and no such effect at higher doses (600-900 mg; Linares et al., 2019; Zuardi et al., 2017).

8.3.6 Potential adverse health effects of cannabidiol

Regarding unwanted effects of CBD, included studies in our literature reviews (**Chapters 2 and 3**) reported no severe adverse events after CBD administration, except for drowsiness and piloerection in rats at extremely high dosages that in humans would be equivalent to ~1000 times the dose employed in our RCT in patients (**Chapters 4, 5 and 6**)). In addition, no serious adverse events were reported in our RCT. Our findings thus suggest that CBD is safe to use at thus far commonly used dose ranges, and they are in line with relatively low toxicity of CBD according to the World Health Organization (WHO, 2018). However, a recent review of toxicological effects of orally administered CBD concluded that CBD has potential for inducing adverse health effects in humans, such as liver damage and adverse effects on the male reproductive system (Gingrich et al., 2023). This review was aimed at highlighting potential dose-dependent adverse effects for future studies to be aware of. Importantly, the authors did not investigate at which levels of drug exposure no adverse effects occurred.

8.3.7 Who will need an alternative or augmented treatment?

In the prospective study in **Chapter 7** we investigated who may become treatment refractory, by characterizing patients prior to the start of exposure therapy using the results of a fear conditioning task. The inconclusive findings of this investigation add to the findings of other human studies. In these studies, that also included a clinically relevant fear or extinction retention phase (Forcadell et al., 2017; Lange et al. 2020; Lubin et al., 2023; Raeder et al., 2020), associations of pretreatment fear conditioning parameters with outcomes of clinical exposure therapy (analogues) were largely variable. Better fear extinction as measured by less CS+CS- discrimination on US expectancy (but not fear potentiated startle or SCR) was associated with greater fear reduction on one outcome of an exposure therapy analog in participants with moderate to strong fear of spiders (n=50; Forcadell et al., 2017); higher ventromedial prefrontal cortex activation upon CS- presentation during early extinction was associated with better exposure

therapy response in individuals with spider phobia (n=25; Lange et al., 2020), while subjective fear ratings were not significantly associated with therapy response (Lange et al., 2020); in another study fear extinction and retention as CS+CS- discrimination on SCR and US expectancy ratings were not associated with cognitive-behavioral treatment response in patients with social anxiety disorder (n=59; Lubin et al., 2023); lastly, lower US expectancy at fear or extinction memory retention (but not SCR) was associated with exposure completion in a study in individuals with spider phobia (n=53; Raeder et al., 2020).

Given the absence of direct replications, the usefulness of fear conditioning paradigms to predict who may or may not benefit from exposure therapy is as of yet unclear.

8.4 Clinician- and patient relevant conclusions

In this section I put forward clinician- and patient relevant conclusions that follow from this thesis. In my opinion, the currently available evidence is insufficient to justify a recommendation that CBD should be approved for treating anxiety disorders.

To date, the field is only beginning to investigate how CBD may be applied in patients with anxiety disorders. Nevertheless, it can be expected that some health care professionals and patients may still consider prescribing/taking CBD for anxiety complaints, especially when there are no better alternatives available to traditional anxiolytic drug treatment. Vital questions may arise: "Is there any benefit of taking CBD?" Who exactly can benefit from CBD? What is the right dose? Is the drug safe?" Currently, there are no clear-cut answers to the question whether CBD can be a bliss for those suffering from anxiety disorders, nor how it should be applied to be effective. While I feel the obligation to communicate as clearly as possible what we have learnt and now know more about the added benefit of CBD in the treatment of anxiety disorders, further investigation is still needed.

Our study in patients with social anxiety disorder and panic disorder with agoraphobia showed that 300 mg oral CBD before weekly exposure therapy sessions did not improve therapy outcome compared to placebo (**Chapter 5**). We can therefore conclude that this specific augmentation application is not effective. A different treatment regimen, for example repeated daily use, may be more promising (Masataka, 2019). With respect to drug dose, anxiety reducing effects in humans have been observed with 300-600 mg orally administered CBD. These observations were predominantly after single dosages of CBD; it is too early to determine what would be the most effective dose range with a daily dosing schedule. Due to variable drug concentration-effect associations across species, however, at this point in time a straightforward dosing recommendation is out of place (**Chapter 3**). Alternative applications, for example us-

ing other routes of administration such as inhalation or sublingual (under the tongue) administration, direct entering of the bloodstream leads to high bioavailability of CBD relative to oral administration (Fleisch, and Woodbridge, 2022). The practice of titration (increasing the dose until a therapeutic effect is achieved) is currently common practice for cannabis-based products including CBD (MacCallum and Russo, 2018). Because pharmacokinetic data are scarce for CBD, what constitutes an 'optimal dose' for the different routes of administration needs further investigation.

Preliminary findings of potentially disadvantageous effects of CBD in a fear conditioning task (**Chapter 6**) seem to contradict the assumption that if it does not help, it does not hurt either. Although our literature reviews showed that adverse effects after CBD administration are mild to moderate at most (**Chapters 2 and 3**), potential health hazards related to CBD consumption are becoming clearer, especially with high and/or chronic dosing, in women of reproductive age, and patients who are on other over the counter or prescription medications (Gingrich et al., 2023). Fortunately, discontinuing CBD use should not be difficult. Rodent and human studies showed that CBD has low potential for abuse (WHO, 2018) and does not lead to withdrawal symptoms after discontinuation (Taylor et al., 2020).

8.5 Suggestions for further research

Despite the less than satisfying answers to clinically relevant questions, our work is a step forward in narrowing down potential clinical applications of CBD and other endocannabinoid enhancing compounds. In this section I offer suggestions for expansion upon the current state of knowledge. This list is non-exhaustive; it is focused on research that has the potential to bring a therapeutic application of CBD in anxiety disorders closer.

As introduced in **Chapter 1**, cognitive behavioral therapy (CBT) relies on two strategies: one aimed at restructuring dysfunctional cognitions and one directly targeted at behavioral change. We investigated CBD augmentation of exposure therapy, a treatment that puts emphasis on approaching fear-eliciting situations (**Chapter 5**). Theoretically, based on the results of our fear conditioning task on threat expectancies (**Chapter 6**), it could be hypothesized that CBD enhances cognitive restructuring of the threat and harm overpredictions that are characteristic for anxiety disorders (Beck et al., 1987; Beck and Emery, 1985). Ultimately, as a result of change in dysfunctional cognitions, overcoming avoidance may also become more feasible to the patient. An interesting research question, therefore, may be whether CBD could enhance (interim) outcomes of this first cognitive pillar of CBT.

In a fear conditioning task CBD differentially affected AD users and non-users, and men

and women (**Chapter 6**). These findings, although very preliminary, have potential clinical significance. To effectively examine these potentially important variables including sex and AD use, larger sample sizes are needed. This is especially important because drug efficacy is likely to be affected not only by AD use and sex, but by a plentitude of factors that make up a patient's past and current biopsychosocial environment (King et al., 2019). Some of these factors may affect CBD's pharmacokinetics, e.g., diet (**Chapter 3**), sex (Gandhi et al., 2004) and include sex-specific factors such as the use of oral contraceptives (Hägg et al., 2001). Other factors have in common with CBD that they affect the endocannabinoid system (ECS), e.g., repeated stress exposure (Patel et al., 2005; Rademacher et al., 2008). These effects of stress exposure have been mainly studied in animals. Research into the effects of stress on the ECS in humans and potential implications for the treatment of anxiety disorders, is still in its infancy (e.g., Leen et al., 2022).

In **Chapter 7** the fear conditioning paradigm was used as a tool to predict who may need an augmentation strategy for exposure therapy. This line of research started with the study by Duits et al. (2021). We have not yet looked into prediction of CBD augmentation efficacy by differences in pre-treatment fear conditioning responses yet. However, this approach seems promising (Lubin et al., 2023).

In our randomized controlled trial single CBD administrations preceded 8 weekly exposure therapy sessions (**Chapter 5**). Also the large majority of preclinical studies employed single doses of CBD (**Chapter 2 and 3**), which is the approach most often seen in the broader anxiolytic drug discovery literature (Griebel and Holmes, 2013). Exceptions are two RCTs that investigated 2 weeks of daily CBD administration in in individuals with high trait worry (Gournay et al., 2023) and 4 weeks of daily CBD administration in patients with social anxiety disorder (Masataka, 2019). Currently ongoing RCTs study the effect of CBD ingested for 2-8 weeks in patients with posttraumatic stress disorder (Telch et al., 2022; and an ongoing study initiated by Utrecht University). It would be worthwhile to further investigate the efficacy of daily CBD administration in anxiety disorders. Pharmacokinetic measurements are highly needed to work towards dose recommendations in humans with various administration schedules.

Although we did not find a concentration-effect relation for single CBD doses across species (**Chapter 3**), we do expect an anxiety-reducing effect of CBD to be related to concentration at the site of action (which, in turn, is related to plasma concentration). Further, whereas 300 mg has been coined the 'goldilocks' dose for anxiety reduction by CBD (Gournay et al., 2023) and the currently available data suggest anxiety-reducing effects of 300-600 mg oral CBD in humans, it is not yet evident if these doses are at top of the presumed inverted U-shaped dose-response

curve (see also 8.3.5) in humans. Since data regarding the right leg of such an inverted U is largely missing (**Chapter 3**), studies that apply multiple doses including doses > 600 mg are important. Studies with experimentally induced anxiety in healthy volunteers could also be considered. In any case, monitoring for potential adverse effects of CBD that are known from the literature (Gingrich et al., 2023) remains warranted.

Our meta-analytic evidence of anxiety-reduction in tests of unconditioned and conditioned anxiety in animals by CBD and related compounds (**Chapter 2**) argues for inquiry of direct symptom reduction as well as effects on fear extinction and related learning mechanisms, which often have a delayed benefit. In line with these preclinical findings, we investigated in a clinical sample the synergistic effects of CBD and exposure therapy (**Chapter 5**), whereas others applied CBD as a monotherapy (Gournay et al., 2023; Masataka, 2019; Telch et al., 2022). Again others have started investigating other AEA enhancing compounds in humans that were previously only studied in animals (e.g., Mayo et al., 2020; Schmidt et al., 2021). This research requires follow-up to consolidate and expand upon the current evidence for anxiolytic effects of CBD and related compounds in humans. Further, preclinical research provided a sound basis for expecting an anti-compulsive effect of CBD in patients with OCD (**Chapter 2** and 8.3.3). We believe that the moment has come to specifically start further inquiry in this direction.

8.6 In conclusion

Anxiolytic drug discovery is a research field in progress. The endocannabinoid system has fascinated researchers the field for many decades. In the future, critical translational steps still need to be taken. The work in this thesis could be considered a stepping stone towards therapeutic applications of CBD that go beyond placebo effects.

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Addendum

A.1 Nederlandse samenvatting (Dutch Summary)

A.1.1 Introductie

Angststoornissen komen veel voor en kunnen voor flinke beperkingen zorgen in belangrijke levensgebieden. Angst is een nuttige emotie wanneer er werkelijk gevaar dreigt. Voor patiënten met angststoornissen doet deze emotie echter meer kwaad dan goed. Je steeds maar weer angstig voelen kost veel energie, en kan daarnaast een verstorende factor zijn in werk, ontspanning en relaties. Veel gedragingen om angstklachten onder controle te krijgen, zoals vermijding van angstopwekkende situaties en pogingen om in je in deze situaties veilig te voelen, werken helaas averechts. Sociale rollen kunnen niet meer naar behoefte en behoren worden vervuld en bovendien voorspellen deze gedragingen een ongunstig beloop. Een angststoornis (of angststoornissen) is voor veel patiënten helaas een chronisch probleem.

Pavloviaanse angstconditionering is een veel gebruikte experimentele methode om te bestuderen hoe zoogdieren, waaronder mensen, leren wat gevaarlijk is. Tijdens angstconditionering (A) wordt een context of stimulus (bijvoorbeeld een plaatje) herhaaldelijk gecombineerd met de toediening van een vervelende prikkel (bijv. een elektrische prikkel). In een dergelijk experiment kan ook de mogelijkheid worden gegeven om te leren wat veilig is, door middel van een veiligheidssignaal (B), bijvoorbeeld een plaatje dat nooit wordt gecombineerd met een elektrische prikkel. Angstextinctie biedt een andere gelegenheid om te leren wat veilig is (in dit geval wat veilig is geworden) (C). Angstextinctie bestaat uit de herhaalde blootstelling aan de context of stimulus uit (A), maar nu zónder vervelende prikkel. Kenmerkend voor patiënten met angststoornissen is een moeite om onderscheid te maken tussen situaties waarin een werkelijk gevaar dreigt (A), en situaties die in feite veilig zijn (B en C).

De huidige richtlijnen voor het behandelen van angststoornissen beschrijven twee opties. De eerste optie is psychotherapie en in het specifiek cognitieve gedragstherapie (CGT)). CGT bestaat uit primair cognitieve technieken om de bij angststoornissen typische overschatting van (dreiging van) gevaar naar beneden bij te stellen, en uit gedragsmatige technieken waarbij patiënten angstopwekkende situaties opzoeken in plaats van ze te vermijden. Dit wordt ook wel 'exposure' (letterlijk vertaald als 'blootstelling') genoemd. Er wordt verondersteld dat tijdens exposure dezelfde vorm van inhibitie-leren plaatsvindt als bij angstextinctie in een angstconditioneringstaak, waardoor moet zijn angstvermindering kan plaatsvinden. CGT is uitgebreid onderzocht in gerandomiseerde gecontroleerde studies. De conclusie van dit onderzoek is dat CGT effectief is bij het behandelen van angstklachten. De tweede behandeloptie is medicatie. Als eerste keus worden selectieve serotonine heropname remmers (SSRI's) of

selectieve serotonine-en-noradrenaline-heropnameremmers (SNRI's) voorgeschreven. Ook deze middelen zijn uitgebreid onderzocht en er is sterk bewijs dat ze effectief zijn voor het behandelen van verschillende angststoornissen.

Tegenwoordig wordt de voorkeur gegeven aan CGT t.o.v. medicatie, omdat de eerste het beter lijkt te doen op de lange termijn. Beide behandelvormen kennen echter nadelen, waarvan de belangrijkste onvoldoende klachtenvermindering is, of terugval in klachten na een initiële verbetering. Dit treft ruim de helft van de patiënten. Bij deze patiënten kan intensivering van CGT, combinatie van CGT en medicatie, en het aanbieden van alternatieve behandelingen worden geprobeerd. Het huidige behandelaanbod verhelpt het probleem van onvoldoende respons en/of terugval echter niet voor iedereen.

Nieuwe medicatie voor het verminderen van angstklachten werd ontwikkeld, vanuit andere neurofarmacologische aanknopingspunten. Omdat de onderliggende pathologische processen kunnen verschillen tussen patiënten, ook wanneer deze dezelfde diagnose hebben, is dit een aan te moedigen ontwikkeling. Een andere veelbelovende strategie is medicatie die synergistisch werkt met psychotherapie. Een voorbeeld hiervan is het toevoegen van d-cycloserine (DCS), een partiële agonist van de glutamaterge N-methyl-D-aspartate (NMDA) receptor, aan exposure therapie sessies. Ondanks initieel veelbelovende resultaten, lieten recentere studies meer bescheiden en weinig klinisch relevante effecten zien.

Cannabisproducten zijn in veel landen toegestaan voor medicinale en onderzoeksdoeleinden. Dit onderzoek heeft geleid tot de ontdekking van cannabinoïde receptoren en lichaamseigen cannabinoïde liganden anandamide (AEA) en arachidonoylglycerol (2-AG), zogenaamde endocannabinoïden. Daarnaast is uit onderzoek met niet-menselijke dieren gebleken dat endocannabinoïde transmissie nodig is voor succesvolle angstextinctie. Er zijn aanwijzingen in de literatuur dat deze processen ook van toepassing zijn in mensen. Een interessante kandidaat voor therapeutische toepassingen is cannabidiol (CBD). Dit bestanddeel van cannabis kan endocannabinoïde transmissie bevorderen en heeft daarnaast relatief weinig nadelige effecten.

A.1.2 Doel van deze thesis

Toen dit promotietraject startte was er onvoldoende wetenschappelijk bewijs dat cannabisproducten kunnen zorgen voor klachtvermindering bij patiënten met angststoornissen. Cannabisproducten zijn dan ook nog niet opgenomen in Europese en Amerikaanse richtlijnen voor de behandeling van angstklachten. Centraal in deze thesis staat het onderzoek naar een therapeutische toepassing van CBD in patiënten met angststoornissen die onvoldoende van eerdere behandelingen hebben geprofiteerd. Dit klinisch toegepaste onderzoek naar CBD als augment-

atie strategie bij exposure therapie wordt beschreven in het tweede deel van deze thesis. Het eerste deel omvat synthesen van eerder onderzoek naar CBD en angst en in het derde en laatste deel pogen wij (on)voldoende respons op exposure therapie te voorspellen aan de hand van een angstconditioneringstaak.

A.1.3 Resultaten

In **Hoofdstuk 2** zochten wij al de bestaande onderzoeken naar angstreducerende effecten van AEA verhogende middelen volgens een systematische manier, en we voegden de resultaten hiervan vervolgens te samen door middel van een meta-analyse. Het doel hiervan was het onderzoeken van het therapeutische potentieel van het bevorderen van endocannabinoïde transmissie, en het identificeren van mogelijke condities waaronder deze strategie werkzaam zou kunnen zijn.

Onze synthese van 120 studies bestond voornamelijk uit dieronderzoek (114 studies). Onderzochte middelen waren CBD (61 studies), de fatty acid amide hydrolase (FAAH) inhibitoren URB597 (39 studies) en PF-3845 (6 studies), en de AEA heropname inhibitor AM404 (14 studies). Angstreductie door CBD, AM404 en PF-3845 (maar niet URB597) trad op in testen van ongeconditioneerde angst, en angstreductie door CBD, URB597 en AM404 in testen van geconditioneerde angst in dieren. Ook verminderde CBD experimenteel opgewekte angst in mensen (CBD was het enige middel waarvoor voldoende studies in mensen beschikbaar waren om een meta-analyse mee te doen). In de meeste studies waarin veiligheidsaspecten geassocieerd met gebruik van het middel werden bekeken (n=17), werden geen bijwerkingen gerapporteerd die aan het gebruik van het middel konden worden herleid.

We onderzochten moderatoren van de angstreducerende effecten met Bayesiaanse geregulariseerde meta-regressie. We vonden dat publicatiejaar, de aan-/afwezigheid van een reeds bestaande angstaandoening en type angsttest samenhingen met de grootte van de effecten van CBD op ongeconditioneerde angst in dieren. Meer recente publicaties waren geassocieerd met kleinere effecten van CBD. Testen van repetitief-compulsief gedrag waren geassocieerd met grotere effecten van CBD dan testen van toenaderings-/ontwijkingsgedrag, en CBD had grotere effecten in dieren met een reeds bestaande angstaandoening dan in dieren zonder.

Voor URB597 was de sociale interactie test geassocieerd met kleinere effecten dan testen van toenaderings-/ontwijkingsgedrag.

Het in zijn algemeenheid positieve meta-analytische bewijs voor angstreductie door deze AEA verhogende middelen moet in het licht worden gezien van de lage kwaliteit van het bewijs. Het is te verwachten dat de daadwerkelijke angstverminderende werking van de middelen kleiner is dan in onze meta-analyse werd geschat.

Het doel van **Hoofdstuk 3** was om uit te vinden bij welke doseringen van CBD een angstreducerend effect verwacht zou kunnen worden in mensen, gebaseerd op preklinische studies. We combineerden data van systematisch literatuuronderzoek naar farmacokinetiek en angstreductie door CBD met de IB-de-risk tool. Deze tool is geschikt om gegevens samen te vatten uit een ' Investigator's Brochure' (IB) of uit een literatuuronderzoek, en kan helpen bij het vertalen van deze gegevens naar een dosis-aanbeveling in mensen. In onze studie integreerden we met de tool farmacokinetiek-, angst- en veiligheid gerelateerde uitkomsten van geïncludeerde studies met een enkele CBD toediening (n=87), om de doseringen te schatten die veilig zijn en het gewenste effect sorteren in mensen. Bij de meeste gerapporteerde effecten (70.3%) had CBD geen effect op angstuitkomsten. CBD plasma concentratie-effect relaties verschillenden tussen diersoorten (rat, mens, muis) en er was geen consistent moet zijn lineair effect van CBD concentraties wat betreft angstreductie. Deze resultaten laten zich niet vertalen naar een eenduidige aanbeveling voor dosering. Er waren enige aanwijzingen voor een dosis-respons relatie met een omgekeerde U-vorm, wanneer er binnen diersoorten werd gekeken. Dit komt overeen met studies in mensen die met name angstreducerende effecten laten zien in het bereik van 300-600 mg, en minder bij lagere doseringen. Tot nu toe pasten weinig studies in mensen hogere doseringen toe. In de toekomst zouden dit soort studies meer duidelijkheid kunnen geven over de meest geschikte dosering in mensen.

In **Hoofdstuk 4** wordt het studieprotocol gepresenteerd voor ons multicentrum gerandomiseerde gecontroleerde onderzoek naar de toepassing van CBD in patiënten met een paniekstoornis met agorafobie of met een sociale angststoornis, die onvoldoende van eerdere behandelingen hadden geprofiteerd.

In **Hoofdstuk 5** worden dit onderzoek naar CBD als augmentatie strategie bij exposure therapie, en de resultaten ervan, beschreven. 300 mg CBD of placebo werd in capsules toegediend voorafgaand aan 8 wekelijkse exposure therapiesessies. We onderzochten of CBD augmentatie zou leiden tot snellere, sterkere of meer aanhoudende verbetering van symptomen van angst, vergeleken met placebo geaugmenteerde exposure therapie. Ook exploreerden we of CBD binnen therapiesessies angstextinctie zou kunnen bevorderen en of dit een effect zou hebben op symptomen in de daarop volgende sessies. We maten CBD plasma niveaus om een uitspraak te kunnen doen over therapietrouw. Dit alles werd gedaan bij 43 patiënten met paniekstoornis met agorafobie en 37 patiënten met sociale angststoornis die werden geïncludeerd in deze studie.

Zoals verwacht waren patiënten en therapeuten niet beter dan kansniveau in het inschatten van de toegewezen conditie (CBD/placebo). CBD plasma niveaus wezen er bovendien op dat alleen patiënten in de CBD conditie CBD hadden ingenomen. Bijwerkingen en ongewenste

voorvallen waren niet ernstig, en gelijk verdeeld over de CBD en placebo condities. De resultaten lieten zien dat ongeacht toegewezen conditie angstklachten verminderden tijdens en tot 6 maanden na de therapie. Op geen van de primaire of secundaire uitkomstmaten leidde CBD tot significant betere behandeluitkomsten dan placebo. Exploratieve analyses naar angstextinctie binnen therapiesessies en effect op symptomen in de daaropvolgende sessies lieten geen voordeel zien van CBD ten opzichte van placebo. In deze eerste gerandomiseerde, gecontroleerde studie naar de toepassing van CBD als augmentatiestrategie bij exposure therapie, leidde CBD niet tot een snellere, sterkere of meer aanhoudende therapierespons.

Het doel van **Hoofdstuk 6** was om experimenteel te onderzoeken of CBD het ophalen van angstige herinneringen zou verminderen en angstextinctie zou bevorderen. Dit onderzoek werd uitgevoerd in een deel van dezelfde groep patiënten met sociale angststoornis en paniekstoornis met agorafobie die onvoldoende van eerdere behandelingen hadden geprofiteerd. Hiervoor werd een angstconditioneringstaak afgenomen waarbij angst geconditioneerd werd aan een bepaald gezicht, terwijl een ander gezicht (het veiligheidssignaal) zonder vervelende prikkels werd gepresenteerd. Vervolgens werden veiligheidssignalen én geconditioneerde stimuli zonder vervelende prikkels werden gepresenteerd tijdens angstextinctie (zie A, B en C in de introductie van deze samenvatting). Na een enkele toediening van ofwel 300 mg CBD of placebo volgden een geheugentest en nogmaals angstextinctie (C). Zowel subjectief ervaren angst, verwachte dreiging en fysieke angstsymptomen werden gemeten. De resultaten lieten zien dat CBD tijdens de geheugentest verwachtingen van gevaar verminderde, onder verschillende niveaus van dreiging. Andere angstuitkomsten werden tijdens deze geheugentest niet beïnvloed. Er werd geen bewijs gevonden voor bevordering van angst re-extinctie door CBD. Exploratieve analyses lieten mogelijk nadelige effecten van CBD zien op de akoestische schrikreflex bij veiligheidssignalen tijdens angstextinctie in vrouwen die serotonerge antidepressiva gebruikten. Daarnaast interfereerde CBD in gebruikers van serotonerge antidepressiva met extinctie van subjectief ervaren angst voor de geconditioneerde stimulus. Deze laatste bevindingen zijn gezien de kleine groepsgroottes preliminair, en replicatie in grotere, onafhankelijke steekproeven is nodig om vast te stellen of ze ook klinisch relevant zijn.

Het doel van **Hoofdstuk 7** was om te onderzoeken of therapierespons voorspeld zou kunnen worden voorafgaand aan exposuretherapie. Hiertoe werden patiënten gekarakteriseerd aan de hand van de resultaten op een angstconditioneringstaak. Als dit bevestigd zou worden, zou voorafgaand aan behandeling al duidelijk kunnen worden gemaakt wie een augmentatiestrategie nodig zou hebben. Concreet onderzochten we of het aantal mogelijk dysfunctionele latente trajecten van angstextinctie en veiligheidsleren tijdens angstconditionering voorspellend zou zijn voor behandelrespons in twee onafhankelijke steekproeven. Onze bevindingen

leidden niet tot duidelijke conclusies. Er was zowel steun in de data voor de hypothese dat het aantal dysfunctionele latente trajecten ongerelateerd was aan behandelrespons, als voor de hypothese dat het hoogst mogelijke aantal dysfunctionele trajecten geassocieerd was met relatief slechte behandelrespons. Op dit moment is het dus nog onduidelijk of resultaten op een angstconditioneringstaak voorspellende waarde hebben voor behandelrespons.

A.1.4 Conclusies

Onze samenvattingen van eerder onderzoek naar CBD en andere AEA verhogende middelen suggereren dat CBD een klinische toepassing zou kunnen hebben bij angststoornissen. De resultaten van dit werk brachten kansrijke toepassingsgebieden aan het licht. In vervolgonderzoek zal voor deze gebieden moeten worden bepaald of de vertaalslag kan worden gemaakt naar de klinische praktijk. In ons eigen klinisch toegepaste onderzoek naar CBD als augmentatiestrategie bij exposure therapie in patiënten met een sociale angststoornis, of met paniekstoornis met agorafobie, werden geen verbeterde behandeluitkomsten gezien in vergelijking met placebo augmentatie. Dit is één toepassing van CBD, die uitgaat van een synergistische werking met exposuretherapie. CBD als monotherapie behoeft nog meer onderzoek. Er is nog veel onduidelijkheid over de juiste dosering van CBD. Inclusie van farmacokinetiek uitkomsten in toekomstig onderzoek is daarom van groot belang. Met deze thesis zijn cruciale stappen gezet in de vertaling naar mogelijke therapeutische toepassingen van CBD in angststoornissen. Dit veld is echter volop in ontwikkeling en er zijn nog vele klinisch relevante vragen die beantwoord moeten worden.

A.2 List of publications

Related to this thesis

Kwee, C.M.B., Baas, J.M.P., van der Flier, F.E., Groenink, L., Duits, P., Eikelenboom, M., van der Veen, D.C., Moerbeek, M., Batelaan, N.M., van Balkom, A.J., Cath, D.C., 2022. Cannabidiol enhancement of exposure therapy in treatment refractory patients with social anxiety disorder and panic disorder with agoraphobia: A randomised controlled trial. Eur Neuropsychopharmacol. 59,58-67.

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Other

Kwee, C.M.B., van den Hout, M.A., 2019. Anxiety sensitivity does not predict treatment outcome or treatment length in obsessive-compulsive disorder and related anxiety disorders. J Obsessive Compuls Relat Disord. 21, 18-25.

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A.4 Curriculum Vitae

Caroline Marianna Bernadette Kwee was born on the 4th of July in Roermond, the Netherlands. She attended high school at the Valuascollege in Venlo, where she combined the school curriculum with professional dance training (ArtEZ Dance Preparatory Course). She graduated cum laude in 2005 and continued her dance training at ArtEZ in Arnhem and at dance company Introdans. She graduated in 2008 with a Bachelor of Arts degree and continued working and performing as a dancer at Introdans until 2012. She transitioned from dance into a new career with support from her parents and Omscholing Dansers Nederland. Caroline wanted to do 'something with people', and started a premaster in Humanistic Studies at the University of Humanistic Studies in 2012-2013. After graduating she studied psychology at Utrecht University from 2013-2016, where she expanded upon her knowledge about mental illness and treatment. She participated in the honors program of the Faculty of Social and Behavioral sciences and graduated cum laude with a Bachelor of Science degree in 2016. She continued with a research master Social and Health Psychology at Utrecht University, which also included Clinical Psychology courses. Her research and clinical internship were done at the Altrecht Academic Anxiety Center (AAA). After finishing the research master's degree in 2018 and a master's degree in Clinical Psychology (cum laude) in 2019, Caroline started as a treating psychologist at the AAA and stayed on as a research assistant, working on the multicenter randomized controlled trial laid out in the second part of this thesis (known as the 'CBD study' by patients and clinicians). After Febe van der Flier handed over the baton, Caroline seized the opportunity and started working as a PhD student on the project. This thesis is the result of that work.