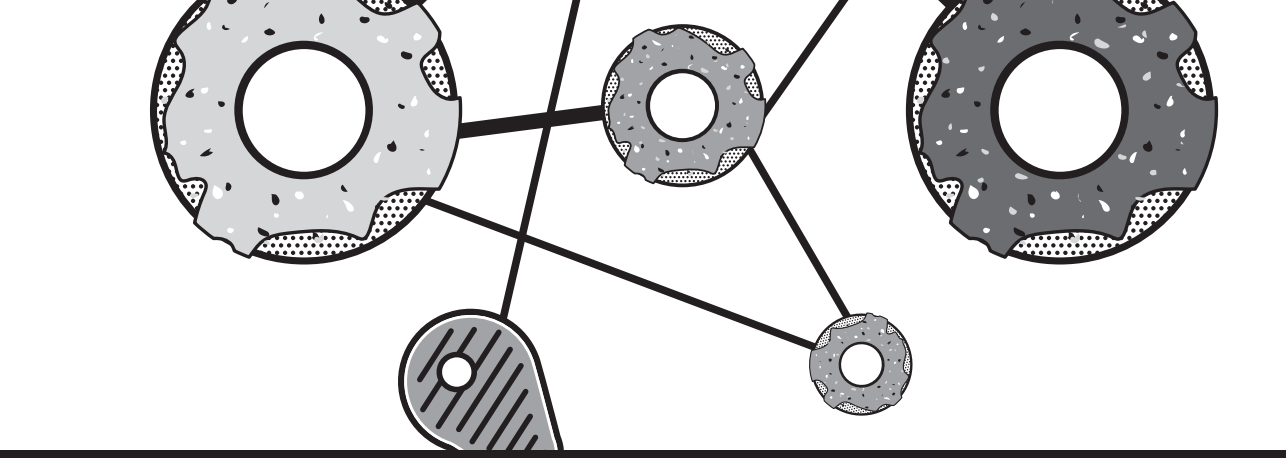
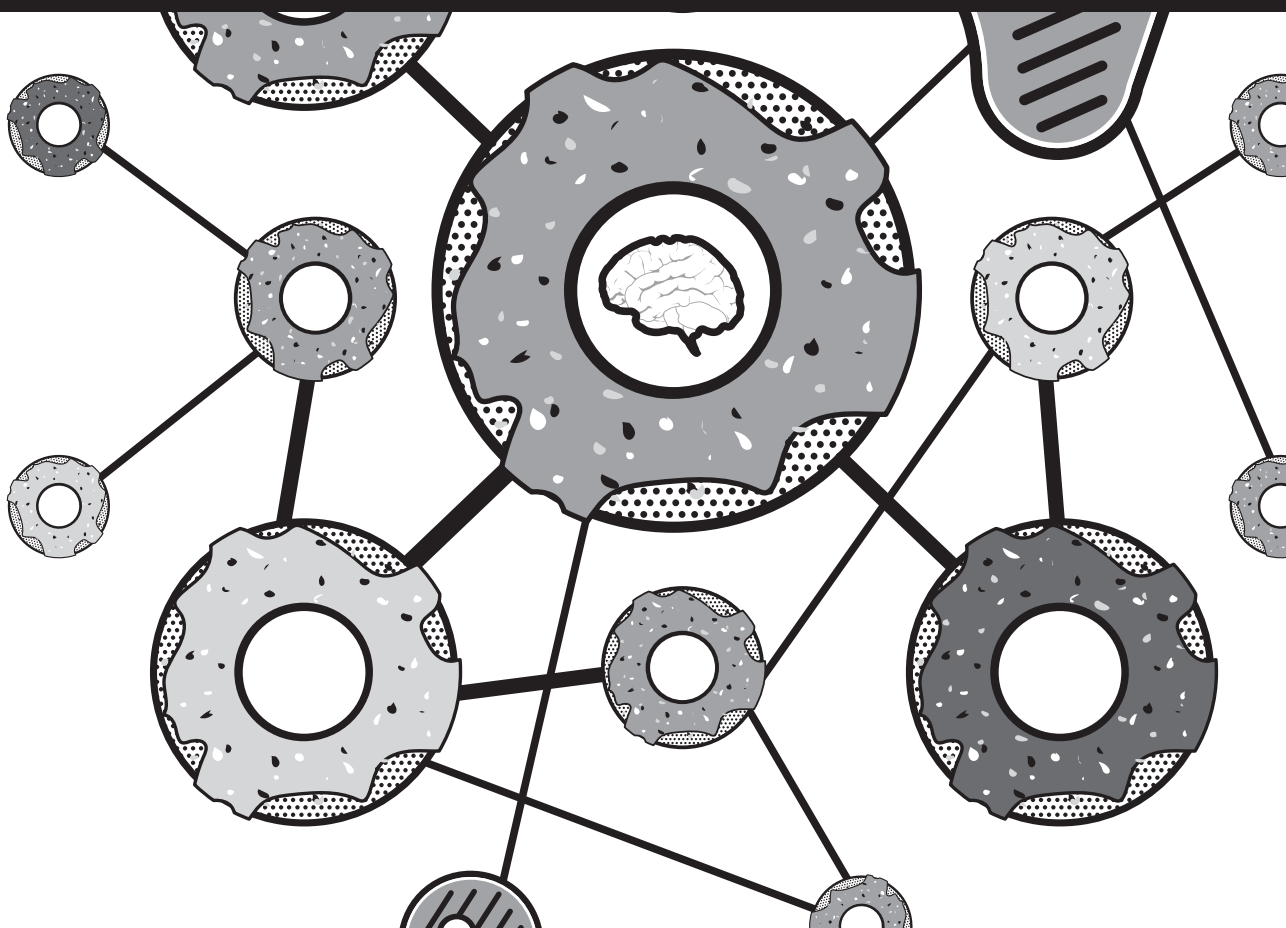


EATING ADDICTION?

THE NERVES AND FIBERS THAT CONTROL FOOD INTAKE

JOHANNES W. DE JONG



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EATING ADDICTION?

THE NERVES AND FIBERS THAT CONTROL FOOD INTAKE

EETVERSLAVING?

DE NEURONEN EN VEZELS IN CONTROLE VAN VOEDSELINNAME

(MET EEN SAMENVATTING IN HET NEDERLANDS)

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Universiteit Utrecht
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Promotoren: Prof.dr. R.A.H. Adan
Prof.dr. L.J.M.J. Vanderschuren

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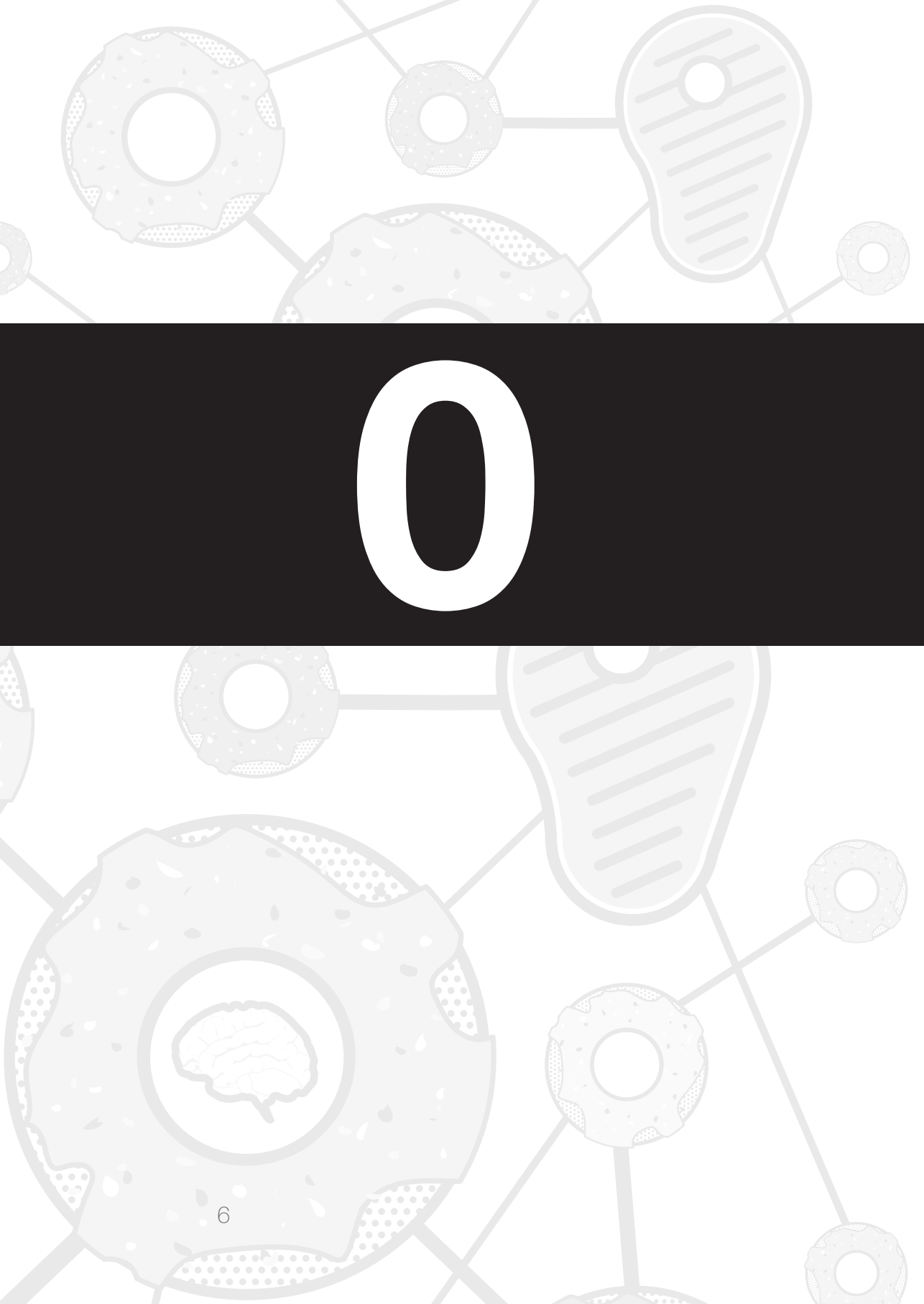

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INTRODUCTION:

NERVES, AND FIBRES, AND SLOWLY BUILT-UP CELLS

INTRODUCTION: NERVES, AND FIBRES, AND SLOWLY BUILT-UP CELLS.

BESIDES, DORIAN, DON'T DECEIVE YOURSELF. LIFE IS NOT GOVERNED BY WILL OR INTENTION. LIFE IS A QUESTION OF NERVES, AND FIBRES, AND SLOWLY BUILT-UP CELLS IN WHICH THOUGHT HIDES ITSELF AND PASSION HAS ITS DREAMS. YOU MAY FANCY YOURSELF SAFE AND THINK YOURSELF STRONG. BUT A CHANCE TONE OF COLOUR IN A ROOM OR A MORNING SKY, A PARTICULAR PERFUME THAT YOU HAD ONCE LOVED AND THAT BRINGS SUBTLE MEMORIES WITH IT, A LINE FROM A FORGOTTEN POEM THAT YOU HAD COME ACROSS AGAIN, A CADENCE FROM A PIECE OF MUSIC THAT YOU HAD CEASED TO PLAY I TELL YOU, DORIAN, THAT IT IS ON THINGS LIKE THESE THAT OUR LIVES DEPEND.

- THE PICTURE OF DORIAN GRAY, OSCAR WILDE, 1891

I want to start my thesis with this quote because the quote is about something that I find absolutely fascinating and that is also the topic of this thesis, namely: how environmental cues influence behavior. In the book, Lord Henry Wotton speaks this quote in defense of a hedonistic lifestyle. His reasoning is that you are not free to choose your own life (he regards free will as an illusion) but that life is just a consequence of the sensations and stimuli you encounter. Not many people would agree with Lord Wotton that this removes personal responsibility for you actions, but I think that he does have a good point in that behavior is for a large part guided by emotional responses to stimuli in our environment.

This is especially true for food. Anybody who has ever tried to diet or made the decision to start making healthier food choices will recognize that it is especially hard to control urges for food when we are exposed to food-related stimuli. To illustrate this, three recognizable examples are included in figure 1. In Figure 1a, Louis CK describes his difficulty in abstaining from eating a whole plate of cookies; he even has difficulty in shifting his attention to other things besides the dish, which is obviously an emotionally loaded stimulus to him. Figure 1b is a Youtube video of small children who are asked to abstain from eating a marshmallow that is presented right in front of them for 20 minutes. A task that is particularly difficult to these children because they are left alone in a room and thus have to inhibit themselves. Figure 1c is a well-known example of a food-associated signal that elicits 'wanting' for food in most people. What is interesting about figure 1c is that it contains food-cues that are completely artificial and man-made. It is unlikely that we are born with a natural sensitivity to cues like these because there is nothing that looks like this in nature. Instead, any emotional value that these cues have, they will have gained through conditioning (i.e. the association of these cues with the food in question). All three are recognizable examples of food-related signals (cues) that can evoke 'wanting' and even craving of food.

Figure 1

Food related-cues, their incentive value and influence on behavior. a. The comedian Louis CK describes his difficulty in managing his food intake, especially when he is confronted with a powerful food-related stimulus (a plate of cookies). b. In a famous 1960 experiment at Stanford, young children are asked to abstain form eating a palatable treat (a marshmallow in the case of this video) while they are exposed to the treat for 20 minutes. c. A characteristic advert from the 80s for a chocolate bar (Twix), which includes the dripping of caramel and chocolate over shortbread. This is such a powerful incentive cue that the company in question has never stopped using it in their adverts since. Hyperlinks to the videos:
a: https://www.youtube.com/watch?v=qSbpyxFC24k
b: https://www.youtube.com/watch?v=x3S0xS2hdi4
c: https://www.youtube.com/watch?v=WGYEOSRJrcg

Our sensitivity to food-related stimuli must have been an evolutionary advantage in prehistoric times when food was scarce, but has become problematic in modern times when highly palatable energy-rich food is omnipresent (Davis, 2014). The result is the ongoing obesity epidemic. The way in which we deal with food-related stimuli, and need top-down control to constrain urges induced by palatable food, is reminiscent of addiction. There are countless mentions of 'food addiction', 'sugar addiction' and even 'chocolate addiction' in pop culture and the idea has gained traction among scientists, although the theory that addiction-like behavior might be contributing to the obesity epidemic remains a controversial opinion (Blundell and Finlayson, 2011; Gearhardt and Corbin, 2009; Volkow *et al*, 2012; Ziauddeen *et al*, 2012).

Outline of the thesis

The general question that this thesis aims to answer is: Does food addiction exist and does it resemble drug addiction in a behavioral and neurobiological sense? To this aim, we review the literature on food addiction in chapter 1 and assess 'control over food intake' in a novel animal model in chapter 2. The contributions of the dopamine system to motivation for and control over food intake are investigated in chapter 3 and 4. In chapter 5 we explore how polysaccharides contribute to the incentive value of food.

Chapter 1

There are similarities between drug addiction (Substance dependence in the DSM-IV, substance use disorder in the DSM-V) and eating disorders such as binge eating disorder (BED) (de Jong *et al*, 2012; Volkow and O'Brien, 2007), notably in how the brain deals with food and drug-related signals (Tomasi *et al*, 2014) (but see: (Ziauddeen *et al*, 2012)). There are also several animal models available that measure food binging and addiction-like behavior (Corwin *et al*, 2011). Several researchers have compared prolonged sucrose seeking to cocaine seeking whereby cocaine seeking showed notable differences with sucrose seeking in that animals that seek sucrose do not become resistant to punishment (electric shock) and sucrose seeking is not reinstated by stress as is cocaine seeking (Ahmed and Koob, 1997; Buczek *et al*, 1999; Pelloux *et al*, 2007; Vanderschuren and Everitt, 2004) (But see: (Johnson and Kenny, 2010; Latagliata *et al*, 2010; Oswald *et al*, 2011)). Note that continued use despite aversive consequences is a diagnostic criterion for substance dependence / substance use disorder in both the DSM-IV and V (American Psychiatric Association, 2000; 2013). Here we propose that an animal model based on

the DSM-IV criteria for substance abuse might be used to assess control over food intake, and possibly food addiction.

Chapter 2

Several authors have reported that specific diets, usually consisting of alternating periods of access to palatable food and food restriction/deprivation, might promote addiction-like behavior for food (Avena *et al*, 2008; Boggiano *et al*, 2005; Corwin *et al*, 2011; Cottone *et al*, 2008). In this chapter, we explored whether such a binge diet promotes uncontrolled food intake, using the animal model we proposed in chapter 1. In this model rats are exposed to several operant tasks where they had to press a lever in order to obtain a highly palatable chocolate flavored reward.

Chapter 3

It is known that dopamine plays a central role in motivation for food and that dopamine is released in response to food cues (Flagel *et al*, 2011; Salamone and Correa, 2012). Dopamine neurons in the ventral tegmental area (VTA) project to (among other brain regions) the striatum (nucleus accumbens (NAcc), caudate and putamen), prefrontal cortex (PFC), amygdala and hippocampus. Especially the projection to the NAcc has been implicated in motivation (Salamone and Correa, 2012). Dopamine neurons are themselves inhibited by dopamine via the dopamine D2 receptor (D2R) that is expressed on their cell bodies (Aghajanian and Bunney, 1977). Interestingly, decreased expression of the D2R on dopamine cells has been associated with impulsive behavior and (in animal models) with extended exposure to drugs of abuse (Bello *et al*, 2011; Buckholtz *et al*, 2010; Calipari *et al*, 2014). In chapter 3 we measure the effect of local knockdown of the D2R in the VTA on motivation for food and cocaine as well as reinstatement of food and drug seeking and compulsive cocaine seeking.

Chapter 4

The mesocortical and mesolimbic projections are two neuronal projections originating in the VTA. These pathways are thought to be very differently involved in behavior (Bassareo *et al*, 2002; Lammel *et al*, 2012). In chapter 4, we describe preliminary results obtained by using a method involving designer receptors exclusively activate by designer drugs (DREADD) and a retrograde-traveling canine adenovirus (CAV2-CRE) to selectively activate the mesocortical and mesolimbic projections in-vivo. These results contribute to our understanding of the role of individual VTA efferent pathways in motivated and aversive behavior.

Chapter 5

An important question is why unhealthy sugar-rich foods have such a big influence on our brain. Arguably, there are lots of reasons to be fond of salad, but it would be quite hard to find examples such as displayed in figure 1 about cravings for lettuce. There are convincing arguments in literature that sucrose has strong reinforcing qualities and promotes dopamine release independent of its sweet taste (Gottfried and de Araujo, 2011). Intragastric infusions of glucose (a component of sucrose), for instance, are reinforcing in that animals will work to obtain them and that they promote flavor conditioning (Elizalde and Sclafani, 1990; Jouhaneau and Le Magnen, 1980; Sclafani and Ackroff, 2006). In chapter 5, we explore the contribution of glucose content to intake and incentive value of food. To address this, we compare motivation for a sweet non-caloric solution (comparable to diet coke) to motivation for a not so sweet, but caloric polysaccharide solution.

Discussion

In the discussion I will come back to these questions by integrating the contributions from this thesis into the current literature. I will discuss how sucrose is able to promote the association of certain stimuli with food intake and consider arguments from literature that sucrose directly influences the dopamine system to promote learning about food cues and motivation for food.

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1

TOWARDS AN ANIMAL MODEL OF FOOD ADDICTION

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Abstract

The dramatically increasing prevalence of obesity, associated with potentially life-threatening health problems, including cardiovascular diseases and type II diabetes, poses an enormous public health problem. It has been proposed that the obesity epidemic can be explained by the concept of 'food addiction'. In this review we focus on possible similarities between Binge Eating Disorder (BED), which is highly prevalent in the obese population, and drug addiction. Indeed, both behavioral and neural similarities between addiction and BED have been demonstrated. Behavioral similarities are reflected in the overlap in DSM-IV criteria for drug addiction with the (suggested) criteria for BED and by food addiction-like behavior in animals after prolonged intermittent access to palatable food. Neural similarities include the overlap in brain regions involved in food and drug craving. Decreased dopamine D2 receptor availability in the striatum has been found in animal models of binge eating, after cocaine self-administration in animals, as well as in drug addiction and obesity in humans. To further explore the neurobiological basis of food addiction, it is essential to have an animal model to test the addictive potential of palatable food. A recently developed animal model for drug addiction involves three behavioral characteristics that are based on the DSM-IV criteria: 1. Extremely high motivation to obtain the drug. 2. Difficulty in limiting drug seeking even in periods of explicit non-availability. 3. Continuation of drug-seeking despite negative consequences. Indeed, it has been shown that a subgroup of rats, after prolonged cocaine self-administration, scores positive on these three criteria. If food possesses addictive properties then food-addicted rats should also meet these criteria while searching for and consuming food. In this review we discuss evidence from literature regarding food addiction-like behavior. We also suggest future experiments that could further contribute to our understanding of behavioral and neural commonalities and differences between obesity and drug addiction.

Introduction

The obesity epidemic has become a major threat to public health with 1 in 3 individuals being obese in the United States (Flegal *et al*, 2010). Obesity, and its comorbidities that include cardiovascular disease, type 2 diabetes and several cancers are now the number one preventable cause of premature death in the USA (Haslam and James, 2005). The notion that food addiction may contribute to the high prevalence of obesity is gaining attention among scientists and mental health professionals (Corsica and Pelchat, 2010; Volkow and O'Brien, 2007; Wilson, 2010). To further investigate the addictive potential of food and its neurobehavioral underpinnings, an animal model of food addiction is essential. In this paper, we will briefly discuss behavioral and neurobiological similarities between overeating and drug addiction before we address the main question of this article: 'What is a valid animal model to determine whether food addition exists and if so, whether it resembles drug addiction on a neurobiological level?' We will briefly highlight several useful, widely employed models from the drug addiction field on which we base our proposed model to study food addiction.

Although the overarching aim of our research is to understand the neural and behavioral mechanisms of obesity in general, we here focus on Binge Eating Disorder (BED), because of its high prevalence (2.0% in males and 3.5% in females (Hudson *et al*, 2007)) and possible behavioral resemblance to addiction. Clearly, similar arguments may be used to link addiction to other eating disorders or obesity in general (Volkow and O'Brien, 2007). We are, indeed, well aware of the fact that not all cases of obesity are caused by BED, that BED and obesity are not synonymous and that BED and obesity have a distinct, if overlapping, neurobiological background (Davis *et al*, 2009).

Although it has become very prominent in recent years, the question whether overeating is a form of addiction is not new. Previously, several authors have theorized about whether food (or food components) can have addictive qualities, akin to drugs of abuse (Davis and Carter, 2009; Davis and Claridge, 1998; Volkow and O'Brien, 2007; Wilson, 2010). Proponents point out that both obesity and addiction involve similar neurobiological substrates and that there are several clinical and behavioral similarities. Opponents, on the other hand, indicate that addiction and overeating have a distinct etiology and that their treatment requires different strategies. Should food indeed be able to induce addiction-like behavior in vulnerable individuals, then this can have far-reaching consequences

for the prevention and treatment of obesity. Obesity prevention programs could, for example, benefit from the success achieved by anti-smoking campaigns in Europe and North America and potential treatments for obesity could include addiction treatments such as pharmacological interventions, cognitive behavioral therapy and 12-step programs (although a 12-step program for obesity can, of course, not aim for complete abstinence of food) (Volkow and Wise, 2005).

Similarities between bed and addiction in humans

BED is an eating disorder characterized by recurrent episodes of uncontrolled eating (binges). It is highly prevalent in obese individuals (Hudson *et al*, 2006). BED differs from other eating disorders in that no effort is made to compensate for the excess of energy intake by purging or intense exercise (American Psychiatric Association, 2000). BED is currently not described in the DSM-IV itself, but in its appendix B, which deals with possible new diagnostic categories. Suggested diagnostic criteria for BED are listed in table 1. An analysis of the DSM-IV criteria reveals similarity with drug addiction (which is termed substance dependence in the DSM-IV; see table 1). Note, however, that the potential DSM criteria for BED remain subject to debate (Cooper and Fairburn, 2003; Fairburn and Cooper, 2011).

According to DSM-IV (American Psychiatric Association, 2000), substance dependence is diagnosed when 3 of 7 criteria listed in table 1 have been met. The occurrence of either withdrawal symptoms or tolerance is indicative of physical dependence to drugs. Although physical dependence may not be a useful concept in the context of obesity, since all animals are physically dependent on food, it has been shown in animal experiments that tolerance and withdrawal may arise after extended intermittent access to palatable food, as will be discussed in the following sections (Avena *et al*, 2008; Cohen *et al*, 1984; Colantuoni *et al*, 2002; Cottone *et al*, 2009). The remaining five DSM-IV criteria for substance dependence relate to loss of control over drug intake. As can be seen in Table 1, some of the criteria for BED markedly overlap with criteria for addiction, and loss of control over food intake is also a major element in BED. For one, in both drug addiction and BED the subject persists in destructive food- or drug-directed behavior while consciously aware of its deleterious consequences(American Psychiatric Association, 2000).

Table 1 lists the diagnostic criteria for addiction (according to DSM-IV) and the corresponding diagnostic criteria for BED. This approach is based on Volkow and O'Brien (Volkow and O'Brien, 2007), who designed a similar table comparing addiction to obesity in general.

Substance Dependence (Addiction)	Binge Eating Disorder
- Withdrawal symptoms	
- Tolerance	
- Taken larger amount and longer than intended	- During episodes: a sense of lack of control - Eating until uncomfortably full - Eating large amounts of food when not feeling hungry - Eating larger than normal amounts in a short period of time
- Persistent desire, repeated unsuccessful attempts to quit	
- Much time spend to obtain, use and recover from use	- Binge eating occurs at least two days per week
- Social, occupational or recreational activities given up in favor of use	- Eating alone because being embarrassed by how much one is eating
- Continued use despite knowledge of adverse consequences	- Marked distress regarding binge eating is present - Feeling disgusted with oneself, depressed or very guilty after overeating. - The binges are not associated with any type of compensatory mechanism such as purging

Behavioral similarities

As can be gleaned from Table 1, addiction shares several behavioral characteristics with overeating, and especially with BED. In both drug addiction and BED, subjects lose control over intake (Corwin and Grigson, 2009). Gearhardt *et al*. have recently developed a questionnaire, the Yale Food Addicton Scale (YFAS), to assess food addiction (Gearhardt *et al*, 2009). The group of individuals that scored high on the YFAS also scored high on measures for BED, childhood Attention Deficit/Hyperactivity disorder (ADHD) and severe depression (Davis *et al*, 2011). It is interesting to note that ADHD and depression have also been associated with substance dependence (Davis *et al*, 2008; Frodl, 2010; Hasin *et al*, 2002; Wilens, 2004; Wilens *et al*, 1997).

Not all individuals exposed to drugs of abuse (or palatable food) lose control over behavior. Highly addictive drugs like cocaine are used on a regular basis in certain environments, but not all the involved individuals become addicted

(Reboussin and Anthony, 2006; Tossmann *et al*, 2001). The same is, of course, true for palatable food. The entire western population is exposed to an environment where palatable (energy dense) food is constantly available, but only a subgroup of individuals will lose control over food intake and become obese and/or develop BED. Several risk factors for addiction have been identified, including genetic factors (reviewed in (Kreek *et al*, 2005)), and impulsivity.

Individual displaying high levels of impulsivity have an increased risk to become addicted to drugs and to develop obesity (Audrain-McGovern *et al*, 2009; Braet *et al*, 2007; Jentsch, 2008; Jentsch and Taylor, 1999; Nederkoorn *et al*, 2006b; Nigg *et al*, 2006). Interestingly, the relationship between addiction and impulsivity is bidirectional, as indicated by the fact that prolonged exposure to drugs results in impaired impulse control (Jentsch and Taylor, 1999; Perry and Carroll, 2008). It has been shown that impulsivity is a predictor of the treatment outcome in obese children (Braet *et al*, 2007; Nederkoorn *et al*, 2006a; 2006b; 2007) and in addiction (Bowden-Jones *et al*, 2005; Krishnan-Sarin *et al*, 2007; Mitchell, 1999; Moeller *et al*, 2001; Paulus *et al*, 2005). One potential behavioral mechanism underlying the relationship between impulsivity and addiction is the fact that impulsive individuals can be more sensitive to immediate gratification (in fact, intolerance to delay of reward is a prominent form of impulsivity) and less sensitive to long-term adverse consequences of behavior, which may contribute to losing control over food and/or drug intake.

Neurobiological similarities

Both food and drugs can be the subject of intense craving. Below, we discuss several examples of functional neuroimaging studies investigating food and drug craving, the neural substrates of which display remarkable overlap. For an extensive review on the neurocircuitry of drug craving and addiction see: (Everitt and Robbins, 2005; Koob and Volkow, 2010).

In studies of food and drug craving, measures of brain activity have been obtained using either Positron Emission Tomography (PET) (Kilts *et al*, 2001) or functional magnetic resonance imaging (fMRI) (Filbey *et al*, 2009; Kober *et al*, 2010). In these studies, craving was provoked using a visual presentation of the craved substance (Kober *et al*, 2010), a tactile drug cue (e.g. a marihuanna pipe, as used by Filbey *et al*. (Filbey *et al*, 2009)) or an verbal recount of a drug-related experience of the participant (Kilts (Kilts *et al*, 2001)).

Several studies have shown involvement of the orbital frontal cortex (OFC), prefrontal cortex (PFC), anterior cingulate, nucleus accumbens, amygdala and insula in drug craving (Childress *et al*, 1999; Filbey *et al*, 2009; Kilts *et al*, 2001; Kober *et al*, 2010). These regions involved in drug craving likely also mediate craving for natural rewards, including sex (Garavan *et al*, 2000) and food (Kober *et al*, 2010). Indeed, there is striking overlap between the regions activated during drug and food craving, since the insula, nucleus accumbens, anterior cingulate, amygdala and OFC have also been implicated in food craving. When craving was self-induced by subjects, increased activity was found in the hippocampus, caudate and insula (Pelchat *et al*, 2004). Craving for chocolate (using pictures of chocolate or letting participants taste chocolate) has been associated with increased activity in the ventral striatum, subgenual cingulate and OFC (Rolls and McCabe, 2007).

Suppression of craving, which will aid in remaining in control over intake, involves the dorsolateral PFC (DLPFC), which has been widely implicated in cognitive control over behavior (Miller and Cohen, 2001). When subjects were asked to suppress food or tobacco craving, activity in the accumbens, VTA, amygdala and cingulate cortex decreased while activity in the DLPFC increased (Kober *et al*, 2010). Interestingly, both the fMRI data and the behavioral (craving) data from this study showed striking similarities in the modulation of craving for drugs and food. The DLPFC was implicated in food addiction in a study by Gearhardt that investigated the neural correlates of food addiction as measured with the recently developed YFAS scale (see above). This study found increased activation in the DLPFC as well as in the caudate in individuals with a high food addiction score, during the anticipation of the receipt of palatable food (Gearhardt *et al*, 2011). The role of the DLPFC in eating disorders has been explored in a clinical study in which participants with bulimic disorders where exposed to repetitive transcranial stimulation (TMS) of the DLPFC. These patients reported decreased craving immediately after TMS and fewer binge eating episodes in the 24 hours following TMS, as compared to patients who received sham-TMS (van den Eynde *et al*, 2010). Thus, craving for food and drugs appears to involve comparable neural substrates (Pelchat, 2002).

Regarding similarities in the neurobiological background of food and drug addiction, dopamine D2 receptor (D2R) availability may play a role in both (Volkow and Wise, 2005). Indeed, PET studies have shown decreased D2R availability in the striatum in both drug-addicted individuals and morbidly obese

patients (Volkow and Wise, 2005; Volkow *et al*, 2008a; 2008b; Wang *et al*, 2001) An explanation for these findings is that decreased D2R availability results in a hypofunctioning reward system and 'addicted' individuals compensate for this effect by consuming large amounts of rewarding substances such as food and drugs. The question remains whether this decreased D2R availability is an effect or the cause of addiction. Apart from the animal studies investigating this question (discussed in the following sections), there is some human data correlating genetic predisposition with decreased D2R availability and reward hypofunction in obesity (Stice *et al*, 2008) and addiction (Noble, 2003), but not necessarily with BED (Davis *et al*, 2009). There is also evidence to suggest that decreased D2R density in the striatum both contributes to the development of addictive behavior (Dalley *et al*, 2007; Morgan *et al*, 2002; Volkow *et al*, 1999; 2002), and is a consequence of prolonged drug use (Nader *et al*, 2002; Porrino *et al*, 2004a). Human studies have shown that decreased D2R availability in the striatum can be a predictor of self-reported 'liking' of an intravenous injection of methylphenidate (Volkow *et al*, 1999; 2002). Conversely, decreased D2R availability was shown to be a consequence of prolonged drug use in studies investigating cocaine self-administration in non-human primates (Nader *et al*, 2002; Porrino *et al*, 2004a).

Brain opioid neurotransmission is also involved in drug addiction and BED. Opioids play an important role in hedonic appreciation ('liking') of food and they have particularly been implicated in the intake of palatable food (Kelley *et al*, 2002; Peciña and Smith, 2010). The opioid receptor antagonist naloxone reduces appetite, in particular in patients with a history of binging (Drewnowski *et al*, 1995; Yeomans and Gray, 1997). In addition, there is an increased prevalence of the A118G polymorphism of the mu-opioid receptor in BED patients (Davis *et al*, 2009). This indicates an important role for the opioid system in BED, mimicking its role in drug addiction, where the mu-opioid receptor has been shown to mediate the rewarding aspects of opioids, ethanol, nicotine and probably psychostimulants (as reviewed in: (Trigo *et al*, 2010)). Furthermore, the opioid system has been implicated in withdrawal for both drugs and food, as discussed in the following sections.

Animal models of drug addiction

There has been a plethora of experiments investigating several aspects of drug addiction, including the motivation to obtain a drug and the process of relapse to drug seeking after extinction of self-administration (Panlilio and Goldberg, 2007;

Shaham *et al*, 2003). This has tremendously contributed to our understanding of the neural and behavioral underpinnings of drug seeking and taking (Bossert *et al*, 2005; Everitt and Robbins, 2005; Koob and Volkow, 2010). In this review we focus on recently developed animals models that incorporate multiple DSM-IV criteria to identify animals that express addiction-like behavior.

Animals will readily self-administer and respond at high levels for food or drugs of abuse (Panlilio and Goldberg, 2007; Richardson and Roberts, 1996) but being extremely motivated to obtain a reward is only one aspect of the addiction syndrome(American Psychiatric Association, 2000). Experimental approaches of addiction, or loss of control over intake (see below), also involve setups in which seeking and/or taking rewards is met with aversive consequences. Examples of such approaches include punishing reward seeking with mild electric shock (Deroche-Gamonet *et al*, 2004; Pelloux *et al*, 2007), adulterating an ingested reward with the bitter tastant quinine (Hopf *et al*, 2010; Lesscher *et al*, 2010; Wolffgramm and Heyne, 1995) (Hopf *et al*, 2010; Lesscher *et al*, 2010; Wolffgramm and Heyne, 1995)or exposing the animal to a cue that has previously been associated with electric shock (Vanderschuren and Everitt, 2004). In addition, models for compulsive drug use usually take two more points into account. First: addiction develops after chronic drug use. Although a drug may be rewarding and evoke motivated behavior on initial contact, loss of control and compulsive behavior only arises after prolonged excessive drug use (Hopf *et al*, 2010; Lenoir and Ahmed, 2007; Lesscher *et al*, 2010; Vanderschuren and Everitt, 2004; Wolffgramm and Heyne, 1995). Second: there is substantial variability in the susceptibility to addictive behavior in animals and humans. Thus, even after extend access to a reward, only a subgroup of the exposed individuals (humans or animals) will lose control over intake (Belin *et al*, 2008; Deroche-Gamonet *et al*, 2004; Lenoir *et al*, 2007; Pelloux *et al*, 2007; Reboussin and Anthony, 2006). Several studies have tried to identify neural or behavioral traits that predict whether or not an individual is likely to lose control over intake and become addicted.

Pioneering work by Piazza *et al*. (Piazza *et al*, 1989) identified so-called 'high' and 'low' responders to novelty, in which high responders showed a stronger psychomotor response to a novel environment. High responders acquired amphetamine self-administration (SA) faster then low responders, and showed enhanced cocaine self-administration in a subsequent study (Piazza *et al*, 2000). Studies in recent years have focused on impulsivity as a predictive factor for

addictive behavior (reviewed in: (Dalley *et al*, 2011; Perry and Carroll, 2008)). Thus, Dalley *et al*. (Dalley *et al*, 2007) used the five choice serial reaction time task to identify impulsive rats. They showed that impulsivity in this task predicts escalation of cocaine intake (but not heroin intake (McNamara *et al*, 2010)). High impulsive rats also displayed decreased D2R availability in the ventral striatum. Belin *et al*. (Belin *et al*, 2008) subsequently showed that impulsive rats in the five choice serial reaction time task were more prone to develop addiction-like behavior for cocaine when addiction criteria based on DSM-IV were used (see below). Interestingly, impulsivity did predict addiction-like behavior, but not acquisition of cocaine self-administration, whereas the locomotor response to novelty (which did not correlate with impulsivity) predicted the acquisition of cocaine self-administration but not addiction-like behavior (Belin *et al*, 2008; Molander *et al*, 2011). Together, these studies (Belin *et al*, 2008; Dalley *et al*, 2007) also indicate that low D2R availability is a predictor of escalated cocaine use ultimately culminating in addictive behavior (Nader *et al*, 2002). The predictive value of impulsivity for addictive behavior is supported by other studies demonstrating that enhanced impulsive behavior is associated with different aspects of cocaine, nicotine and ethanol (but not heroin) self-administration (Diergaarde *et al*, 2008; Perry *et al*, 2005; Poulos *et al*, 1995; Schippers *et al*, 2011). Another approach to identify an addiction-susceptible subgroup of animals has been developed by Ahmed and colleagues (Cantin *et al*, 2010; Lenoir *et al*, 2007). These researchers used a choice paradigm to show that, even after chronic exposure to cocaine, 90% of all Wistar rats prefer a sweet saccharin solution over a cocaine infusion (Cantin *et al*, 2010; Lenoir and Ahmed, 2008; Lenoir *et al*, 2007). This appears in contrast to the behavior of addicted individuals who (by definition) sacrifice non-drug rewards (like palatable food, or social interaction) in favor of drug-related activities (American Psychiatric Association, 2000). Interestingly, about 10% of the animals in these studies did express a preference for cocaine over saccharin, which is comparable to the proportion of human cocaine users who will go on to meet the criteria for addiction. It remains to be demonstrated, of course, whether the 10% cocaine-preferring animals will also show 'addiction-like' behavior for cocaine.

Based on the DSM-IV criteria for substance dependence (American Psychiatric Association, 2000) and the research previously described, three criteria, that relate to escalated drug intake and the failure to exert control over intake (Deroche-Gamonet *et al*, 2004), have been proposed for addiction-like behavior in animals. First: an extremely high motivation to seek the drug. Second: difficulty limiting

drug intake. Third: continuation of drug seeking despite aversive consequences. Deroche-Gamonet *et al*. (Deroche-Gamonet *et al*, 2004) showed that a subgroup of rats, after chronic cocaine self-administration, scored positive on these three criteria. These animals differ from addiction-resistant animals in that they are highly impulsive (Belin *et al*, 2008) and have a persistent impairment in NMDAR-mediated long term depression (LTD) in the nucleus accumbens (Kasanetz *et al*, 2010). In order to assess the functional similarity in drug and food addiction, a similar group of 'food addiction-like behavior' expressing animals should be identified. Subsequently, this group of 'food-addicted' animals can be compared to drug 'addicted' animals to evaluate whether or not the biochemical and cellular changes in food and drug addiction-like behavior are similar.

Models for aspects of food addiction-like behavior and neurochemical changes resembling addiction

There is ample evidence from animal studies to suggest that addiction-like behavior for food exists. Here, we briefly review experimental models that can be used to capture food addiction-like behavior. We will first discuss the seven DSM-IV criteria for addiction. Five of these relate to loss of control over drug intake. Loss of control can be studied in animals using the, previously described, 3-criteria model designed by Deroche-Gamonet *et al*. (Deroche-Gamonet *et al*, 2004). We will therefore also discuss these 3 criteria and how they can been assessed.

Tolerance

In the context of drug addiction, tolerance refers to the fact that after repeated drug use, a larger quantity of the drug is needed to obtain the desired subjective effect, or that the (positive) effect of a given drug dose decreases with repeated drug use. Interestingly, after extended access to a palatable diet, it has been shown that rats indeed increase their food intake (La Fleur *et al*, 2010), although mechanisms other than tolerance could also explain this finding. There is also data to suggest the existence of cross-tolerance between sweet solutions and opioids (Cohen *et al*, 1984; Lieblich *et al*, 1983). Reward tolerance has also been studied measuring reward thresholds in an intracranial self-stimulation setup. This reward threshold is defined as the minimum electrical current needed to maintain stable self-stimulation (Esposito *et al*, 1978; Knapp and Kornetsky,

1994). Acute treatment with drugs of abuse lowers the reward threshold, indicative of the rewarding properties of drugs (Wise, 1996). However, the reward threshold is increased during withdrawal after extended drug treatment, likely as a result of desensitization of brain reward pathways (Esposito *et al*, 1978; Markou and Koob, 1991). A similar effect on self-stimulation thresholds has been demonstrated after withdrawal from a highly palatable 'cafeteria style diet' (Johnson and Kenny, 2010). The increase in self-stimulation threshold after withdrawal from palatable food or drugs has been associated with decreased D2R activity (Johnson and Kenny, 2010; Markou and Koob, 1992).

Withdrawal

Pioneering work of Hoebel and colleagues provided evidence for withdrawal phenomena in rats that were exposed to 12h/12h cycles of food deprivation and access to a sweet solution (Avena and Hoebel, 2003; Avena *et al*, 2008). When denied access to sucrose, rats exposed to these diet cycles, will binge and display signs of withdrawal such as increased anxiety (as assessed in an elevated plus maze) and increased teeth chattering (Colantuoni *et al*, 2002). These withdrawal symptoms were shown to be inducible by treatment with the opioid receptor antagonist naloxone and to be associated with an increase in D1R and μ-opioid receptor binding and a decrease in D2R binding (Colantuoni *et al*, 2001; 2002). A decrease in D2R binding following intermittent sucrose administration, was also observed by others (Bello *et al*, 2002).

Withdrawal from drugs of abuse and the associated changes in behavior have been suggested to depend on activation of brain stress mechanisms (Koob, 2008). Conversely, stress can play an important role in the development of overeating (Dallman, 2009; Parylak *et al*, 2011). Indeed, there is an important interaction between food binging and stress. Binge eating can be triggered by footshock stress (Hagan *et al*, 2002b; 2003) or the frustrating presence of an unreachable (but easily visible) palatable treat (Cifani *et al*, 2009). Food restriction itself is also stressful and this may promote binge eating (Pankevich *et al*, 2010). In addition, animals withdrawn from intermittent access to palatable food show withdrawal signs (Increased anxiety and motivational deficits) that are attenuated by treatment with a CRF receptor antagonist (Cottone *et al*, 2009). Stress is also widely used to reinstate extinguished drug seeking in an animal model of relapse to drug use (Ahmed and Koob, 1997; Buczek *et al*, 1999). Intriguingly, in these models stress does not reinstate sucrose seeking. This may indicate that mere sucrose self-administration does not result in the same behavioral changes as

drug self-administration or intermittent palatable food intake coupled with food restriction does.

Extremely high motivation to obtain the reward

A widely used method to measure the motivation to obtain food or drugs is the so-called progressive ratio schedule of reinforcement, in which animals have to make an increasing number of operant responses for every subsequent reward (Richardson and Roberts, 1996). Indeed, after prolonged cocaine or heroin self-administration, the motivation for drugs under a progressive ratio schedule of reinforcement has been shown to increase (Deroche-Gamonet *et al*, 2004; Orio *et al*, 2009; Paterson and Markou, 2003; Wee *et al*, 2008) (But see: (Liu *et al*, 2005; Morgan *et al*, 2006)). Likewise, it has been shown that rats show an increased motivation to obtain a sucrose reward under a progressive ratio schedule after chronic exposure to a high-fat high-sucrose (HFHS) choice diet (la Fleur *et al*, 2007). Other studies have shown that limited (1h, 3days a week) access to fat also increases the motivation for food (Wojnicki *et al*, 2006).

Difficulty stopping use or limiting intake

This aspect of addictive behavior can be investigated using a so-called 'time-out' model. In this paradigm, seeking responses are measured in a designated period of an operant self-administration session when the non-availability of a reward is explicitly signaled to the animals. It has been shown that rats, after extended access to cocaine, continue to seek cocaine when this is not available (Deroche-Gamonet *et al*, 2004). Likewise, Ghitza *et al*. (Ghitza *et al*, 2006) demonstrated that with prolonged training, animals exposed to a palatable diet increase their food seeking responses during time-out periods, indicating that they develop a 'difficulty limiting' food seeking.

Continued use despite adverse consequences

Recent studies have demonstrated that this characteristic of addictive behavior also occurs in laboratory animals. Vanderschuren *et al*. (Vanderschuren and Everitt, 2004) showed that after prolonged (but not limited) cocaine self-administration, rats will continue to seek cocaine in the presence of an aversive conditioned stimulus (a tone previously paired with footshock). However, after prolonged sucrose self-administration, suppression of sucrose seeking by the footshock conditioned stimulus still occurred. Comparable results were obtained in devaluation experiments (in which an ingested reward is paired with lithium chloride-induced illness). In these studies, sucrose seeking was sensitive to

lithium chloride-induced devaluation, whereas responding to alcohol (Dickinson *et al*, 2002) or cocaine was not (Miles *et al*, 2003). Again, these data show that self-administered sucrose does not have the same addictive potential as drugs of abuse. Comparable conditioned aversion paradigms have, however, been used on several occasions to show that seeking palatable food (usually a combination of fat and sugar, instead of just sugar) can become resistant to punishment (Johnson and Kenny, 2010; Latagliata *et al*, 2010). For example, Johnson and Kenny (Johnson and Kenny, 2010) showed that after extended access to a 'cafeteria-style diet', food seeking in rats became insensitive to presentation of a conditioned aversive stimulus. Using a conditioned suppression paradigm akin to that used by Vanderschuren *et al*. (Vanderschuren and Everitt, 2004), Latagliata *et al*. (Latagliata *et al*, 2010) showed that food restricted animals continue to seek food regardless of its aversive consequences. Interestingly, they showed that noradrenaline depletion of the medial PFC prevented the occurrence of food seeking despite aversive consequences, i.e. restored conditioned suppression. These data are consistent with the notion that the PFC mediates 'top-down' inhibitory influence over maladaptive, addictive behavior (Kober *et al*, 2010).

In addition to *conditioned* aversion, several models have been developed that measure sensitivity to unconditioned punishment. Pelloux *et al*. (Pelloux *et al*, 2007) showed that a subgroup of rats, after chronic cocaine self-administration, continue to seek cocaine whilst taking the risk of receiving a footshock as a consequence. Oswald *et al*. (Oswald *et al*, 2011) designed a model in which animals have a choice between standard chow and palatable food paired with footshock. It appeared that rats that easily binge when exposed to palatable food (Binge Eating Prone (BEP) rats) were significantly less sensitive to the aversive effect of footshock and continued to consume the palatable food, as compared to Binge Eating Resistant (BER) rats.

Using a related punishment setup, Heyne *et al*. (Heyne *et al*, 2009) showed that inflexible intake of palatable food occurs after lengthy intake of a choice diet. In these experiments, rats were given the choice between a 'cafeteria diet' (consisting of bacon, sausage, cheesecake, pound cake, frosting and chocolate) and standard chow. After several weeks, a subgroup of animals continued to ingest the cafeteria diet even when it was adulterated with quinine (a bitter tasting substance). This can be interpreted as inflexible behavior (Lesscher *et al*, 2010; Wolffgramm, 1991), which is a defining characteristic of addictive behavior, in the sense that subjects are unable to shift their thoughts and

Table 2

Measuring addiction-like behavior:	
What to measure?	How to measure?
Tolerance	Cross tolerance with opioids, increased self-stimulation threshold. Drugs: (Markou and Koob, 1991; 1992) (Among many others.) Food: (Cohen et al, 1984; Johnson and Kenny, 2010; Lieblich et al, 1983)
Withdrawal	Observing withdrawal symptoms, e.g. teeth chattering and increased anxiety. Drugs: (Sarnyai et al, 1995)(Among many others.) Food: (Colantuoni et al, 2002; Cottone et al, 2009)
Extremely high motivation	Progressive ratio schedule, in which animals have to exert increasingly more work to obtain a reward. Drugs: (Lenoir and Ahmed, 2007; Orio et al, 2009; Paterson and Markou, 2003; Wee et al, 2008) Food: (la Fleur et al, 2007; Wojnicki et al, 2006)
Difficulty limiting intake	Limited access paradigm, in which seeking responses during signaled non-availability are measured. Drugs: (Deroche-Gamonet et al, 2004) Food: (Ghitza et al, 2006)

behavior away from drugs, but continue to seek the drug despite knowledge of aversive consequences (American Psychiatric Association, 2000). Interestingly, the animals that did cease to eat the cafeteria diet after quinine adulteration displayed another form of, perhaps, inflexible behavior in that they did not compensate for decreased energy intake by taking more of the standard chow. This 'inflexible' behavior is not indicative of 'addictive' behavior, but it is a form of 'inflexible behavior' in that these animals do not adequately respond to a changing environment (i.e. the adulteration of their preferred food), by acquiring their daily caloric ration from another source.

Relapse and cue-induced feeding

Addiction is a chronic, relapsing disorder. In fact, the high risk of relapse to addictive behavior that former drug addicts run, and that remains present after years of abstinence, is perhaps the most insidious aspects of addiction. Animals can not, in the strict sense relapse, since they are not consciously aware of the disadvantages of drug seeking and taking. They can, however, reinstate responding for food or drugs, which is widely employed as an animal model for relapse (Shaham *et al*, 2003). Food seeking can be reinstated by non-contingent presentation of food, or response-contingent presentation of food-associated

conditioned stimuli. Although reinstatement to food or drug seeking does not equate to addiction-like behavior, it has been shown that animals that had lost control over cocaine intake (as assessed using the 3-criteria model), were more prone to reinstatement of cocaine seeking (Deroche-Gamonet *et al*, 2004), and the neural substrates of reinstatement of food and drug seeking overlap to some degree (Nair *et al*, 2009).

Besides provoking reinstatement of food seeking, food-associated cues can promote food intake itself. In so-called cue-induced feeding models, sated animals ingest chow following exposure to a food-associated conditioned cue. This was first demonstrated by Weingarten (Weingarten, 1983), who showed that sated rats resumed eating when exposed to a stimulus previously associated with meal delivery during food restriction. Another possibility involves exposing the animals to cues associated with palatable food or a tiny morsel of the palatable food itself (Boggiano *et al*, 2009). It has been suggested that overeating in a western society may be mediated by a similar process caused by conditioned craving in response to food cues in our environment (Jansen, 1998; Pelchat, 2009).

Conclusion and future perspectives

The data from animal studies discussed above support the notion of addiction-like behavior directed at food. Both neurobiological (e.g. D2R down regulation) and behavioral (increased intake, loss of control) similarities with drug addiction have been demonstrated.

Chronic ingestion of (large quantities of) palatable food may result in addiction-like behavior, as it occurs with drugs. Clearly, food addiction-like behavior may be dependent on the type of diet and the type of food reward the animals obtain. Especially relevant are limited access models, including the one used by Hoebel *et al*. to show withdrawal, and the one by Corwin *et al*. to show both withdrawal and increased motivation for food (Colantuoni *et al*, 2002; Wojnicki *et al*, 2006). When rats are exposed to cycles of alternating periods of food restriction (dieting) and periods of exposure to (palatable) food they will start to display binges on palatable food (Hagan and Moss, 1997). Indeed, alternating periods of dieting and binging on palatable food are highly prevalent in humans with BED (Hagan *et al*, 2002a). Hagan *et al*. have suggested that a diet cycle model has face and construct validity for BED. The binges are characterized by increased intake of palatable food but not standard chow intake. Therefore, they

may be mediated by hedonic, but not homeostatic, control (Hagan *et al*, 2002b; 2003). Cifani *et al*. (Cifani *et al*, 2009) provided support for the predictive validity of the diet cycle model by showing that several psychoactive drugs (sibutramine, fluoxetine, topiramate and midazolam) have similar effects in the model and in patients with BED.

We propose that animals, after extended access to a limited-access paradigm, be tested on the three criteria for addiction-like behavior comparable to the procedure employed for cocaine addiction by Deroche-Gamonet *et al*. (Deroche-Gamonet *et al*, 2004). If a subgroup of animals that is more likely to lose control over intake (based on these three criteria) can be identified, these animals can then be characterized to see whether or not their neural and behavioral makeup resembles drug addiction-prone animals. Several behavioral aspects should be taken into account, to test whether food addiction-prone animals express the same altered behavior that drug addiction-prone animals do. For instance: in rats, impulsivity is a predictor for cocaine intake and addiction-like behavior (Belin *et al*, 2008; Dalley *et al*, 2007; 2011; Perry *et al*, 2005) nicotine and ethanol self-administration (Diergaarde *et al*, 2008; Poulos *et al*, 1995) and sucrose seeking (Diergaarde *et al*, 2009). Is impulsivity also predictive of addiction-like behavior for food? Also, addiction-prone rats have a distinct pattern of drug intake when the drug is freely available, even before they display clear-cut signs of addiction-like behavior (Belin *et al*, 2009). It would of interest to see if this is also the case for food intake. Last, the expression of addiction-like behavior for cocaine has also been associated with increased reinstatement of cocaine seeking after extinction. It would therefore also be relevant to test if food 'addiction' is associated with augmented reinstatement of food seeking (Duarte *et al*, 2003; Ghitza *et al*, 2006).

In this review we briefly alluded to neurobiological changes in (food) addiction, including differences in D1R, D2R and mu-opioid receptor expression. Once animals that express food addiction-like behavior have been identified, these systems can be further studied in the context of food addiction. As an example, neuronal activity following palatable food administration or anticipation to a palatable treat can be measured using immunohistochemistry for immediate early genes (Angeles-Castellanos *et al*, 2007) or using (in-vivo) electrophysiology (REF). Using these techniques, it can be investigated whether food-addicted animals rely on different neural networks for the expression of food-oriented behavior compared to addiction-resistant animals. Indeed, there is human data

that indicates that food addicts rely differently on the dorsal lateral PFC and the caudate during anticipation of food (Gearhardt *et al*, 2011). Moreover, studies in non-human primates have shown that the brains of primates with a long history of cocaine administration respond differently to cocaine then animals with only limited experience with cocaine. One prominent neural change that has been identified is a shift in metabolic activity from the ventral to the dorsolateral striatum during cocaine self-administration in animals that self-administered cocaine for 1.5 years as compared to animals with limited self-administration experience (Porrino *et al*, 2004a; 2004b). Likewise, it has been shown that the neural response to a methylphenidate challenge differs between cocaine addicts and control subjects (Volkow *et al*, 2005). Since the development of addictive behavior relies on concerted neural changes in the VTA, striatum, amygdala and PFC (Koob and Volkow, 2010; Pierce and Vanderschuren, 2010), these circuits should be investigated accordingly.

In conclusion, the behavioral and neurobiological similarities between addiction and overeating (in particular BED) warrant further investigation. Of particular interest is the question whether the 'loss of control' over intake for both food and drugs involves comparable behavioral and neurobiological processes. To do this, applying pertinent models from the drug addiction field to the eating disorder field may provide vital information.

Conflict of interest

The authors declare no conflict of interest.

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2

LOW CONTROL OVER PALATABLE FOOD INTAKE IN RATS IS ASSOCIATED WITH HABITUAL BEHAVIOR AND RELAPSE VULNERABILITY:

INDIVIDUAL DIFFERENCES

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Abstract

The worldwide obesity epidemic poses an enormous and growing threat to public health. However, the neurobehavioral mechanisms of overeating and obesity are incompletely understood. It has been proposed that addiction-like processes may underlie certain forms of obesity, in particular those associated with binge eating disorder. To investigate the role of addiction-like processes in obesity, we adapted a model of cocaine addiction-like behavior in rats responding for highly palatable food. Here, we tested whether rats responding for highly palatable chocolate Ensure would come to show three criteria of addiction-like behavior, i.e., high motivation, continued seeking despite signaled non-availability and persistence of seeking despite aversive consequences. We also investigated whether exposure to a binge model (a diet consisting of alternating periods of limited food access and access to highly palatable food), promotes the appearance of food addiction-like behavior. Our data show substantial individual differences in control over palatable food seeking and taking, but no distinct subgroup of animals showing addiction-like behavior could be identified. Instead, we observed a wide range extending from low to very high control over palatable food intake. Exposure to the binge model did not affect control over palatable food seeking and taking, however. Animals that showed low control over palatable food intake (i.e., scored high on the three criteria for addiction-like behavior) were less sensitive to devaluation of the food reward and more prone to food-induced reinstatement of extinguished responding, indicating that control over palatable food intake is associated with habitual food intake and vulnerability to relapse. In conclusion, we present an animal model to assess control over food seeking and taking. Since diminished control over food intake is a major factor in the development of obesity, understanding its behavioral and neural underpinnings may facilitate improved management of the obesity epidemic.

Introduction

Obesity is a major threat to public health, because it increases the risk for diabetes, cardiovascular disease and cancer (Kral *et al*, 2012; Wang *et al*, 2011). Prevalence rates of obesity have been steadily increasing with an expected increase by 2030 of 65 million and 11 million obese adults in the USA and UK, respectively (Wang *et al*, 2011). The current prevalence of obesity (defined as a body mass index > 30 kg/m2) is about 33% in the US and more than half of the member states of the EU have obesity levels >20% (Flegal *et al*, 2010; Fry and Finley, 2005). Despite its high prevalence, the neural and behavioral underpinnings of obesity are incompletely understood.

It has been suggested that certain forms of excessive food intake associated with obesity are mediated by an addiction-like process(Avena, 2011; Avena and Gold, 2011; Avena *et al*, 2012b; Davis *et al*, 2011; Gearhardt *et al*, 2012; Kral *et al*, 2012; Volkow *et al*, 2011; 2012; Wang *et al*, 2011). Although the extent to which food addiction could explain the obesity epidemic is subject to intense debate (Blundell and Finlayson, 2011; Wang *et al*, 2011; Ziauddeen and Fletcher, 2012; Ziauddeen *et al*, 2012). In support of a role of addiction-like processes in obesity, there is overlap between the DSM-IV criteria for substance dependence and the proposed criteria for binge eating disorder (Davis and Carter, 2009; de Jong *et al*, 2012; Flegal *et al*, 2010; Fry and Finley, 2005) and obesity (Kral *et al*, 2012; Volkow and O'Brien, 2007; Wang *et al*, 2011). Furthermore, the comorbidity between eating disorders and substance abuse disorders may be as high as 40% (Conason *et al*, 2006). In this respect it has been suggested that (over) eating and drug use rely on similar neural circuitry(Hoebel, 1985). One possible shared neural mechanism is a decrease in dopamine D2 receptor availability in the striatum that is found in both disorders(Fetissov and Meguid, 2009; Stice *et al*, 2008; Volkow *et al*, 2001; 2002; Wang *et al*, 2004), a finding that was confirmed in an animal model of compulsive eating(Johnson and Kenny, 2010). Other similarities include a similar brain activity pattern following craving and suppression of craving (Gearhardt *et al*, 2011; Kilts *et al*, 2001; Kober *et al*, 2010; Pelchat *et al*, 2004; Rolls and McCabe, 2007) and co-occurrence with an impulsive personality or Attention Deficit Hyperactivity Disorder (Braet *et al*, 2007; Jentsch, 2008; Mitchell, 1999; Nederkoorn *et al*, 2006; Perry and Carroll, 2008; Zhang and Kelley, 2002).

We have previously argued that recently developed models from the drug addiction field may be useful to investigate the concept of food addiction (de Jong *et al*, 2012). In 2004, Deroche-Gamonet *et al*. developed a model for addiction-like behavior in rats, based on loss of control over cocaine intake (Deroche-Gamonet *et al*, 2004). In this model, rats self-administered cocaine daily for several months. The animals were tested for three behavioral parameters based on the DSM-IV criteria for substance dependence, i.e. 1) Difficulty limiting seeking during signaled non-availability. 2) Extremely high motivation to seek and take the drug. 3) Continued seeking of the drug despite aversive consequences. It was found that a subgroup of rats (17,2 %) scored within the upper tertile for each criterion, which is far more than would be expected by chance (i.e., 3,6%). In addition, these addiction-like behavior-expressing animals appeared to be more vulnerable to reinstatement of extinguished drug seeking, a model for relapse to drug abuse after detoxification (Shaham *et al*, 2003).

In the present study, we tested whether addictive behavior directed at food can be demonstrated using a similar approach as Deroche-Gamonet *et al*.. In order to facilitate the appearance of food addiction-like behavior we exposed animals to a binge-model consisting of alternating periods of food restriction and access to palatable food. Binge eating models consisting of either intermediate access to palatable food (Hagan *et al*, 2002; Wojnicki *et al*, 2008) or alternating (12h/12h) access to sucrose and food deprivation have been shown to mediate bingeing (Avena *et al*, 2008) and certain aspects of addiction such as withdrawal symptoms (Colantuoni *et al*, 2002; Cottone *et al*, 2009) as well as changes in dopamine signaling that are also seen after prolonged drug exposure (Bello *et al*, 2002; 2003).

It has been proposed that the development of addiction is facilitated by a switch from outcome-driven, goal-directed behavior to a habitual, stimulus-response structure of behavior (Everitt and Robbins, 2005; Pierce and Vanderschuren, 2010). In order to test the role of habitual behavior in our proposed model of food addiction-like behavior, we also tested responding for food after devaluation of the palatable food reinforcer (Dickinson, 1985). Moreover, since addiction-like behavior is associated with increased vulnerability to reinstatement of drug seeking (Deroche-Gamonet *et al*, 2004), we hypothesized that animals with less control over their food intake would be more prone to cue and food-induced reinstatement of food seeking after extinction.

Materials and methods

Ethics statement

Experiments were approved by the Animal Ethics Committee of Utrecht University and were conducted in agreement with Dutch laws (Wet op de Dierproeven, 1996) and European regulations (Guideline 86/609/EEC).

Animals

6 week old male Wistar rats (Charles River, Sulzfeld, Germany) weighing 150-200 grams at the beginning of experiment were individually housed in Macrolon cages (L = 40 cm, W = 25 cm, H = 18 cm) under controlled conditions (temperature 20–21 °C, 55±15 % relative humidity) and under a reversed 12 hour light-dark cycle (lights on at 19.00 h). Chow and water were freely available. All experiments were conducted during the dark phase of the day-night cycle.

Experimental overview

In adapting the Deroche-Gamonet model for loss of control of cocaine seeking to palatable food seeking, we found in a pilot study that even mild electric footshock suppressed all food seeking. We therefore chose to measure 'continued seeking despite punishment' using quinine adulteration of the palatable food(Lesscher *et al*, 2010). This pilot experiment compared 4 diets (described below) for their potency to evoke food addiction-like behavior. In this case 24 animals (n=6 per group) were trained and tested on the three behaviors as described by (Deroche-Gamonet *et al*, 2004). Interestingly, when the animals were tested for the third criterion (resistance to mild electric footshock), a complete suppression of chocolate seeking was found, even when the shock intensity was lowered to 0.35 mA. No difference in responding under the shock paradigm was found between the different diet groups (ANOVA p=0.1146 F=2.243 df=23). Additionally, we did not observe a significant difference in responding under a progressive ratio schedule of reinforcement between the four diet groups (data not shown). We did, however, observe a trend towards an increase in addiction-like behavior in animals exposed to the binge model when we took all three criteria into consideration. Since electric footshock suppressed all reward seeking, we chose to measure the criterion of resistance to adversity in a different way, i.e. by exposing the animals to the palatable food adulterated with 2 mM quinine. In the main experiment described in the present study, we compared a group exposed to the binge model (n=36) to a chow-fed control group (n=12). For this experiment, the animals were pre-trained on the three criteria for 5 weeks followed by 8 weeks of access to the diet. We did not observe a difference in

operant responding between the diet groups before the diet. We then continued by retraining and testing on the three criteria followed by 10 extinction sessions and two reinstatement (cue- and chocolate induced) sessions.

Diets

Four different diets were used in this study, and animals were exposed to the respective diets for 8 weeks. The control diet consisted of ad libitum chow (SDS, 3.3kcal/g, 77.0% carbohydrate, 2.8% fat, 17.3 % protein). The restricted access diet consisted of ad libitum chow supplemented with 3h access to chocolate EnsureTM (Abbott Laboratories, Abbott Park, IL, USA), for 5 days a week (from 12.00-15.00h). The high-fat high-sucrose choice diet consisted of ad libitum chow in combination with ad libitum saturated fat (Beef tallow (Ossewit/Blanc de Boeuf), Vandemoortele, Belgium, 9.1kcal/g) and a 30% sucrose solution (commercial grade sucrose in tap water, 1.2kcal/ml). The binge diet consisted of 4 days of 15.0-15.5g chow/day alternated with 3 days of ad libitum chow supplemented with ad libitum Oreo cookies (Nabisco, East Hanover, NJ, USA, 4.7kcal/g, 74% carbohydrates, 21% fat, 3% protein). In this case the Oreo cookies were available for 24h/day for three days. The 15g chow/day was based on previous work by Hagan *et al*. where animals were restricted to 66% of ad-lib chow. This model is a modified version of Hagan *et al*. without the stress component of the binge-model (Hagan and Moss, 1997; Hagan *et al*, 2002). Tap water was available at all times, except during testing. A pilot study compared all four diets. Animals were tested before and after 8 weeks of access to the diets. The main experiment of this article compares 8 weeks of binge diet to 8 weeks of ad-lib chow. We continued with the binge diet because data from literature, as well as our own pilot data suggested that a binge diet as described above is most likely to evoke food addiction-like behavior (Hagan *et al*, 2002).

Apparatus

Rats were trained in operant conditioning chambers (30.5 x 24.1 x 21.0 cm; Med Associates Inc, St. Albans, VT, USA). Each chamber was equipped with two retractable levers (4.8 x 1.9 cm). Above each lever a cue light was located (ENV-221M stimulus light for rats, 28V, 100mA; Med Associates Inc) and a house light (ENV-215M house light for rat chambers, 28 V, 100mA; Med Associates Inc) was placed on the opposite wall. The floor of the chamber was covered with a metal grid with bars separated by 1 cm. The chamber was placed in a sound attenuating cubicle equipped with a ventilation fan to minimize external noise. Chocolate Ensure was delivered to a food receptacle, located in between the

two levers, via nylon tubing attached to a single speed syringe pump (PHM-100-3.33; Med Associates Inc) placed outside the chamber. The operant chamber was controlled by MED-PC (version IV) Research Control & Data Acquisition System software.

Acquisition of chocolate ensure self administration

Animals were trained to respond for food as described before (la Fleur *et al*, 2007; Veeneman *et al*, 2012). The rats first received 10 operant training sessions lasting 1 h. During these sessions, two levers were present, one of which was designated as active. The position of the active and inactive levers was counterbalanced between animals. A session started with insertion of both levers and illumination of the house light. During the first session, a fixed ratio (FR) 1 schedule of reinforcement was used, meaning that each active lever press resulted in the delivery of 0.2 ml chocolate Ensure, retraction of both levers for 20 sec and illumination of the cue light above the active lever for 10 sec during which the house light was turned off. The response requirement was increased to a FR2 schedule of reinforcement during the second and third session. From the fourth session onwards, a FR5 schedule of reinforcement was enforced.

Time-out responding

The time-out procedure was based on (Deroche-Gamonet *et al*, 2004), although a shorter session duration was used to prevent effects of satiety on responding. Sessions consisted of 5 blocks of 10 min chocolate Ensure availability interchanged with 4 blocks of 5 min during which chocolate Ensure was unavailable. During availability blocks, the response-contingent presence of the reward was indicated to the animals by illumination of the house light. The self-administration procedure during availability blocks was the same as described above, i.e., an FR5 schedule of reinforcement was used. During an unavailability block the house light was off and responses on both levers were without scheduled consequences. Responding became more variable during the latter blocks in the session, likely as a result of satiety. We therefore used the amount of responses made during the first 5 min unavailability block as the critical parameter, because this block was flanked by two availability blocks in which animals always obtained the maximum amount of rewards within the time available. The animals received 10 sessions before the diet and 15 sessions after the diet. The mean number of responses during the first unavailability block of the last 4 sessions was used as the time-out score of the animal.

Progressive ratio schedule of reinforcement

Under the progressive ratio schedule of reinforcement, the animals had to meet a response requirement on the active lever that progressively increased after every earned chocolate Ensure reward (1, 2, 4, 6, 9, 12, 15, 20, 25, etc; (Richardson and Roberts, 1996)). The session started with illumination of the house light (signalling availability of the reward) and insertion of both the active and the inactive lever. Meeting the response requirement on the active lever resulted in retraction of both levers, illumination of the cue light above the active lever for 10 sec and delivery of 0.2 ml chocolate Ensure. After a 20 sec timeout, a new cycle started. The session ended when the animals failed to earn a reward within 60 min. Animals received 4 PR sessions before and 4 PR sessions after the diet. In both cases the average of the active lever responses over the 4 sessions was used as the PR score of the animal.

Punished responding

The procedure was adapted from Deroche-Gamonet *et al*. (2004). During this procedure, the animals were tested in operant conditioning chambers that were different from those used during the training, time-out and PR sessions. The session started with illumination of the house light and presentation of both levers. During these sessions, animals responded under a FR5 schedule of reinforcement, in which each 1st lever press resulted in the presentation of a tone and each 4th and 5th lever press resulted in presentation of an electric foot shock (0.35mA, 2sec), administered via the grid floor. Each 5th lever press resulted in delivery of 0.2 ml chocolate Ensure. The tone was turned off after the 4th lever press or when the animals failed to make 4 responses within 1 minute, in which case a new FR5 cycle started. The outcome measure was the amount of lever presses that animals made during a session as a percentage of baseline responding (the average of 4 FR5 sessions the days before). We assessed responding under this paradigm in a pilot study (described above), in which electric footshock nearly completely suppressed responding for food in all animals.

Quinine adulteration

Animals were given free access to either unadulterated or adulterated (using 2 mM quinine; Sigma, The Netherlands) chocolate Ensure in the home cage for 30 min on different days. A pilot experiment showed that a concentration of 2 mM quinine resulted in substantial individual variability, while higher concentrations suppressed intake in almost all animals, and lower concentrations had very

little effect on chocolate Ensure intake. The suppression ratio was calculated as follows: ((un-adulterated consumption - adulterated consumption) / un-adulterated consumption) * 100, so that a suppression ratio of 100 comprised full suppression of intake, and a ratio of 0 meant no suppression at all.

Reward devaluation

Animals were given 2 h of free access to chocolate Ensure in the home cage immediately before an operant session of 20 min, during which the house light was illuminated and both levers were present throughout the session. Both active and inactive lever responses were without scheduled consequences. The devaluation score was calculated as the amount of active lever presses made by the animal after devaluation. Results were compared to the amount of lever presses during a normal 20 min non-devalued FR5 session the day before.

Extinction and reinstatement

Animals received 12 daily 1 h operant sessions during which lever presses were without scheduled consequences. The house light (that previously signalled reward availability) was turned on throughout the session. On day 13, cue-induced reinstatement was tested as follows. The session started with illumination of the cue light above the active lever for 10 sec. During this session, meeting the FR5 requirement on the active lever resulted in retraction of both levers and illumination of the cue light for 10 sec, but no reward was delivered. Animals received normal extinction sessions on day 14 and 15. On day 16, chocolate Ensure-induced reinstatement was tested. The session started with delivery of 0.6 ml of chocolate Ensure. Lever presses during this session were without scheduled consequences.

Data analysis

Based on the three criteria, an 'addiction score' was calculated according to Belin *et al*. (Belin *et al*, 2009). Normalization was done by subtracting the mean of all animals from every individual animal and dividing by the standard deviation of the whole group. This resulted in a criterion score with an average of 0 and a standard deviation of 1 for each criterion. The addiction score was then calculated as the sum of three normalized scores. We also categorized the animals according to Deroche–Gamonet *et al*., meaning that we counted the number of criteria for which the animal scored between the 66th and 99th percentile of the distribution (Deroche-Gamonet *et al*, 2004). The two diet groups were compared to each other using Student's t-tests. The criteria groups were

compared using one-way ANOVAs followed by Turkey's multiple comparison post-hoc tests, where appropriate. Raw data sets are available upon request.

Results

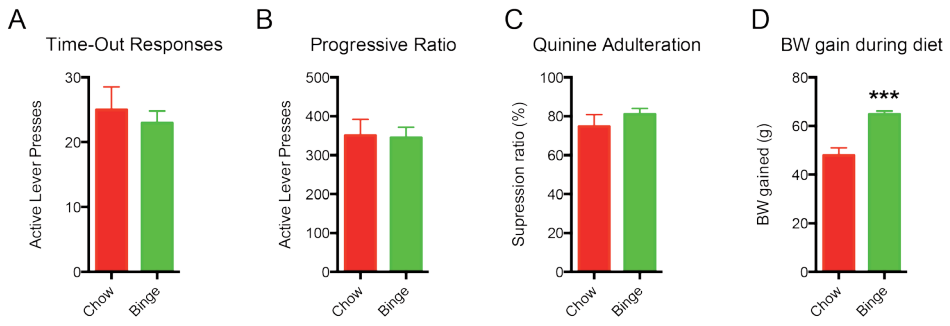
A cohort of animals (n=48) was tested for the three criteria of addiction-like behavior. In order to provoke the development of uncontrolled eating, a subgroup (n=36) was exposed to a binge model. No significant differences on any of the three individual criteria between control and binge animals were observed (time out responding (TO): p=0.6 t=0.53 df=46; progressive ratio (PR): p=0.9 t=0.1128 df=46; quinine: p=0.3 t=1.048 df=46) (fig. 1A-C). The binge model did, however, result in a significant increase in body weight gain (p<0.0001 t=6.105 df=46) (Fig. 1D).

Next, we divided all animals into 4 subgroups based on the amount of criteria for which they scored between the 66th and 99th percentile, according to Deroche-Gamonet *et al*. (2004). In our case, the 3-critt subgroup was not larger than expected by chance (i.e., 3,6%)(Fig. 2).

This was true for both the binge group (Fig 2A) as well as the whole cohort (Fig 2B). The criteria subgroups differed from each other on each criterion (ANOVA TO: p<0.0001 F=11.42 df=47; PR: p<0.0001 F=9,850 df=47; quinine: p=0.0006 F=6.932 df=47) (Fig. 3A-C). In the binge group we assessed if decreased control predicted body weight gain during the diet, which was not the case (Fig 3D).

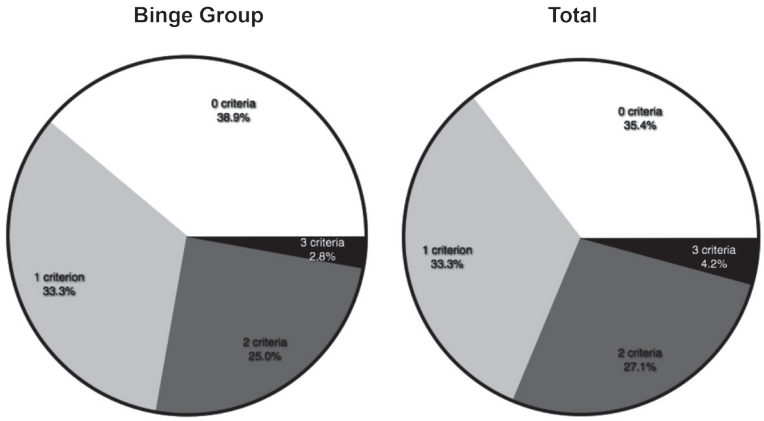
It has been suggested that the formation of aberrant, drug-directed stimulus-response habits is a critical step in the development of addictive behavior (Everitt and Robbins, 2005; Pierce and Vanderschuren, 2010). To assess if the behavior expressed by the animals was goal-directed or habitual, we devalued the chocolate Ensure reward by giving the animals 2 h of free access in their home cage prior to a 20 min operant testing session during which lever presses where not reinforced. The animals made on average 63% less responses when the chocolate was devalued compared to a 20 min session in which lever presses where reinforced and the chocolate was not devalued (Mean difference is 104.0, 95%c.i.= 92.06 to 115.9) (Fig. 6A). Lever presses made after devaluation correlated with addiction score (r2=0.2, p<0.001) (Fig. 6B). No difference between binge and control group was observed (data not shown).

Figure 1



The effect of the binge diet on operant responding and body weight gain. Panel A and B show mean lever presses (+SEM) (y-axis) per diet group (x-axis) during the first time-out during a time-out paradigm (A) or while working under a progressive ratio schedule of reinforcement (B). Panel C shows the mean suppression ratio (+SEM) (y-axis) of chocolate consumption caused by adulteration with 2mM quinine. Panel D shows the mean increase in body weight in grams (+SEM) (y-axis) during the 8 weeks of the diet. *** Indicates significant difference between the groups ($p<0.0001$).

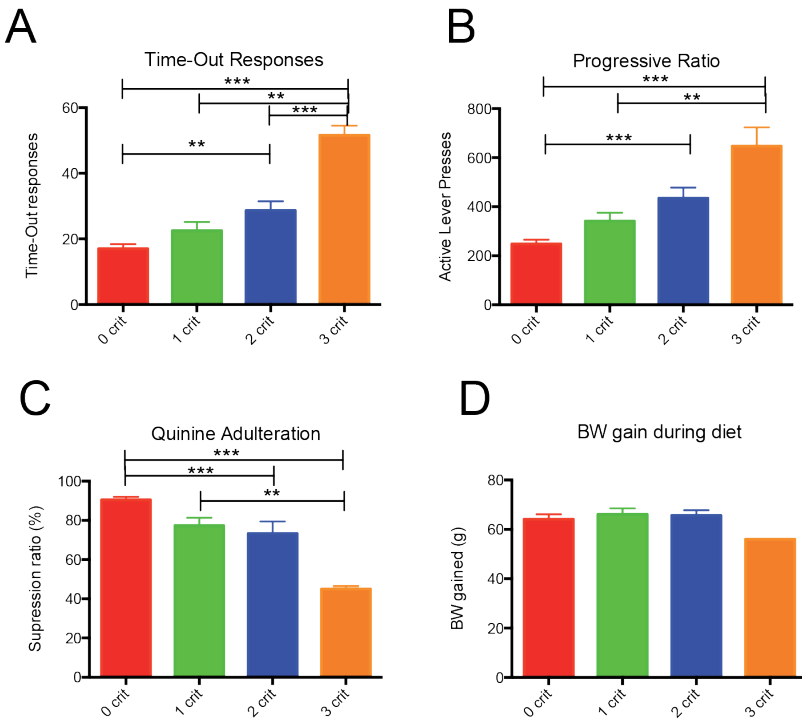
Figure 2



The distribution of the different criteria groups. Animals were assigned to a criteria subgroup based on the amount of criteria for which they scored between the 66th and 99th percentile. The left panel shows the distribution in the animals that were exposed to the binge diet, whereas the right panel shows the distribution throughout the whole cohort.

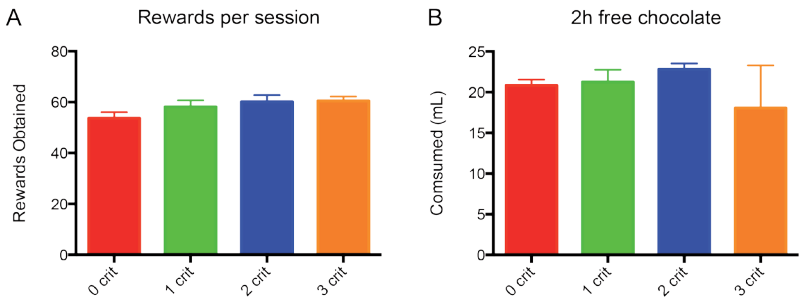
Next, we assessed if animals with diminished control over eating were more prone to reinstate extinguished responding. We measured 2 types of reinstatement. As compared to responding during extinction (Fig. 7A), response-contingent presentation of the chocolate Ensure-associated cues engendered significant ($p=0.0035$ $t=3.077$ $df=47$) reinstatement of responding over the whole cohort, but there was no difference between the criteria groups (ANOVA $p=0.865$ $F=0.2442$ $df=47$) (fig. 7B). During chocolate Ensure-induced reinstatement, we observed significant reinstatement ($p<0.0001$ $t=12.35$ $df=47$) and a significant difference in reinstatement between groups, with the 2 criteria group showing higher levels of responding than the 0 and 1 criteria animals (ANOVA $p=0.01$ $F=4.225$ $df=47$) (Fig. 7C).

Figure 3



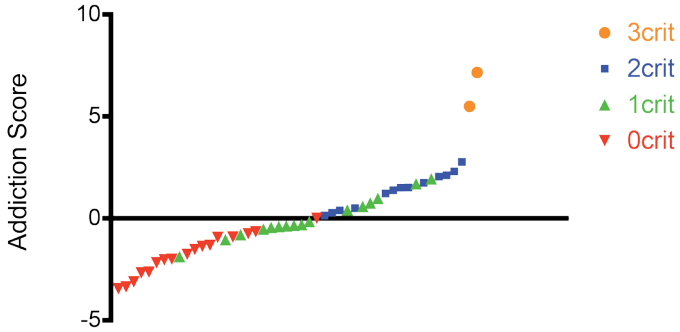
Differences in operant responding between the criteria subgroups. Panel A and B show mean operant responses (+SEM) (y-axis) per criteria subgroup (x-axis) either during the first time-out (A) or under a progressive ratio schedule of reinforcement (B). Panel C represents the mean suppression ratio (+SEM) (y-axis) by 2mM quinine adulteration per criteria subgroup (x-axis). Panel D depicts the mean body weight gain (+SEM) from the animals in the binge group during the diet. **: P<0.001, ***:P<0.0001.

Figure 4



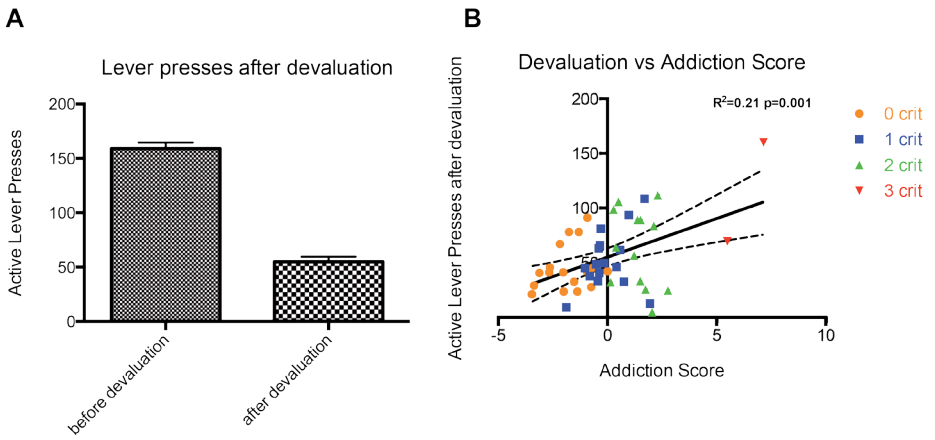
Chocolate consumption. Panel A shows the mean rewards obtained in a normal Time-Out session (+SEM) (y-axis). No difference is observed between criteria groups (x-axis). Panel B shows mean chocolate intake in ml (+SEM) (y-axis) during 2h of ad libitum access in the home cage. No difference was observed between the criteria groups (x-axis).

Figure 5



Range of addiction scores divided by criteria group. The addiction score (sum of the normalized scores for three criteria) is indicated on the y-axis. Animals are ranked from low to high addiction score and divided by criteria group as indicated by the symbols.

Figure 6



The effect of satiety-induced devaluation on responding in extinction. Panel A shows active lever presses made (+SEM)(y-axis) either during a 20min FR5 session (before devaluation) or during a 20min operant session in which lever presses where non-reinforced that was preceded by 2h of ad libitum access to chocolate ensure (after devaluation). Panel B shows the active lever presses made during the session after devaluation (y-axis) as a function of the addiction score (x-axis). A black line indicates the best fit of a linear regression analysis; dashed lines indicate the 95% confidence interval of the best fit.

Figure 7

Propensity to reinstate per criteria group. This figure shows the mean lever presses (+SEM) (y-axis) made during the last 60min extinction session (panel A), cue-induced reinstatement session (panel B) or chocolate induced reinstatement session (panel C) divided per criteria group (x-axis). * Indicates significant difference between the groups ($p<0.05$).

Discussion

In the present study, we adapted an animal model of addiction-like behavior for cocaine to assess the occurrence of addictive behavior directed at palatable food. In order to facilitate the development of uncontrolled eating, a subgroup of the animals (n=36) was exposed to a binge-type model consisting of 4 days of 66% of ad libitum chow alternated with 3 days access to ad libitum chow in combination with Oreo Cookies. After testing for the three criteria of loss of control, we also measured responding after devaluation and the propensity to reinstate extinguished responding induced by response-contingent presentation of the food reward-associated cue or the chocolate Ensure reward itself.

A binge model does not affect control over food seeking – We did not observe an effect of the binge model on any on the three criteria for addiction-like behavior (Fig. 1 and 2). We did, however, observe an increase in body weight gain after exposure to the binge model. The current diet is based on a study by Hagan *et al*., who showed increased bingeing on palatable food of animals who had been exposed to a comparable diet even after they had been withdrawn from this diet for 30 days (Hagan and Moss, 1997). In contrast to Hagan *et al*., we used male rats. We can therefore not exclude that we might had obtained more pronounced effects of the binge diet had we used female rats. Indeed, BED is more prevalent in human females then in males (Kessler *et al*, 2013). On the other hand, it has been repeatedly shown that, given the right circumstances, both male and female rats will binge on palatable food (Corwin *et al*, 1998; 2011; Dimitriou *et al*, 2000). Another commonly used binge model, that causes bingeing in both sexes of rats, uses alternating 12h/12h periods of food deprivation combined with access to a 10% sucrose solution (Avena and Hoebel, 2003; Avena *et al*, 2012a). Previous research has also shown that constant access to a high fat-high sucrose diet increases responding under a PR schedule and responding under a PR schedule before access to the diet positively correlates with abdominal fat storage after 4weeks of access to a high-fat high-sugar diet in male rats (la Fleur *et al*, 2007). Thus, exposure to certain types of obesogenic diets can lead to bingeing and increased motivation for food. However, our data indicate that prolonged exposure to a binge diet is in itself not sufficient to evoke clear-cut addiction-like behavior.

No evidence for 'food-addiction', but high individual variability in control over palatable food intake – Contrary to what has been found for cocaine, the subgroup

of rats that performed in the upper tertile for all three criteria was not larger than expected by chance (3,6%). Therefore, it is reasonable to conclude that no clear-cut signs of addiction-like behavior directed at chocolate Ensure developed in our study. Even in the absence of such an 'addicted-subgroup', the range of control over food seeking observed in the present study is highly relevant. That is, diminished control over food intake in humans, even in the absence of clear addiction-like behavior, may cause overeating and prolonged mild overeating leads to obesity in some individuals. In the present study, decreased control over palatable food intake did not predict body weight gain, which is likely due to the fact that rats (in contrast to humans) do not try to prevent body weight gain. Thus, the neural mechanisms behind this continuum of control over food seeking and taking are important to investigate and our current model provides the behavioral tools to do so.

Animals showing diminished control over food intake are less sensitive to reward devaluation – We observed a significant decrease in responding after devaluation on a group level (Fig. 6a). Interestingly, there were large individual differences regarding the impact of devaluation, which correlated with the addiction score (Fig. 6b). It has been proposed that the development of addiction is facilitated by a switch from goal directed outcome-driven behavior towards habitual stimulus-driven behavior (Everitt and Robbins, 2005). The former is thought to be mediated by ventral and medial parts of the striatum, whereas the latter is depends on the dorsolateral striatum (Balleine *et al*, 2009). Indeed, it has been repeatedly shown that prolonged cocaine self-administration recruits dorsolateral striatal mechanisms underlying drug seeking (Belin and Everitt, 2008; Jonkman *et al*, 2012; Porrino *et al*, 2004; Vanderschuren *et al*, 2005) and that lesions or inactivation of the dorsolateral striatum reduces habitual behavior (Faure *et al*, 2005; Yin *et al*, 2004; 2006; Zapata *et al*, 2010). Since animals that show less control over food intake express more habitual behavior, these findings suggest that reduced control over food intake is associated with a greater dorsolateral striatal involvement in the control over eating.

Low control animals are more prone to reinstate extinguished food-seeking - A prominent feature of addiction is the high risk of relapse (Brandon *et al*, 2007; Hunt *et al*, 1971). This can be investigated using animal models that study the propensity of an animal to reinstate drug seeking following extinction of the operant response. Drug seeking can be reinstated using a drug-associated cue, a small 'priming' amount of the drug or by stress (Shaham *et al*, 2003). To assess

if animals with less control over their food seeking were more likely to reinstate extinguished food-seeking, we tested the animals for both cue- and reward-induced reinstatement. As seen in figure 7C, only priming the animals with the chocolate-flavored reward induced significant difference in reinstatement between the 4 criteria groups. In this case 2 criteria animals responded significantly more during reinstatement. It is likely that the 3 criteria animals are also more likely to reinstate, but this was difficult to demonstrate statistically because of the small number of animals in this group.

In conclusion, we present a model that can be used to measure changes in the control over eating behavior. The model produces a continuum of behavior ranging from very high to low control, the extreme of which might be termed food addiction, but at least in the current experiment, no clear boundary between 'addicted' and 'non-addicted' animals can be drawn, nor is the subgroup of animals that can potentially be classified as showing addiction-like behavior greater than expected by chance. On the other hand, we found that low control over food intake was associated with a high propensity of palatable food-induced relapse and increased habitual responding for chocolate, indicating that behavioral changes associated with addictive behavior can be seen in animals with low control over palatable food intake. The model therefore provides a valuable tool to study control over eating and its neural underpinnings. This is highly relevant when we consider that diminished control over eating, even without the strict classification of food-addiction, may result in severe health problems.

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3

REDUCING VENTRAL TEGMENTAL DOPAMINE D2 RECEPTOR EXPRESSION SELECTIVELY BOOSTS INCENTIVE MOTIVATION

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Abstract

Altered mesolimbic dopamine signaling has been widely implicated in addictive behavior. For the most part, this work has focused on dopamine within the striatum, but there is emerging evidence for a role of the auto-inhibitory, somatodendritic dopamine D2 receptor (D2R) in the ventral tegmental area (VTA) in addiction. Thus, decreased midbrain D2R expression has been implicated in addiction in humans. Moreover, knockout of the gene encoding the D2R receptor (Drd2) in dopamine neurons has been shown to enhance the locomotor response to cocaine in mice. Therefore, we here tested the hypothesis that decreasing D2R expression in the VTA of adult rats, using shRNA knockdown, promotes addiction-like behavior in rats responding for cocaine or palatable food. Rats with decreased VTA D2R expression showed markedly increased motivation for both sucrose and cocaine under a progressive ratio schedule of reinforcement, but the acquisition or maintenance of cocaine self administration were not affected. They also displayed enhanced cocaine-induced locomotor activity, but no change in basal locomotion. This robust increase in incentive motivation was behaviorally specific, since we did not observe any differences in fixed ratio responding, extinction responding, reinstatement or conditioned suppression of cocaine and sucrose seeking. We conclude that VTA D2R knockdown results in increased incentive motivation, but does not directly promote other aspects of addiction-like behavior.

Introduction

Both obesity and substance addiction are enormous socioeconomic and public health problems (European Commission, 2007; Gustavsson *et al*, 2011). Estimates are that 27 million people around the world are addicted to illicit drugs, and 76 million are addicted to alcohol (United Nations Office on Drugs and Crime, 2012). Indeed, substance addiction has been calculated to be the most financially costly of all major neuropsychiatric disorders (Effertz and Mann, 2013). Obesity prevalence is increasing and its comorbidities, i.e., type 2 diabetes, cardiovascular disease and cancer are a major cause of death in the western world (Flegal *et al*, 2010). Interestingly, it has been suggested that there is overlap in the neural and behavioral processes of these disorders, as addiction-like processes may underlie certain forms of obesity (Gearhardt and Corbin, 2009; Potenza, 2014; Volkow *et al*, 2013). Moreover, addictive behaviors have also been proposed to play a role in the psychopathology of eating disorders that do not necessarily lead to obesity such as bulimia nervosa and binge eating disorder (Gearhardt *et al*, 2014; Kessler *et al*, 2013).

All addictive substances directly or indirectly target the mesolimbic dopamine (DA) system (Di Chiara and Imperato, 1988; Nestler, 2005), and DA signaling has also been implicated in (maladaptive) intake of palatable food (Baik, 2013; Meye and Adan, 2014). The cell bodies of the mesolimbic DA system are located within the midbrain ventral tegmental area (VTA). These DA neurons project throughout the forebrain, and the most dense projection reaches the ventral striatum (nucleus accumbens (NAcc) core, shell and olfactory tubercle). Five types of DA receptors have been identified. These can be divided into two classes: the DA receptor D1-like (D1 and D5) which activate adenylyl cyclase and the DA receptor D2-like (D2, D3 and D4) which have an inhibitory influence on this enzyme (Sibley and Monsma, 1992). Here we focus on the DA D2 receptor (D2R) which is expressed postsynaptically on (among others) GABAergic medium spiny neurons in the striatum, as well as presynaptically and somatodentritically on DA neurons, where they act as auto-inhibitory receptors (Usiello *et al*, 2000). Stimulation of the D2R on midbrain DA neurons inhibits firing as well as DA production and release (Aghajanian and Bunney, 1977; Anzalone *et al*, 2012; Tepper *et al*, 1997). In the striatum, neurons expressing the D2R are thought to work in concert with DA D1 receptor-expressing neurons to mediate action selection, salience, motivation and incentive learning (Isomura *et al*, 2009; Robinson and Berridge, 1993; Salamone *et al*, 2009).

Both the pre- and post-synaptic D2R have been associated with addictive behavior. Decreased D2R availability in the striatum is associated with drug addiction and severe obesity (de Weijer *et al*, 2011; Volkow *et al*, 2011; Wang *et al*, 2001) (but see (Eisenstein *et al*, 2013)). Decreased somatodendritic D2R availability has recently been implicated in novelty seeking and impulsivity in humans (Buckholtz *et al*, 2010; Zald *et al*, 2008) and rodents (Tournier *et al*, 2013). These character traits have been associated with drug addiction (Jupp and Dalley, 2014) and obesity (Nederkoorn *et al*, 2007). The notion that somatodendritic D2R play a role in addictive behavior is also supported by animal studies. Thus, rats that exhibit enhanced cocaine self-administration show sub-sensitivity of D2 somatodendritic autoreceptors (Marinelli and White, 2000). Likewise, mice lacking the D2 autoreceptor display elevated DA release and are hypersensitive to the psychomotor effect of cocaine (Bello *et al*, 2011) and midbrain D2R down-regulation has been shown to be involved in the effects of cocaine on plasticity of VTA glutamate signaling (Madhavan *et al*, 2013). In addition, amphetamine self-administration as well as prolonged exposure to cocaine results in decreased midbrain D2R sensitivity (Calipari *et al*, 2014b; Henry *et al*, 1989). Conversely, administration of the D2R agonist quinpirole into the VTA inhibits cocaine-induced reinstatement of cocaine seeking (Xue *et al*, 2011). Taken together, these data indicate that that decreased midbrain D2R availability promotes addictive behavior.

Previously, a DA cell-type specific approach has been used to ablate the D2R in DA neurons in mice. This resulted in an enhanced locomotor response to cocaine and increased motivation for sucrose (Bello *et al*, 2011). Since this approach ablated the D2R in all midbrain DA neurons, it remains unclear if these behavioral effects were mediated by altered function of DA neurons in the VTA or the substantia nigra. Furthermore, constitutive absence of the D2R throughout development may evoke compensatory functional adaptations. Here, we therefore opted for an shRNA-mediated approach to reduce the expression of the D2R specifically in the VTA of adult animals. Since previous experiments have suggested a role for decreased midbrain D2R availability in addiction, we hypothesized that knockdown of VTA D2R promotes behaviors associated with addiction, i.e. high motivation, resistance to punishment and high vulnerability to relapse (see (Deroche-Gamonet *et al*, 2004)). First, we tested the psychomotor effect of a low dose of cocaine. Next, animals were trained to self-administer sucrose or cocaine, and we probed their motivation for sucrose or cocaine under a progressive ratio (PR) schedule of reinforcement. Subsequently, we

investigated conditioned suppression as a model of compulsive cocaine seeking (Vanderschuren and Everitt, 2004). Last, we investigated the propensity of animals to reinstate sucrose or cocaine seeking after extinction by priming with sucrose, cocaine or a cocaine-associated cue, as a model for relapse.

Methods

Animals

Six-week old male Wistar rats (Charles River, Sulzfeld, Germany) weighing 150-200g at the start of the experiment were individually housed in macrolon cages (37.8 x 21.7 x 18.0 cm) under a reversed 12:12 light/dark cycle (lights on at 19:00h). They had ad-libitum access to chow and water and a wooden block was provided as home cage enrichment. All experiments were approved by the Animal Ethics Committee of Utrecht University and were conducted in agreement with Dutch laws (Wet op de Dierproeven, 1996) and European regulations (Guideline 86/609/EEC).

Production of the D2R shRNA

Four sequences were selected based on homology to Rat D2R mRNA (NM_012547.1). To prevent aspecific binding, we assessed the sequence using the NCBI Basic Local Alignment Search Tool. These were: 1: CATCGTCACTCTGCTGGTCTA, 2: CAACCTGAAGACACCACTCAA, 3: TGGTGGGTGAGTGGAAATTCA & 4: AGGATTCACTGTGACATCTTT. Hairpins were designed and cloned into an miR155-based precursor which was located in the first intron of Enhanced Green Fluorescent Protein (EGFP) (Du *et al*, 2006). The construct containing EGFP and the artificial pre-mRNA was then cloned into an AAV vector behind the enhanced synapsin (eSYN) promoter (White *et al*, 2011). In this way the expression of the shRNA would be driven by RNA polymerase II. We opted for this strategy because U6 or H1 promoter-driven (polymerase II-mediated) shRNA expression has been shown to have toxic effects (van Gestel *et al*, 2014), which we do not observe using the current strategy. In the case of van Gestel *et al*. (2014), damage was assessed by staining for tyrosine hydroxylase (TH) as well as for miR-124 using an LNA *in-situ* hybridization procedure. We observed proper miR-124 and TH expression in animals injected with the D2R knockdown or the control vector. Ex-vivo testing on a dual luciferase assay (Du *et al*, 2006) showed that sequence 2 and 3 resulted in most efficient knockdown (Fig. 1a). These two sequences were therefore selected for in-vivo testing in a pilot experiment (n=12), in which both altered the sensitivity to cocaine (see below). For the final experiments

in this paper we selected sequence 2, since it showed a slightly larger effect in the pilot experiment. Animals injected with the D2R knockdown vector were compared to animals injected with a control vector of equal length that was targeted at a gene (luciferase) that does not have an equivalent in mammals. The sequence of the control shRNA was: AAAGCAATTGTTCAGGAACC.

Surgery

Rats were anaesthetized with Hypnorm (0.315 mg/kg fentanyl, 10 mg/kg fluanisone intramuscular, Janssen Pharmaceutica, Beerse, Belgium) and supplemented with Hypnorm as needed. Rats allocated to cocaine self-administration experiments were implanted with a single intravenous catheter into the right jugular vein aimed at the left vena cava. Catheters (Camcaths, Cambridge, UK) consisted of a 22 g cannula attached to silastic tubing (0.012 ID) and fixed to nylon mesh. The mesh end of the catheter was sutured subcutaneously (s.c.) on the dorsum. Next, the animals were placed in a stereotaxic apparatus (David Kopf), and 1µl of a solution containing AAV vector was injected bilaterally into the VTA (coordinates relative to bregma: anteroposterior -5.40, mediolateral ±2.20, dorsoventral -8.90). Carprofen (50 mg/kg, s.c.) was administrated once before and twice after surgery. Gentamycin (5 mg/kg, s.c.) was administered before surgery and for 5 days post-surgery. Animals were allowed 7-9 days to recover from surgery.

Cocaine-induced locomotor activity

Locomotor activity was assessed as described previously (Veeneman *et al*, 2011). Animals were first habituated to the testing apparatus (plastic boxes measuring 50 x 33 x 40 cm, l x w x h). Horizontal locomotor activity was registered using a camera positioned approximately 2 m above the setup. The data was recorded and analyzed using a video tracking system (Ethovision, Noldus, Wageningen, the Netherlands). A session started with a 15 min habituation period. Next, the animals received an intraperitoneal (i.p.) injection of saline or cocaine (cocaine HCl, Bufa BV, Uitgeest, The Netherlands; 5 mg/kg or 10 mg/kg dissolved in saline). Locomotor activity was measured for 30 min. All locomotor experiments were performed during the light phase of the day-night cycle. The animals for this experiment were used for the sucrose self-administration experiments before testing for cocaine-induced locomotion.

Operant conditioning apparatus

Rats were trained in operant conditioning chambers (30.5 x 24.1 x 21.0 cm; Med Associates Inc., St. Albans VT. USA). The chambers were placed in light- and

sound-attenuating cubicles equipped with a ventilation fan. Each chamber was equipped with two 4.8 cm wide retractable levers, placed 11.7 cm apart and 6.0 cm from the grid floor. The assignment of the left and right lever as active and inactive lever (see below) was counterbalanced across rats. A cue light (28 V, 100 mA) was present above each lever and a house light (28 V, 100 mA) was located on the opposite wall. 45 mg sucrose pellets (SP; 5TUL, TestDiet, USA) were delivered to a receptacle between the two levers. Cocaine infusions were controlled by an infusion pump (PHM-100-3-33; Med Associates Inc.) placed on top of the cubicles. During the cocaine self-administration sessions, polyethylene tubing ran from the syringe placed in the infusion pump via a swivel to the cannula on the subjects' back; in the operant chamber tubing was shielded with a metal spring. Sucrose and cocaine self-administration experiments were conducted in identical chambers. The operant testing apparatus was controlled by MED-PC (version IV) Research Control & Data Acquisition System software. Self-administration sessions were carried out once daily, between 9 AM-6 PM, for 5-7 days a week.

Sucrose self-administration

Animals were trained to respond for sucrose as described previously (la Fleur *et al*, 2007). Operant sessions lasted 1 hr during which the availability of the reward was signaled to the animal by illumination of the house light. Pressing the active lever resulted in the delivery of a sucrose pellet, the illumination of the cue light above the active lever for 5 seconds and retraction of the levers. After a 20 sec time-out period, the levers were reintroduced and the house light illuminated, signaling the start of a new cycle. Pressing on the inactive lever was without scheduled consequences. Animals were trained to respond for sucrose under a fixed-ratio (FR) 1 schedule of reinforcement, meaning that each active lever press resulted in the delivery of one sucrose pellet (45 mg). After acquisition of sucrose self-administration under this schedule, the response requirement was increased to five lever presses (i.e., an FR5 schedule of reinforcement). Subsequently, we assessed motivation for sucrose under a PR schedule of reinforcement, in which the response requirement was progressively increased after each obtained reward (1, 2, 4, 6, 9, 12, 15, 20, 25, etc.; (Richardson and Roberts, 1996). A PR session ended after the animal failed to obtain a reward within 30 min. In this experiment, we assessed FR and PR responding both before and after D2R knockdown; PR testing commenced two weeks after virus injection. After the final PR session (the animals received 6 sessions in total), animals received two more FR5 sessions before extinction. Extinction responding was assessed

under identical circumstances as FR responding, except that responding on either lever was without scheduled consequences. Animals received 10 30-min extinction sessions followed by a reinstatement session that was identical to an extinction session, except that 3 sucrose pellets were delivered at the beginning of the session. All operant behavior for sucrose was assessed in the same cohort of animals (n=23). During the experimental period, animals received ad libitum chow in the home cage.

Cocaine self-administration

A separate cohort of 37 rats was used for the cocaine self-administration experiments. Cocaine self-administration experiments, conducted as previously described (Veeneman *et al*, 2012a; -2012b), started two weeks after surgery. Cocaine HCl (Bufa BV, Uitgeest, The Netherlands) was dissolved in saline. The first two weeks consisted of acquisition of self-administration under an FR1 schedule of reinforcement, followed by 1 week of testing under a PR schedule of reinforcement (which occurred 4 weeks after virus injection). To assess the sensitivity of the animals to acquire cocaine self-administration, we used a unit dose (0.083 mg/infusion) that was threefold lower than our usual training dose during the first 5 self-administration sessions, followed by 5 sessions in which our usual unit dose of cocaine was available, i.e., 0.25 mg/infusion (Baarendse *et al*, 2014). Self-administration training started under a FR1 schedule of reinforcement, in which responding on the lever resulted in delivery of a cocaine infusion (16.7µl/sec, during 6 sec), retraction of the levers and the illumination of a cue light above the active lever for 6 sec. This was followed by a 20 sec time-out period during which the levers remained retracted and both the cue and house light were turned off. A new cycle was then started by insertion of the lever. These FR1 sessions lasted for 60 min. After FR1 responding had stabilized (approximately 10 sessions), a PR schedule of reinforcement was introduced, in which the response requirement increased progressively after each obtained reward (1, 2, 4, 6, 9, 12, 15, 20, 25, etc.; (Richardson and Roberts, 1996)) and reward delivery was followed by a 10 min time-out period to minimize the influence of cocaine-induced psychomotor effects on responding for the next infusion. Animals were tested for 3 PR sessions. Next, the animals were trained under a heterogeneous seeking-taking (ST) chain schedule of reinforcement (Limpens *et al*, 2014; Olmstead *et al*, 2000; Vanderschuren and Everitt, 2004; Veeneman *et al*, 2012b) with a random interval (RI) of 120 seconds on the seeking link (ST(RI-120)). These ST sessions started with the introduction of a new lever ('seeking lever') and the illumination of the house light. The first press on the

seeking lever initiated the RI and pressing this lever was without consequences until the RI had elapsed. When the RI had elapsed, pressing the seeking lever resulted in retraction of the seeking lever and insertion of the taking lever. Next, responding on the taking lever (under the FR1 schedule of reinforcement) resulted in an infusion with cocaine, illumination of the cue light, retraction of the taking lever and the switching off of the house light. This was followed by a 10 min time-out period to minimize the influence of cocaine-induced psychomotor effects on responding for the next infusion. After the time-out period, a new cycle started with the reintroduction of the seeking lever and the illumination of the house light. When the rats had acquired the task under a RI of 2 sec, the RI was progressively increased between sessions until animals had acquired the task under an RI of 120 sec. The program automatically ended after 2 hr or if animals had obtained 10 rewards, whichever occurred first. The conditioned suppression procedure consisted of a conditioning phase in which a subgroup of rats (n=26, 12 control and 14 D2R knockdown) learned to associate a 85 dB, 2900 Hz tone (CS) with footshock (0.35 mA, 1 sec, 20 presentations) in a different environment, as described before (Limpens *et al*, 2014; Vanderschuren and Everitt, 2004). A control group of animals (n=7, 4 control and 3 D2R knockdown) was subjected to the same procedure but without the delivery of footshocks. Rats were then presented with the seeking lever in the operant conditioning chamber. We assessed the amount of seeking lever responses made during 7 2 min blocks. Two-minute intervals in which the tone CS was presented (CS-ON interval) were alternated with two-minute intervals where the tone CS was absent (CS-OFF interval). The total number of lever presses made during presentation of the CS was taken as the outcome measure. Cocaine was not available during conditioned suppression sessions. Subsequently, the rats were exposed to daily 1-hour extinction sessions in which taking lever responses were without scheduled consequences. This continued until the rats made less then 30 responses for three consecutive days. We then assessed reinstatement following an i.p. injection of cocaine (5-10 mg/kg, i.p.) or following the presentation of the cocaine associated cue-light and retraction of the levers contingent with a response on the taking lever. After each 1 hr reinstatement session, rats received extinction sessions until they made less then 30 responses in a session.

Tissue preparation

Animals were euthanized by i.p. injection of pentobarbital. For qPCR experiments, the brains were removed, quickly frozen on dry ice and stored at -80˚C. For immunohistochemistry experiments, animals were given an intracardial perfusion

with cold 4%PFA in PBS. After dissection the brains were post fixed for 24 hr in 4% PFA in PBS and then stored in 30% sucrose in PBS at 4˚C until ready for immunohistochemistry.

Quantitative polymerase chain reaction

Fresh frozen brains were thawed in PBS and the VTA was quickly dissected and dissolved in Trizol (Invitrogen). RNA was obtained using phenol-chloroform extraction followed by ethanol precipitation. All samples were diluted to a concentration of 100 ng/µl, 1 µl of which was used per qPCR measurement. qPCR was performed using QantiTect SYBR Green RT-PCR kit (QIAGEN) according to the manufacturers instruction. The following primers were used: Drd2-forward: CTGTGGCTGATCTTCTGGTG; Drd2-reverse: CACACGGTTCAGGATGCTT; beta-actin-forward: CGTTGACATCCGTAAAGACC; beta-actin-reverse: TAGAGCCACCAATCCACACA.

Immunohistochemistry

Perfused brains were cut at 40 µm and stored in 30% sucrose and 0.02%NaAz in PBS until ready for processing. Sections were than washed in PBS and incubated in blocking buffer (1% normal goat serum, 0.2% Triton X) and incubated overnight at 4˚C with 1:500 chicken anti GFP (Abcam ab13970) and rabbit anti TH (1:500, Millipore, USA). GFP was visualized with Alexa-488-labeled goat anti chicken and TH with Alexa-594-labeled goat anti rabbit (both Molecular Probes, USA, 1:500). After washing in PBS, sections were mounted and embedded in Fluorsave (Merck Millipore).

Statistics

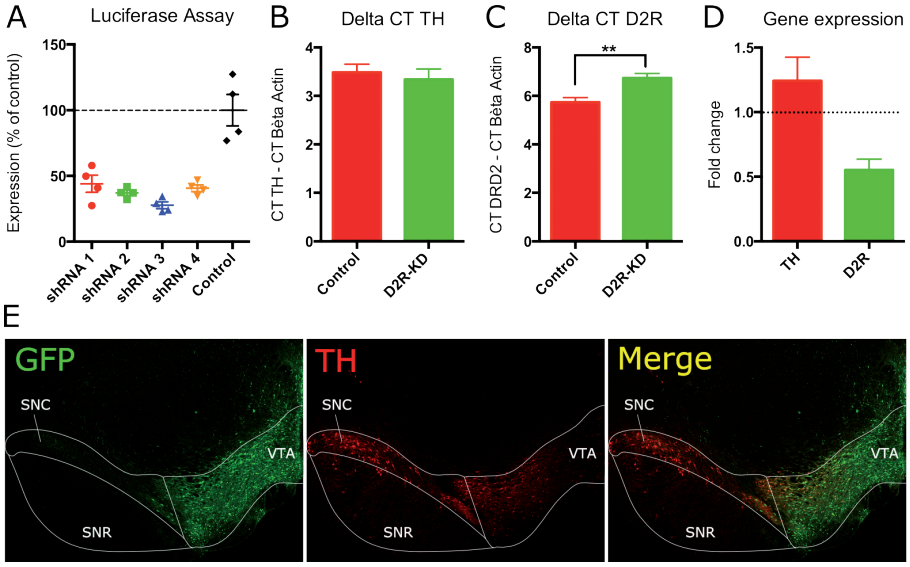
Data was analyzed using Prism 6 (Graphpad Software). Where appropriate (i.e. cocaine locomotion, sucrose self-administration and cocaine-induced reinstatement), the D2R knockdown group was compared to the control group using a two-way repeated measures ANOVA with cocaine dose or before/after surgery as within-subjects factor. Significant main effects were then followed up with Sidak's multiple comparison test. When two groups were compared at one time point (i.e. D2 knockdown qPCR, cocaine self-administration) a Student's t-test was used.

Results

Knockdown of the D2R

To validate knockdown, a fusion construct of renilla-D2R cDNA was transfected into human embryonic kidney cells. These cells where then transfected with one of four shRNA sequences targeted at D2R mRNA or a control sequence. The data was corrected for transfection efficiency based on a co-transfection with luciferase. We observed efficient knockdown in all cases (Fig. 1a). Based on this and a pilot experiment that assessed cocaine-induced locomotor activity (data not shown) we selected sequence 2 for subsequent experiments. Knockdown

Figure 1



Virus expression and D2R knock down. a: The expression of a D2R-Renilla luciferase fusion protein was assessed by measuring light emission following addition of the Renilla substrate, coelenterazine. All 4 constructs decreased light emission compared to a control hairpin. The data were corrected for transfection efficiency by co-transfection with firefly luciferase. b: D2R and TH mRNA expression was assessed using QPCR in VTA dissections. No significant difference in ΔCT value for TH was observed in D2R knockdown animals versus controls. c: ΔCT values for D2R mRNA were significantly increased in D2R knockdown animals compared to controls. d: ΔΔCT values were used to calculate fold change compared to control for both TH and DRD2. We observed a decreased of about 50% in D2R mRNA expression. e: The location of virus expression was confirmed by staining the animals for GFP and TH. GFP expression in the substantia nigra was a criterion for exclusion, but this was not observed. SNC = Substantia nigra pars compacta, SNR = Substantia nigra pars reticulata, VTA = Ventral tegmental area, D2R-KD = D2R knockdown. Data represents mean + S.E.M. **p<0.01.

of D2R mRNA was assessed by QPCR on VTA dissections from animals after completion of the sucrose or cocaine self-administration experiment (n=25) (Fig. 1b-d). D2R and TH mRNA quantification was normalized to beta-actin mRNA. We observed no difference in TH expression (ΔCT mean difference: -0.145 ± 0.269, t=0.5411, df=23, p = 0.59) and an increase in ΔCT value for D2R in the D2R knockdown group (ΔCT mean difference: 1.00 ± 0.272, t=3.680, df=23, p = 0.001). This corresponds to ±50% knockdown. We observed comparable knockdown in a cohort (n=3) that was sacrificed 4 weeks after surgery (Supplementary Figure 1). Efficient virus expression was validated using immunohistochemistry against TH and Green Fluorescent Protein (GFP) (Fig. 1e). GFP expression was confined to the VTA in all cases (n=48).

Cocaine-induced locomotor activity

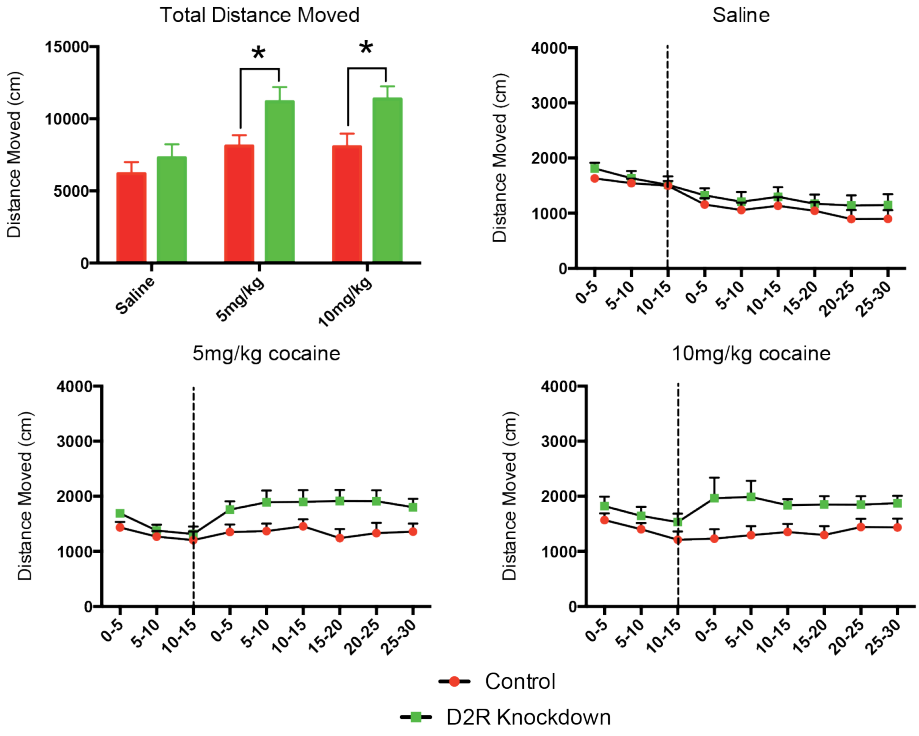
We validated the knockdown behaviorally by determining cocaine-induced locomotor activity (Bello *et al*, 2011; Vanderschuren *et al*, 2000). We found that animals with D2R knock down were hypersensitive to a low dose of cocaine ($F_{(1, 60)\ knockdown}$=11.80, P=0.001) (Fig. 2a). Animals with D2R knockdown showed an increase in locomotor activity after injection with both 5 mg/kg (t=2.508, df=60, P=0.044) and 10 mg/kg cocaine (t=2.509, df=60, P=0.044). We observed a trend towards increase in locomotor activity after D2R knockdown during the 15 min habituation period ($F_{(1, 60)\ knockdown}$=3.697, P=0.059) but there was no effect of D2R knockdown on locomotor activity following a saline injection (t=0.897 df=60, P=0.754). The increase in locomotor activity started within 5min after i.p. injection and lasted for at least 30min (Fig. 2b-d).

Sucrose self-administration

To investigate the effect of VTA D2R knockdown on sucrose self administration, rats (n=23) were tested on FR and PR for sucrose before and after virus injection. We found an increase in FR responding in both groups after surgery ($F_{(1, 19)\ surgery}$=32.58, P=0.0001), which may reflect increased sucrose appetite in older and therefore heavier animals. We did not observe a difference in FR responding between the groups ($F_{(1, 19)\ knockdown}$=0.922, P=0.349; data from two animals were excluded because of a malfunctioning operant chamber) (Fig. 3a). However, D2R knockdown animals made approximately twice as many responses under a PR schedule of reinforcement ($F_{(1, 21)\ surgery\ x\ knockdown}$=8.929, P=0.007) (Fig. 3b). VTA D2R knockdown did not affect inactive lever presses ($F_{(1, 21)\ surgery\ x\ knockdown}$=1.388, P=0.253) (Fig. 3c). Next, we assessed responding under extinction and sucrose-primed reinstatement. We did not observe a

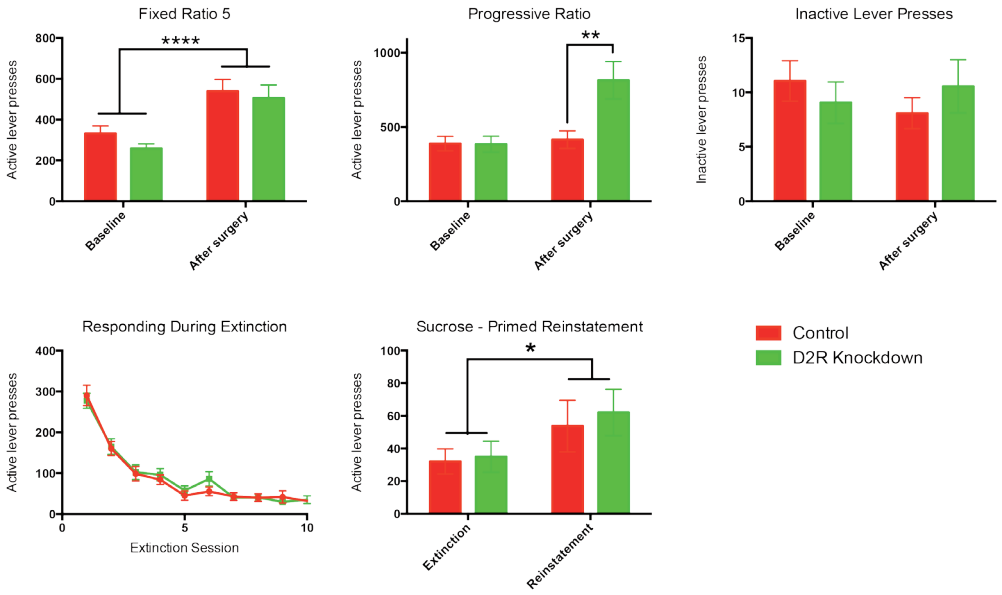
difference between the two groups, neither under extinction ($F_{(1, 21)\ knockdown}$=0.0760, P=0.785) (Fig. 3d) nor during sucrose-primed reinstatement ($F_{(1, 21)\ reinstatement}$=5.304, P=0.032; $F_{(1,21)\ knockdown}$=0.167, P=0.687) (Fig. 3e).

Figure 2



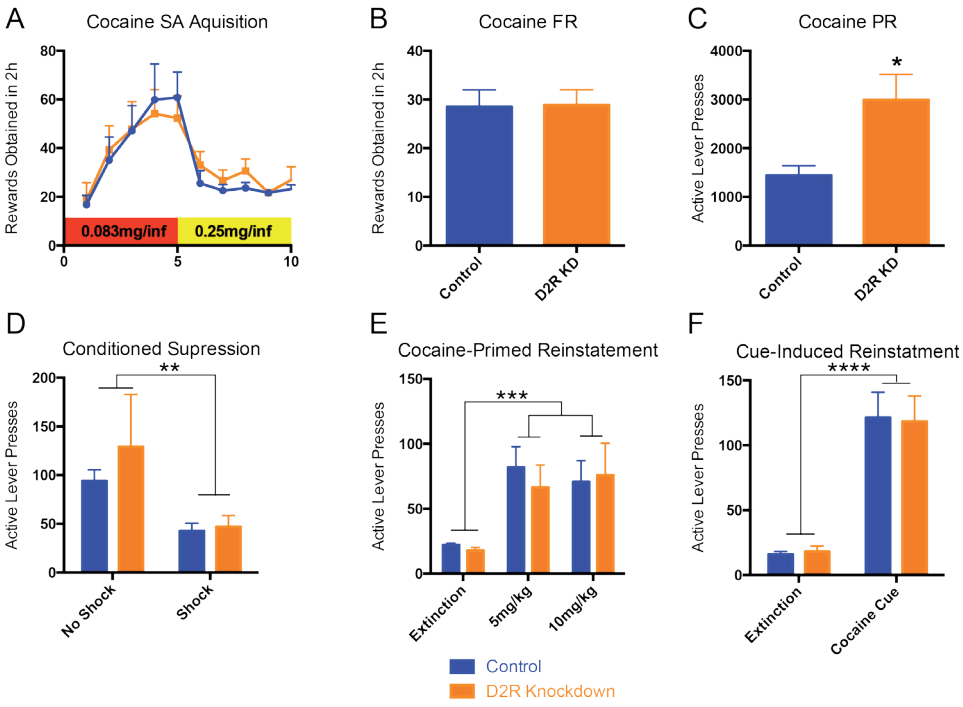
D2R knockdown animals show an enhanced locomotor response to a low dose of cocaine. a: Total distance moved in the 30 min following an injection with saline or cocaine. The same data is displayed in blocks of 5 min for saline (b), 5 mg/kg cocaine (c) or 10 mg/kg cocaine (d). Each session started with a 15 min habituation period, the end of which is indicated by the vertical dotted line. D2R-KD = D2R knockdown. Data represents means + S.E.M. *p<0.05.

Figure 3



Sucrose self-administration under FR and PR schedules of reinforcement, extinction and reinstatement. a: FR5 responding (1 hr session) was increased after surgery with no difference between D2R knockdown and controls. b: PR responding (average of six sessions) approximately doubled in D2R knockdown animals after surgery whereas PR responding in control animals was unaltered. c: D2R knockdown and control animals made a comparable, minimal amount of inactive lever presses during PR sessions. There was no difference between experimental groups. d: Rats were exposed to 10 (1 hr) extinction sessions, in which active lever presses were not reinforced. Extinction of responding occurred in a comparable fashion in D2R knockdown and control animals. e: The non-contingent delivery of 3 sucrose pellets induced significant reinstatement of sucrose seeking in both groups. 'Extinction' represents the number of active lever presses in extinction session 10. Data is presented as means + S.E.M. *p<0.05, **p<0.01, ****p<0.0001.

Figure 4



Cocaine self-administration under FR and PR schedules of reinforcement, conditioned suppression, extinction and reinstatement. a: Rats acquired cocaine self-administration using a unit dose of 0.083 mg/infusion for 5 sessions, followed by 5 sessions in which 0.25 mg/infusion was available. Acquisition of cocaine self-administration was not altered by VTA D2R knockdown. b: Cocaine intake as assessed under a FR1 schedule (2 hr session) did not differ between the groups. The data represents the average cocaine intake during session 6-10 in Fig. 4a. c: When tested under a PR schedule (average of three sessions), D2R knockdown animals made significantly more active lever presses. d: Cocaine seeking was suppressed by the presentation of a footshock-associated CS . There was no different in the magnitude of suppression between control and D2R knockdown animals. Data represents the total number of lever presses during CS presentation. e: Cocaine-primed reinstatement of cocaine seeking. Both 5 mg/kg and 10 mg/kg cocaine induced significant reinstatement of responding in both experimental groups. f: Cue-induced reinstatement. Presentation of the cocaine-paired cues induced reinstatement in both groups. All reinstatement sessions lasted 1 hr. D2R-KD = D2R knockdown. Data represents group means + S.E.M. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Cocaine self-administration

We first tested the acquisition of cocaine self-administration during 5 sessions using a low unit dose of cocaine (i.e., 0.083 mg/infusion; Baarendse *et al*, 2014) (Fig. 4a). During acquisition, we did not observe a difference between the two groups ($F_{(1,19)\ knockdown}$=0.0137, P=0.908), nor did we observe a difference in responding for cocaine during the 5 subsequent FR1 sessions using our usual unit dose (i.e. 0.25 mg/infusion; (Baarendse *et al*, 2014; Veeneman *et al*, 2012a; 2012b) t=0.068, df=35, P=0.95)(Fig. 4b). There was also no difference between the groups in the loading dose of cocaine (i.e., the initial amount of cocaine the animals take to bring their blood cocaine concentration up to a certain level at the beginning of the self-administration session) (Supplementary Fig. 2). However, the D2R knockdown group made about twice as many active lever presses under a PR schedule of reinforcement (t=2.608, df=28, P=0.014)(Fig. 4c).

Conditioned suppression of cocaine seeking

In order to assess compulsive cocaine seeking we employed a conditioned suppression model as previously used (Vanderschuren and Everitt, 2004). Fig. 4d describes the total number of lever presses during presentation of the CS. We observed profound suppression of cocaine seeking during presentation of the footshock-associated CS, but no differences in responding between the groups ($F_{(1,29)\ conditioning}$=31.03, P=0.001; $F_{(1,29)\ knockdown}$=2.691, P=0.29; $F_{(1,29)\ conditioning\ x\ knockdown}$=1.631, P=0.4112) (Fig. 4d).

Reinstatement of cocaine seeking

Significant cocaine-induced reinstatement was observed following injection of either 5 mg/kg or 10 mg/kg cocaine, but VTA D2R knockdown did not change reinstatement of cocaine seeking ($F_{(2,50)\ cocaine\ dose}$=9.90, P=0.0002; $F_{(1,25)\ knockdown}$=0.11 P=0.738.)(Fig. 4e). Response-contingent presentation of the cocaine-associated cue-light in combination with retraction of the levers lead to reinstatement of cocaine-seeking, but there was no effect of VTA D2R knockdown ($F_{(1,25)\ cue}$=55.20, P<0.0001; $F_{(1,25)\ knockdown}$=0.001, P=0 .980) (Fig. 4f).

Discussion

Previous experiments have suggested that decreased D2R availability in the midbrain contributes to addiction-like behavior (Bello *et al*, 2011; Buckholtz *et al*, 2010; Madhavan *et al*, 2013; Marinelli and White, 2000; Zald *et al*, 2008).

Moreover, obese mice have been shown to have desensitized D2 autoreceptors (Koyama *et al*, 2014). To investigate whether decreased expression of the D2R in the VTA promotes addictive behavior directed at food or drugs, we used shRNA-mediated knockdown of the D2R. VTA D2R knockdown increased the psychomotor response to a low dose of cocaine and increased the motivation to respond for sucrose or cocaine. However, there were no differences in sucrose or cocaine self-administration under a FR schedule of reinforcement, conditioned suppression of cocaine seeking or reinstatement of sucrose or cocaine seeking. These data show that VTA D2R down-regulation renders animals more motivated to work for a reward, but that other aspects of addictive behavior are not affected.

Downregulation of VTA D2R increased the psychomotor response to a low dose of cocaine. This is in accordance with previous work, in which the D2R was ablated in all DA neurons (Bello *et al*, 2011). Cocaine increases the synaptic DA concentration by blocking the DA transporter (Ritz and Kuhar, 1989) and in turn, DA inhibits firing of DA neurons by binding to the somatodendritic D2R (Zhou *et al*, 2006). Decreased D2R expression on DA neurons is therefore likely to impair this feedback mechanism, providing a plausible explanation for our results. Indeed, it has previously been shown that decreased D2R autoreceptor activity or Drd2 knockout in DA neurons results in increased DA release in the striatum as assessed with fast scan cyclic voltammetry (Bello *et al*, 2011; Calipari *et al*, 2014b). Interestingly, we did not find a change in locomotor activity after a saline injection, or in inactive lever presses in the self-administration experiments. This result diverges from the findings with Drd2 autoreceptor knockout mice, which did show increased basal locomotor activity (Bello *et al*, 2011). Apart from the species difference, the different findings may be explained by the location of the knockdown, which, in our case, was confined to the VTA. Thus, the effects of Bello *et al*. [23] may be the result of the absence of the D2R in the substantia nigra, which is known to play a role in novelty-induced exploration (Schiemann *et al*, 2012). Alternatively, in our study, knockdown resulted in a 50% reduction in D2R expression, which is likely to evoke more modest behavioral effects than a complete knockout of D2R. We would also like to emphasize that in our study the D2R knockdown was induced in adult animals, whereas in the study of Bello *et al*. (2011) the Drd2 gene was constitutively absent throughout the development of the animals, which may also have induced compensatory functional changes. A limitation of the current approach is that the vector used does not selectively transfect dopamine neurons, but other neuron types as well. Although they form a minority, there are GABAergic neurons in the VTA that are responsive to a

D2R agonist (Margolis *et al*, 2006), and project to e.g. the PFC, amygdala and NAcc (Margolis *et al*, 2008; Swanson, 1982). Likewise there are glutamatergic neurons that are sensitive to a D2R agonist in the medial VTA (Hnasko *et al*, 2012). Although a contribution of VTA GABAergic or glutamatergic neurons to the behavioral changes observed could therefore not be excluded, the available literature strongly suggests a role for VTA DA, as described below. We observed that down-regulation of the D2R in the VTA results in increased motivation to respond for both sucrose and cocaine without changing responding under a FR schedule of reinforcement. This is consistent with the well-established notion that mesolimbic DA mediates incentive motivation and willingness to work for rewards, especially when the effort requirement is high (Salamone and Correa, 2012). The striking difference in how FR responding (which may be more related to reward intake than to incentive motivation) and PR responding (widely accepted as an index of incentive motivation) are affected by VTA D2R knockdown fits well into a large literature that shows markedly different neural substrates of reward consumption vs. processes related to motivation and willingness to perform effort (Berridge *et al*, 2009), whereby DA has been primarily implicated in the latter (Barbano and Cador, 2007). Since we hypothesized that DA release in the NAcc will be increased by VTA D2R knockdown, this might well explain the increased willingness that the animals show to obtain a sucrose or cocaine reward.

We did not find a change in conditioned suppression of cocaine seeking, nor did we find differences in food-, cocaine- or cue-induced reinstatement after VTA D2R knockdown. Thus, decreased VTA D2R activity specifically increased incentive motivation, but does not alter other addiction-like behaviors such as compulsive seeking or reinstatement. Interestingly, it has previously been shown that lengthening daily cocaine access (6h per day) results in escalated cocaine intake, which is interpreted as a sign of addiction-like behavior (Ahmed and Koob, 1998; 1999; Edwards and Koob, 2013). In our study, cocaine intake was not increased in the D2R knockdown group, which is in accordance with the observations that escalation of cocaine self-administration is associated with a decrease in DA release in the ventral striatum (Calipari *et al*, 2014a; Willuhn *et al*, 2014). In this regard, the initial reinforcing effects of cocaine are thought to be mediated by the ventral striatum, whereas the development of habitual and finally compulsive cocaine intake is associated with functional changes in the dorsal striatum and prefrontal cortex (Everitt and Robbins, 2005; Goldstein and Volkow, 2011; Jentsch and Taylor, 1999; Lesscher and Vanderschuren, 2012; Pierce and

Vanderschuren, 2010). It is therefore likely that our manipulation predominantly altered VTA to ventral striatal DA signaling and therefore only influenced the incentive motivational properties of sucrose and cocaine, but did not directly promote other aspects of addiction-like behavior. Of course, even though VTA D2R knockdown did not result in the emergence of multiple signs of addiction-like behavior, this does not exclude a role for VTA D2R in the development of addiction. Humans or animals more motivated for drugs may overcome bigger hurdles to obtain them and thus expose themselves to more drugs over time, which may ultimately lead to full-blown addictive behavior, mediated by other mechanisms than VTA D2R function. Likewise, increased motivation for food may promote increased body weight and obesity in the long run, even without being labeled 'food addiction'. In conclusion, we show that decreased availability of the D2R in the VTA specifically induces increased motivation for both food and drug rewards.

Funding and disclosure

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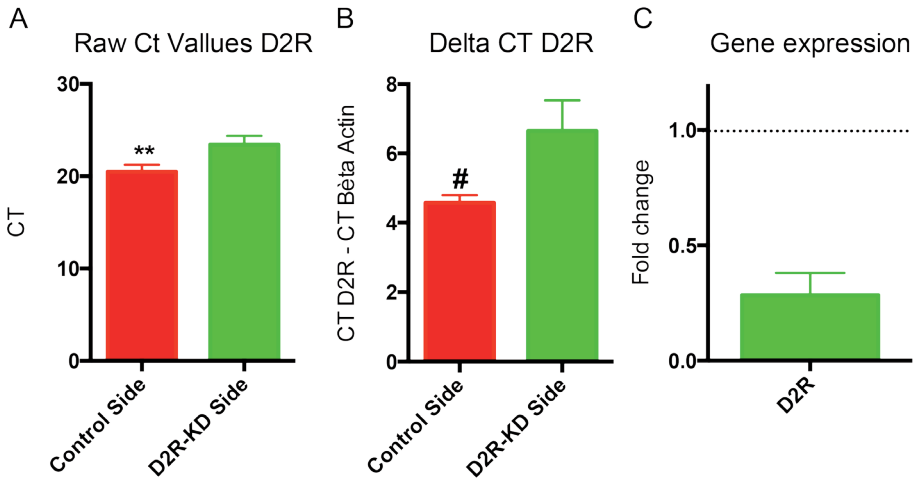
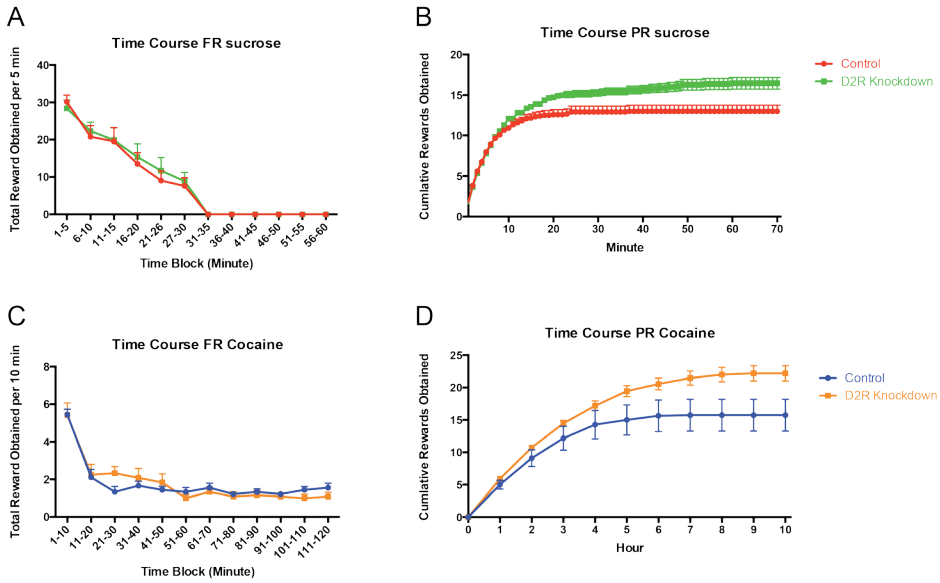
Supplementary figure 1



QPCR on unilateral virus injections 4 weeks after surgery. To confirm stable knockdown throughout the behavioral experiments, 3 animals were injected with the D2R knockdown vector on one side and the control vector on the other side of the VTA. Four weeks after surgery, the animals were sacrificed and unilateral dissections were made. QPCR was performed as described in the methods section. a. We observed a significant increase in raw CT values when comparing the D2R knockdown side to the control side ($T=13.27$, $df=2$, $p=0.006$). b. When ΔCT values (CT D2R - CT Bèta-Actin) were compared there was a trend ($T=3.107$, $df=2$, $p=0.09$). c. The increase in ΔCT corresponded to approximately 75% knockdown. D2R-KD = D2R knockdown. Data represents group means + S.E.M. #$p<0.1$, **$p<0.01$.



Time course of FR and PR responding for sucrose or cocaine. a. Representative example of an FR5 session for sucrose (The last FR5 session, right before extinction). Sucrose intake declined over time, likely due to satiety factors. b. Representative example of the cumulative intake during a PR session for sucrose (i.e., the 2th PR session after surgery). c. Representative example of an FR session for cocaine (i.e., the 4th session where the animals worked for 0.25 mg/inf.). Note the characteristic increased intake in the first 10 min of the session (so-called 'loading phase'), which was not different between the groups. d. Representative example of the cumulative intake during a PR session for cocaine (the first PR session).

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4

DREADD ACTIVATION OF THE MESOLIMBIC AND MESOCORTICAL PATHWAYS DIFFERENTIALLY ALTERS APPETITIVE AND AVERSIVE BEHAVIOR

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Abstract

Mesolimbic and mesocortical dopamine neurons are involved in several psychiatric disorders including mood disorders, addiction, eating disorders, schizophrenia and attention-deficit/hyperactivity disorder. How these projections are functionally involved in behavior has in the past been studied using dopamine-selective lesions or micro-infusions of dopamine receptor agonists and antagonists into the medial prefrontal cortex (mPFC) or nucleus accumbens (NAcc). Here, we selectively activate the mesolimbic or mesocortical pathway *in vivo* using designer receptors exclusively activated by designer drugs (DREADD) in combination with a retrograde traveling canine adeno virus that expresses CRE recombinase (CAV2-CRE). We show that activation of the mesolimbic pathway increases general activity, motivation for palatable food and cue-induced reinstatement of sucrose seeking. Activation of the mesocortical pathway promoted fear extinction and altered the response to a mildly stressful stimulus. These results contribute to our further understanding of how subdivisions of the mesocorticolimbic system are involved in appetitive and aversive behavior.

Introduction

Midbrain dopamine neurons originating in the ventral tegmental area (VTA) are involved in reward-related processes, emotion, memory as well as locomotion and decision making (Berridge, 2007; Kelley, 2004; Rogers, 2011; Salamone and Correa, 2012; Schultz, 2007). Dysfunction of these neurons has been implicated in mood disorders, addiction, eating disorders, schizophrenia and attention-deficit/hyperactivity disorder (Cao *et al*, 2010; Fernando *et al*, 2012; Lammel *et al*, 2014; Mathes *et al*, 2009; Nestler, 2005; Volkow *et al*, 2009). These VTA dopamine project to brain regions including the ventral striatum (nucleus accumbens (NAcc) and olfactory tubercle) as well as the dorsomedial striatum, prefrontal cortex (PFC), hippocampus and amygdala. Of these, the two most widely studied projections are the VTA to NAcc (mesolimbic) and the VTA to PFC (mesocortical) pathways. These pathways are thought to have very different, perhaps sometimes opposite, effects on behavior and have distinct electrophysiological properties and gene expression patterns (Bassareo *et al*, 2002; Lammel *et al*, 2008; 2011; 2012; Margolis *et al*, 2006; Tanda *et al*, 2014).

Until recently, most evidence about the role of specific dopamine projections came from studies using dopamine-selective lesions or pharmacological studies using site-specific microinfusions of dopamine agonists or antagonists (e.g. (Mueller *et al*, 2010; Veeneman *et al*, 2014)). These studies are limited in that they do not simulate dopamine neuronal activation, although they mimic or inhibit activation of dopamine receptors. In fact, a dopamine D2 or nonspecific receptor agonist is likely to inhibit dopamine release via the presynaptic dopamine D2 receptor (D2R), and to stimulate postsynaptic D2Rs at the same time (Aghajanian and Bunney, 1977; Bello *et al*, 2011). The recent development of optogenetics and pharmacogenetics allows for the selective activation or inhibition of individual neuronal pathways *in-vivo* (Aston-Jones and Deisseroth, 2013). Here we employ pharmacogenetics (i.e. designer receptors exclusively activated by designer drugs (DREADD)) in combination with a retrograde traveling canine adenovirus that expresses CRE recombinase (CAV2-CRE) (Boender *et al*, 2014; Soudais *et al*, 2001). DREADDs are G-coupled receptors exclusively activated by an otherwise inert, exogenous ligand, clozapine-N-oxide (CNO), which can be injected systemically (Armbruster *et al*, 2007). The activating DREADD (hM3Dq) stimulates neuronal activation upon binding of CNO (Krashes *et al*, 2011). CAV2-CRE allows for the specific targeting of hM3Dq to individual neuronal projections. DREADD technology is suitable for these experiments because of its ease of

use, which allows for behavioral experiments with a large group size (due to their inherent variability) such as the experiments described in this chapter.

Here we investigate several behaviors that have previously been associated with VTA dopaminergic signaling. Dopamine release in the NAcc is associated with increased motivation to obtain a reward (Berridge, 2007; Kelley, 2004; Salamone and Correa, 2012; Schultz, 2007). We therefore describe the effect of selective activation of the mesolimbic and mesocortical pathways on sucrose self-administration and motivation to obtain a sucrose reward as well as cue-induced reinstatement. We also investigated suppression of sucrose seeking by a previously conditioned aversive stimulus (Vanderschuren and Everitt, 2004). Stimulation of the D2R in the infralimbic cortex (IL) has previously been shown to be required for proper fear extinction (Mueller *et al*, 2010). In order to investigate if activation of the VTA to PFC pathway facilitates fear extinction, animals where repeatedly exposed to the conditioned suppression paradigm. Since prefrontal systems have also been implicated in the effects of stress (Lammel *et al*, 2013; Pezze and Feldon, 2004), we opted to assess responding to a mild stressor in these rats on a sudden onset of silence (SOS) paradigm (Hendriksen *et al*, 2014).

Methods

Animals

Six-week old male Wistar rats (Charles River, Sulzfeld, Germany) weighing 150-200g at the beginning of the experiment were pair housed in macrolon cages (37.8 x 21.7 x 18.0 cm) under a reversed 12:12 light/dark cycle (lights on at 19:00h). They had ad-libitum access to chow and water and home cage enrichment was provided in the form of a wooden block. All animal experiments were approved by the Animal Ethics Committee of Utrecht University and were conducted in agreement with Dutch laws (Wet op de Dierproeven, 1996) and European regulations (Guideline 86/609/EEC).

Surgery

Rats were anaesthetized with fentayl/fluanisone (0.315mg/kg fentanyl, 10 mg/kg fluanisone, Hyponorm, Janssen Pharmaceutica, Beerse, Belgium) i.m. and supplemented with Hypnorm as needed. Next, the animals were placed in a stereotaxic apparatus (David Kopf), and 1µl of a solution containing AAV5-hSyn-DIO-hM3Gq-mCherry (10^9 particles) was injected bilaterally into the VTA (coordinates relative to bregma: anteroposterior -5.40, mediolateral ±2.20,

dorsoventral -8.90). One µl of CAV2-CRE (1.25.10^9 particles) was injected into the PFC (anteroposterior +2.70, mediolateral ±1.40, dorsoventral -4.90) or NAcc (anteroposterior +1.20, mediolateral ±2.80, dorsoventral -7.50). Animals receiving sham injections received CAV2-CRE injections as in one of the other two groups, but were instead injected with AAV5-hSyn-DIO-ChR2-eYFP (10^9 particles) into the VTA. Carprofen (50 mg/kg, s.c.) was administrated once before and twice after surgery. Animals were allowed 7-9 days to recover from surgery. All behavioral experiments started at least 3 weeks after surgery, a which point we have observed proper virus expression (data not shown).

Operant conditioning apparatus

Rats were trained in operant conditioning chambers (30.5 x 24.1 x 21.0 cm; Med Associates Inc., St. Albans VT. USA). The chambers were placed in light- and sound-attenuating cubicles equipped with a ventilation fan. Each chamber was equipped with two 4.8 cm wide retractable levers, placed 11.7 cm apart and 6.0 cm from the grid floor. The assignment of the left and right lever as active and inactive lever (see below) was counterbalanced across rats. A cue light (28 V, 100 mA) was present above each lever and a house light (28 V, 100 mA) was located on the opposite wall. Sucrose pellets (45 mg; SP; 5TUL, TestDiet, USA) were delivered to a receptacle between the two levers. The operant testing apparatus was controlled by MED-PC (version IV) Research Control & Data Acquisition System software. Self-administration sessions were carried out once daily, between 9 AM-6 PM, for 5-7 days a week.

Sucrose self-administration

Animals were trained to respond for sucrose as described previously (la Fleur *et al*, 2007). During the experimental period animals received ad libitum chow. Operant sessions lasted 1 hr, during which the availability of the reward was signaled to the animal by illumination of the house light. Pressing the active lever resulted in the delivery of a sucrose pellet, the illumination of the cue light above the active lever for 5 seconds and retraction of the levers. After a 10 sec time-out period, the levers were reintroduced and the house light illuminated, signaling the start of a new cycle. Pressing on the inactive lever was without scheduled consequences. Animals were trained to respond for sucrose under a fixed-ratio 1 (FR1) schedule of reinforcement, meaning that each active lever press resulted in the delivery of one sucrose pellet (45 mg). After acquisition of sucrose self-administration under this schedule, the response requirement was increased to ten lever presses (i.e. an FR10 schedule of reinforcement).

Thereafter we assessed motivation for sucrose under a progressive ratio (PR) schedule of reinforcement, in which the response requirement was progressively increased after each obtained reward (1, 2, 4, 6, 9, 12, 15, 20, 25, etc (Richardson and Roberts, 1996)) A PR session ended after the animal failed to obtain a reward within 30 min. The animals were trained on FR and PR responding before surgery. After surgery they were retrained on both FR and PR schedules until we observed stable responding. They were then tested after saline or CNO (0.3mg/kg, i.p.) injection in a counterbalanced design. The animals had previously been habituated to i.p. saline injections.

Devaluation procedure

One hour before the operant procedure, animals were individually housed in standard cages where they had ad-libitum access to water and standard chow (non devalued situation) or sucrose pellets (devalued situation). After 30 min, they received an i.p. injection of 0.3mg/kg CNO or saline after which they were placed back in the cage for an additional 30min. The devaluation test comprised 10min of non-reinforced lever pressing whereby pressing on either lever was without scheduled consequences. This was immediately followed by a regular FR5 session. The animals were tested 4 times (devalued/non-devalued, CNO/saline) according to a within-subject counterbalanced design. Each test day was followed by at least 2 days of FR5 training to prevent occurrence of extinction.

Conditioned suppression

For the conditioned suppression procedure (Limpens *et al*, 2014; Vanderschuren and Everitt, 2004) rats, that has previously been trained under an FR10 schedule of reinforcement, learned to associate a tone (85dB, 2900Hz) with the unpredictable delivery of mild electric (0.35mA, 1s) footshocks. The conditioning session consisted of a lead in period of 10 min followed by 10minutes of exposure to the tone and simultaneous delivery of 10 randomly dispersed footshocks. This procedure (10min tone off, followed by 10min tone on and delivery of footshocks) was repeated once after which the session ended with a 10min lead out period during which no tone or shocks were present. The procedure was performed in an operant chamber, which was physically different from the one in which the animals responded for sucrose. They had been habituated to this chamber before (two times for 10 min, while the house light was on, but no levers were present). The suppression session was identical to a regular FR10 session, except that the footshock-associated tone was presented from minute 3-5 of the 60min session. The conditioned suppression session was repeated three

times to induce extinction of the tone-footshock association. CNO was injected during the first two conditioned suppression sessions, but not during the final session. There was within-session as well as between-session extinction of the conditioned tone. Based on a pilot experiment, responding during the first minute of tone presentation was used to assess between-session extinction. This was necessary because an increase in responding during the 2th minute resulted in a ceiling effect since the maximum number of lever presses that the animals could make in one minute is approximately 40.

Extinction and cue-induced reinstatement procedure

Extinction of responding was assessed under identical circumstances as FR responding, except that responding on either lever was without scheduled consequences. Animals received 9 60-min extinction sessions followed by a reinstatement test that was identical to a FR10 session in that every 10th lever press was followed by retraction of the levers and presentation of the cue-light above the active lever, but no rewards were delivered. In addition, the first lever press also resulted in presentation of these cues. CNO was injected at three instances during this procedure. 1. Before the first extinction session to measure the effect on the extinction overshoot. 2. Before the 8th session to measure the effect on extinction responding. 3. Before the cue-induced reinstatement session.

General locomotion and stress of sudden silence

Animals received an i.p. injection of CNO or saline 30min prior to the locomotor test. They were then exposed to a square (72×72×45 cm) arena for 10min. During the first 5min, 85 dB white noise was present, the second 5min were in silence (i.e. sudden onset of silence (SOS)). The test was performed during the dark (active) phase of the animals, in a brightly illuminated room. Horizontal movement of the animal was tracked using a camera placed above the arena that was coupled to a computer running Ethovision 3 (Noldus, Wageningen). Locomotor activity was assessed throughout the session in 10 blocks of 1min. To investigate a possible short-term effect (freezing) after SOS, the time window from 50sec before until 50sec around SOS was analyzed in blocks of 10sec.

Tissue preparation and immunohistochemistry

Animals were euthanized by i.p. injection of pentobarbital followed by an intracardial perfusion with cold 4%PFA in PBS. After dissection, the brains were post fixed for 24h in 4% PFA in PBS and then stored in 30% sucrose in PBS at

4˚C until ready for immunohistochemistry. Brains were cut at 40µm and stored in 30% sucrose and 0.02%NaAz in PBS until ready for processing. Sections were then washed in PBS and incubated in blocking buffer (1% normal goat serum, 0.2% Triton X) and incubated overnight at 4˚C with 1:500 rabbit anti dsRED (Living Colors Cat. No. 632496) and 1:750 mouse anti TH. Secondary antibodies were Alexa-594-labeled goat anti rabbit and Alexa-488 anti mouse (both Molecular Probes, USA), 1:500. After washing in PBS, sections were mounted and embedded in Fluorsave (Merck Millipore).

Statistics

Data was analyzed using Prism 6 (Graphpad Software). The 3 groups were compared using an two-way ANOVA with CNO or Saline treatment as within-subjects factor, except when a between-subjects desing was employed (i.e., conditioned suppression, SOS), in which cases the data was analyzed using a one-way ANOVA. Significant main effects were followed up with Sidak's multiple comparison test.

Results

Virus expression

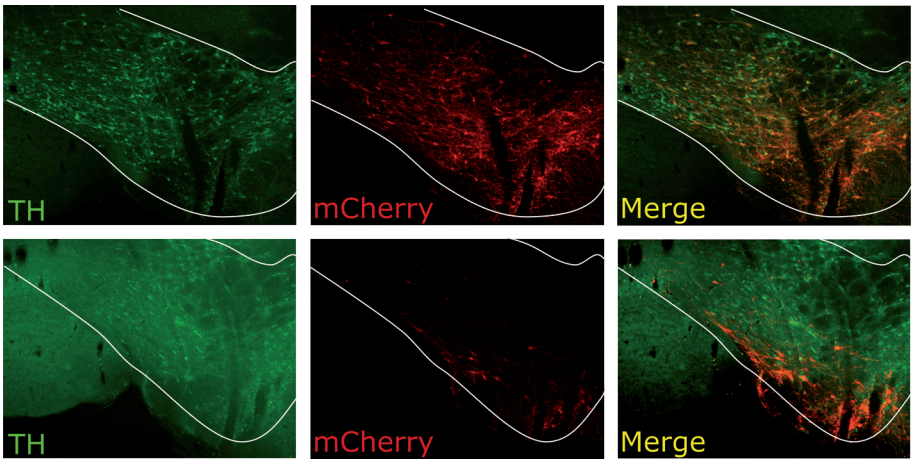
Preliminary data demonstrates DIO-hM3Gq-mCherry expression in both the VTA to NAcc and VTA to PFC group (Fig. 1). We observed coexpression with TH in both groups, but notable differences in number and location of hM3Gq-expressing cells (Fig. 1).

Sucrose-self administration

Rats (n=26, 8 NAcc, 9 PFC, 9 sham) were trained to self-administer sucrose under a FR10 schedule of reinforcement There was a significant (ANOVA) effect of CNO treatment on active lever responding ($F_{(1,23)}$=4.993, p=0.036) but no interaction with the projections ($F_{(2,23)}$=0.7143, p=0.500)(fig. 2a). Post-hoc testing did not show any significant differences per group, (T=2.156, df=23, p=0.120). There was a robust increase in inactive lever responding after CNO in the VTA to NAcc group ($F_{(2,\ 23)\ \text{treatment x projection}}$=4.612, p=0.021, $T_{\text{cno vs. saline}}$=3.995, df=23, p=0.002) (fig. 2b).

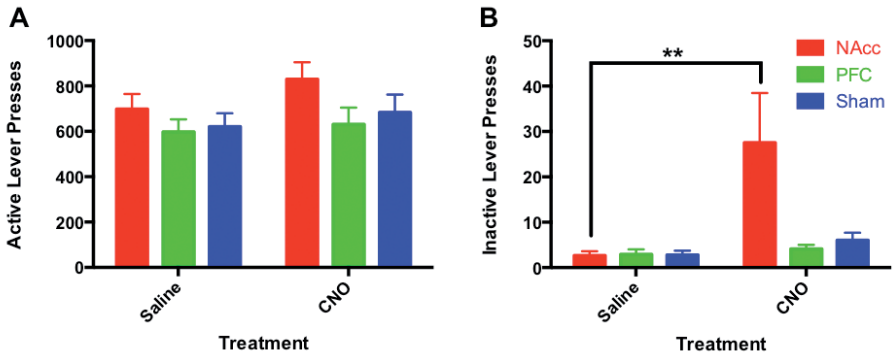
The same rats were tested under a PR schedule of reinforcement. There was a significant interaction between CNO treatment and projection group ($F_{(2,23)}$=10.04, p=0.0007)(fig 3a). In concurrence with our earlier work (Boender *et al*, 2014),

Figure 1



Virus expression in the VTA. Top row: VTA from an animal from the VTA to NAcc group. Bottom row: VTA from an animal from the VTA to PFC group. White outline signifies the contour of the VTA.

Figure 2



Responding for sucrose. Animals responded for sucrose under an FR10 schedule of reinforcement. A. Active lever responding on a FR10 schedule. B. Inactive lever responses while responding under a FR10 schedule. Data represents mean ± S.E.M. **p<0.01.

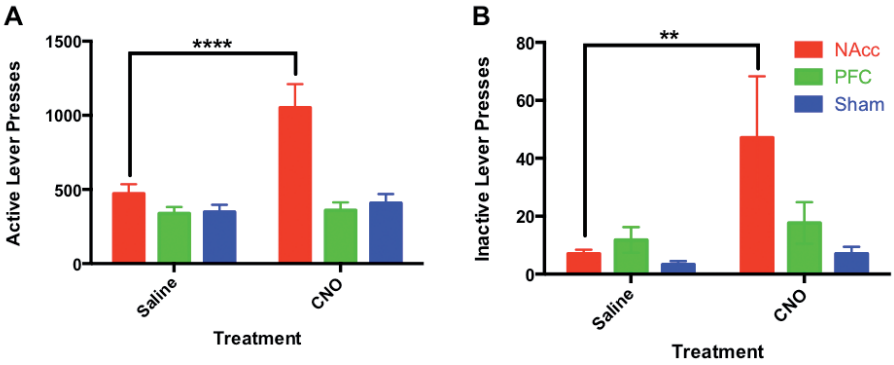
activation of the VTA to NAcc pathway resulted in an increase in responding for sucrose (T=5.776, df=23, p<0.0001)(fig. 3a) that was accompanied by an increase in inactive lever responses (T=3.353, df=23, p=0.008)(fig. 3b). Active and inactive lever presses in the VTA to PFC or Sham group were unaffected by CNO treatment.

Because the increase in active lever responding in the VTA to NAcc group under a PR schedule was accompanied by an increase in inactive lever responding, it is possible that these animals were simply more active and that lever responding in these rats was not goal directed and thus not an accurate reflection of their motivation. We therefore assessed responding for sucrose after devaluation by prefeeding the animals with sucrose. Rats (n=23, 12 NAcc, 11 PFC) were trained to self-administer sucrose under a FR5 schedule of reinforcement. We assessed both non-reinforced (made in a 10min period before a normal FR session in which lever presses were not reinforced) and reinforced responses on the active lever. CNO treatment did not affect the total amount of sucrose or chow consumed during the devaluation procedure. Prefeeding with sucrose robustly decreased non-reinforced and reinforced responses in both groups. CNO did not affect responding after devaluation in either non-reinforced (ANOVA on data from devalued samples $F_{(1, 21)}$=0.433, p=0.518)(fig. 4, left panel) or reinforced responses (ANOVA on data from devalued samples $F_{(1, 21)}$=1.030, p=0.322)(fig. 4 right panel). Neither was there an effect of CNO when sucrose was not devalued and the animals were instead prefed with chow (non-reinforced responses: $F_{(1,21)}$=0.038, p=0.848; reinforced responses: $F_{(1,21)}$=1.474, p=0.238). Within the VTA to NAcc group we did observe an increase in inactive lever presses after CNO injection that was not affected by prefeeding ($F_{(1,11)\ \text{CNO treatment}}$ =7.245, p=0.0210; $F_{(1,11)\ \text{prefeeding}}$=1.561, p=0.237)(Data not shown).

Conditioned suppression of sucrose seeking

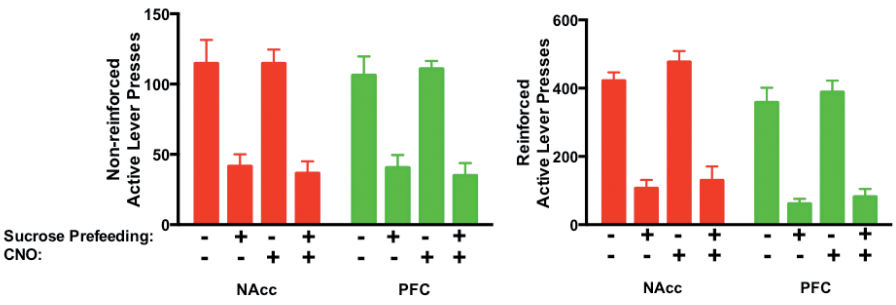
In order to assess compulsive sucrose seeking, the same rats as used for the PR and FR experiments (total n=34, 8 NAcc, 9 PFC, 9 Sham) were tested in a conditioned suppression paradigm (Vanderschuren and Everitt, 2004). Although the conditioned tone suppressed sucrose seeking during initial exposure (CS1), after repeated exposure the animals displayed fear extinction whereby the tone was no longer able to suppress sucrose seeking (fig. 5). To investigate the effect of DREADD stimulation on the extinction of fear, the animals were exposed to the conditioned tone on three consecutive days, of which they only received CNO on the first two. There was a borderline significant interaction between the

Figure 3



PR responding for sucrose. A. Active lever presses while responding under a PR schedule. B. Inactive lever responding during a PR session. Data represents mean ± S.E.M. **p<0.01, ****p<0.0001.

Figure 4



Non-reinforced and reinforced responding after devaluation by prefeeding with sucrose. Left panel: The number of active lever presses made during 10 minutes of non-reinforced lever pressing after prefeeding with either unlimited sucrose or chow. Right panel: Active lever responding during a normal FR5 session after prefeeding with either unlimited sucrose or chow. Data represents mean ± S.E.M.

session and group ($F_{(6, 69)\ projection\ x\ session}$=2.147, p=0.059) whereby the PFC group was different from the sham group during the third CS session, when no CNO was injected (T=2.910, df=120, p=0.026) (fig. 5).

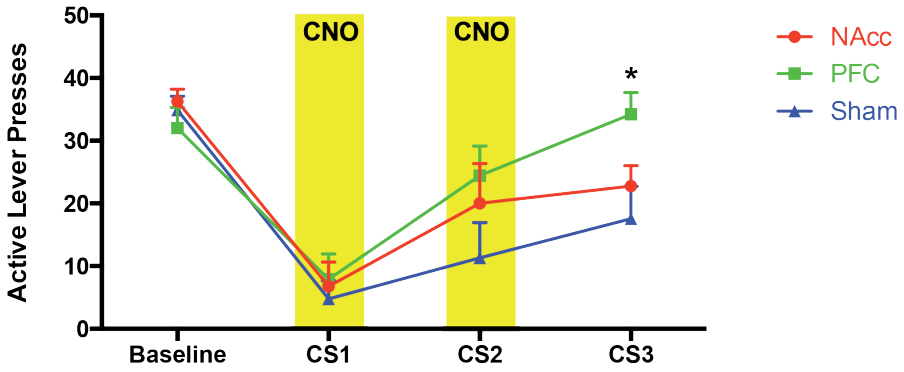
Extinction and cue-induced reinstatement of sucrose seeking

The same cohort of animals was then exposed to an extinction and reinstatement procedure. There was a significant group effect and an interaction between group and session for responding on the active lever ($F_{(2,23)\ group}$=16,80, p<0.0001; $F_{(18,\ 207)\ group\ x\ session}$=14.35, p<0.0001)(fig. 6a). The VTA to NAcc animals pressed significantly more than the sham-injected animals during the first extinction session (T=5.702, df=230, p<0.0001) and during reinstatement (T=13.99, df=230, p<0.0001). There was a trend during the 8th extinction session (T=2.276, df=230, p=0.070). There was a group effect on inactive lever responding ($F_{(2,23)}$=5.66, p=0.01) and an interaction between session and group ($F_{(18,207)}$=3.24, p<0.0001) (fig. 6b). Inactive lever responding was significantly increased in the VTA to NAcc group (compared to sham-injected animals) during the first extinction session (T=3.487, df=230, p=0.002), the 8th extinction session (T=2.771, df=230, p=0.012) and during reinstatement (T=5.665, df=230, p<0.0001)(fig 6b). All groups showed significant cue-induced reinstatement of sucrose seeking (Effect of reinstatement NAcc: $F_{(1,14)}$=35.01, p<0.0001; PFC: $F_{(1,16)}$=6.96, p=0.018; Sham: $F_{(1,16)}$=5.63, p=0.03)(fig. 6c, 6d and 6e). In the VTA to NAcc group, CNO increased active lever responding during reinstatement (T=8.082, df=14, p<0.0001) inactive lever responding in the presence of CNO was equal during extinction and reinstatement (T=0.286, df=14, p=0.779)(fig. 6c).

General locomotion and stress of sudden silence

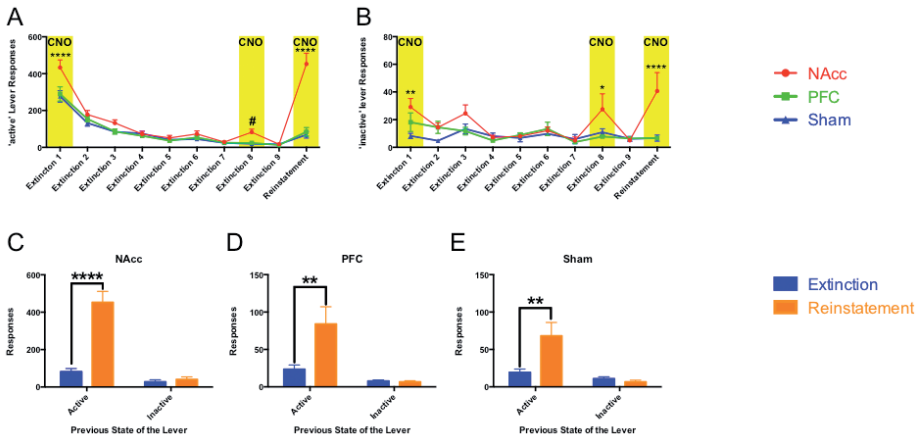
Rats (n=47, 17 NAcc, 15 PFC, 6 Sham surgery, 9 injected with saline) were injected with CNO or saline. They were then exposed to a square arena where we measured horizontal locomotor activity. A session lasted 10 minutes. During the first 5 minutes the animals were exposed to 85dB white noise, while the last 5 minutes were in total silence. Since we did not observe any difference between the saline and sham-injected group, they were combined in the analysis. Throughout the whole session, animals in the VTA to NAcc group were significantly more active ($F_{(2,44)}$=9.67, p=0.0003)(fig. 7a). There was a significant interaction between SOS and experimental group ($F_{(4,\ 88)\ silence\ x\ projection}$=4.514, p=0.0023). The animals in the VTA to NAcc group showed a strong, but very brief (<10s) drop in locomotor activity following the onset of silence (fig. 7b). Whereas the animals in the VTA

Figure 5



Conditioned suppression of sucrose seeking. The number of active lever presses made in 1 minute in the presence of a previously conditioned aversive tone. Baseline refers to the same minute during the session before the first conditioned suppression session (a normal FR10 session). The yellow shading indicates that CNO was injected 30min prior to the conditioned suppression session. Data represents mean ± S.E.M. *p<0.05.

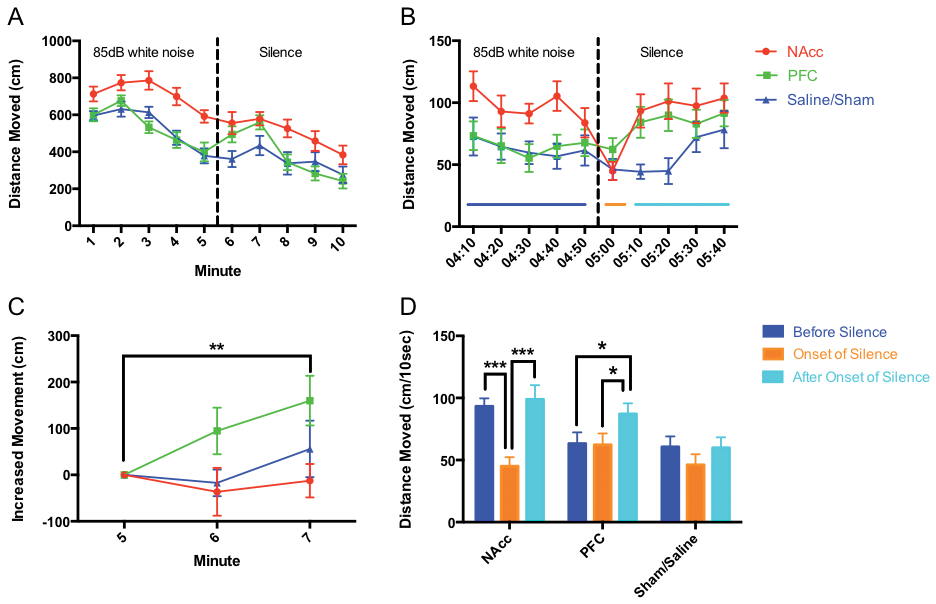
Figure 6



Extinction and cue-induced reinstatement of sucrose seeking. A. Responding on the active lever. B. Responding on the inactive lever. C, D & E. responding on the active and inactive lever during extinction (session 8) and reinstatement when CNO was present. Note that the y-axis of C is different than the ones in D and E. The yellow shading indicates that CNO was injected 30min prior to the extinction session. Data represents mean ± S.E.M. #p=0.07, *p<0.05, **p<0.01, ****p<0.0001.

to PFC groups responded oppositely with an increase in locomotor activity following the onset of sudden silence that lasted for 2min ($T_{\text{minute 5 vs minute 7}}$=3.186, df=88, p=0.006)(fig. 7c). The drop in locomotor activity in the VTA to NAcc group was significant in the 10sec bin directly following the onset of silence ($T_{\text{before vs during}}$=0.967, df=88, p<0.0001)(fig. 7d). They did however not show any effect that lasted longer than 10s ($T_{\text{before vs after}}$=0.613, df=88, p=0.542)(fig. 7d). The increase in locomotor activity in the VTA to PFC group was apparent approximately 10s after the onset of sudden silence ($T_{\text{before vs after}}$=2.463, df=88, p=0.036)(fig. 7d). We did not observe any sudden silence stress in the saline-injected or sham-operated animals.

Figure 7



General locomotion and reaction to sudden silence. A. Distance moved by the animals during a 10min locomotor measurement. During the first 5min the animals were exposed to 85dB of background noise, which was not present during the last half of the session. B. Perievent plot of the 50s before and after the onset of sudden silence. The horizontal lines indicate the data points of which the average was used for the analysis in D. C. The increase in locomotion after the onset of sudden silence, normalized to the distance moved in minute 5 (when the background noise was present). The statistics refer to the PFC group only. D. Average distance moved per 10s before, during and after the onset of silence. Data represents mean ± S.E.M. *p<0.05, **p<0.01, ***p<0.001.

Discussion

Here we employed CAV2-CRE in combination with DREADD to selectively activate the mesolimbic and mesocortical pathways. We showed that activation of the VTA to NAcc pathway strongly increased the motivation to work for a sucrose reward as well as cue-induced reintatement. These same animals also show increased general activity in the form of inactive lever responses and increased general locomotor activity. It is likely that the increased general activity that these animals showed contributed to the increase in responding on a PR schedule and during cue-induced reinstatement. It is unlikely however that increased general activity fully explains these effects for two reasons. 1. Lever pressing after devaluation (by prefeeding of the sucrose reward) was not affected by CNO. If the increase in PR responding was purely caused by increased general activity, one would expect that DREADD activation of the VTA to NAcc pathway would also increase active lever responding when the outcome was devalued, but this was not the case. Thus animals acted in an outcome-dependent manner. 2. CNO increased both active and inactive lever responses during extinction (likely due to increased general activity) but active lever responses were significantly more increased during cue-induced reinstatement whereas inactive lever responses were increased as during extinction (fig. 6b). It seems likely therefore that activation of the VTA to NAcc pathway renders animals more active *and* increases incentive motivation. Others have previously stated that general activity and motivation are very hard to distinguish experimentally as well as conceptually (Kravitz and Kreitzer, 2012; Wise and Bozarth, 1987). There is extensive literature on the role of dopamine in the NAcc and its effects on motivation (e.g. (Nunes *et al*, 2013)) and voluntary locomotion (Graybiel *et al*, 1994). Activation of the medium spiny neurons expressing the dopamine D1 receptor (D1R) in the striatum promotes both locomotion as well as cocaine reward (Kravitz *et al*, 2012; Lobo *et al*, 2010). Conversely, activation of the D2R subpopulation promotes freezing and suppresses cocaine reward (Kravitz *et al*, 2012; Lobo *et al*, 2010). Furthermore, decreased motivation and voluntary movement go hand in hand in disorders such as Parkinson's disease (Czernecki *et al*, 2002).

Neither VTA to NAcc nor VTA to PFC pathway activation influenced conditioned suppression of responding. We did however observe a decrease in conditioned suppression in the VTA to PFC group after multiple exposures to the aversive conditioned stimuli, suggesting accelerated extinction of conditioned fear. In the

current study, we did not distinguish between the prelimbic (PLC) and infralimbic cortex (ILC) with our Cav2-CRE injections, but aimed instead for the mPFC as a whole. Evidence from literature however points to a more prominent role of the ILC to mediate fear extinction (Milad and Quirk, 2002; Peters *et al*, 2009; Russo *et al*, 2000). Furthermore, infusion of the DRD2 antagonist raclopride into the ILC attenuates extinction of the freezing response to an aversive conditioned tone (Mueller *et al*, 2010). Thus our results seem to support a role for dopamine in the ILC in the extinction of fear.

Interestingly, our results on conditioned suppression are in concurrence with the results in chapter 3, where knockdown of the D2R in the VTA, which presumably led to disinhibition of dopamine neurons, did not influence conditioned suppression of cocaine seeking. In both cases we observed a robust increase in motivation (i.e. in the VTA to NAcc group), which was not accompanied with increased compulsive behavior.

The effect on SOS in the VTA to PFC group is difficult to interpret. Traditionally, SOS is associated with decreased motility and a sudden increase in heart rate (Buwalda *et al*, 1992; Hendriksen *et al*, 2014). We did see a sudden drop in locomotor activity in the VTA to NAcc group, although it is possible that the other groups responded in a similar way, but that their decrease in locomotor activity was more difficult to distinguish because they were not very active in the first place. This is in contrast to the VTA to NAcc group, that showed increased activity throughout the session. The protracted (2min) increase in locomotor activity in the VTA to PFC groups hints at an altered response to a stressful stimulus. In this respect it is interesting to note that the SOS model has been suggested to be a model for the dysfunctional stress response in Post Traumatic Stress Disorder (PTSD) (Hendriksen *et al*, 2014). PTSD patients suffer from an aberrant stress response to mildly stressful stimuli, which is associated with altered patterns of activity in fMRI studies (Liberzon and Martis, 2006; Quirk *et al*, 2006). Taken together, the literature on the role of dopamine in the mPFC on fear extinction (Milad and Quirk, 2002; Peters *et al*, 2009; Russo *et al*, 2000) and our data on the increased extinction of conditioned suppression after DREADD activation of the VTA to PFC pathway, are consistent with the view that activation of the mesocortical pathway modulates fear responses to environmental stimuli.

Methodological considerations

With the development of optogenetics it became possible to selectively activate dopamine neurons using TH::CRE or DAT::CRE mice or TH::CRE rats (Tsai *et al*, 2009; Witten *et al*, 2011). It is also possible to target a specific projection by injecting channelrhodopsin 2 (DIO-ChR2) in the VTA and placing the optic fiber trough which laser light is delivered in a specific projection site. A limitation of this approach is that although synaptic release of dopamine is mimicked, there is no neuronal firing starting at the neuronal soma; in fact there is a risk of antidromic activation. A more practical disadvantage is that not all labs have optogenetics (which is expensive and requires significant technical know-how) available and that optogenetics in freely moving animals is technically challenging in that animals (especially rats) have a natural aversion to any sort of cable fixed to their head and (as many researchers have found) seem to be quite apt at demolishing fragile glass fiber cables. This makes the successful attempts with optogenetics in freely moving rats all the more laudable (e.g. (Chen *et al*, 2013; Witten *et al*, 2011)). Especially in experiments that require a large group size, DREADD technology in combination with CAV2-CRE is a very feasible and attractive alternative.

A possible limitation of this study is that the current approach is not cell-type specific. Therefore, our results might me explained by other (presumably GABAergic or glutamatergic) neurons projecting from the VTA to the PFC or NAcc. It should be noted that the vast majority of neurons projecting from the VTA to NAcc are dopaminergic, although this is not necessarily true for the mesocortical pathway, which are for a large part GABAergic (Carr and Sesack, 2000; Duvarci *et al*, n.d.; Margolis *et al*, 2008; Swanson, 1982). A role for dopamine in the present behavioral findings is, however,consistent with the available literature, as reviewed above. Currently, Cav2-CRE technology does not allow for both cell-type and projection specificity, but the imminent development of CRE-depended Cav2-flip in combination with CRE-expressing rat and mice lines as well as the development of cell-type specific minimal promoters will allow for this in the near future.
Another limitation, that was already briefly discussed, is that our injections were aimed at the entire mPFC and NAcc, whereas these brain structures consist of anatomically and functionally distinct sub-regions (ILC and PLC, NAcc Core, lateral and medial Shell) (Cardinal *et al*, 2002; Floresco, 2015; Heidbreder and Groenewegen, 2003; Peters *et al*, 2009; Voorn *et al*, 2004). Future experiments using microinjections of CAV2-CRE in very specific brain regions will further pinpoint the neural correlates of the behaviors described in this chapter.

In conclusion, we show that activation of the VTA to NAcc pathway increases both general locomotion and incentive motivation and that activation of the VTA to PFC pathway facilitates fear extinction and affects the response to a mildly stressful stimulus. These results shed further light on the heterogeneity of the mesocorticolimbic system and the involvement of individual VTA efferents in motivated and aversive behavior.

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5

SWEET TASTE AND POSTINGESTIVE EFFECTS DIFFERENTIALLY AFFECT THE MOTIVATION FOR FOOD:

THE ROLE OF INCENTIVE LEARNING

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Abstract

Sugar-rich foods are known to engage brain reward systems, which may promote unhealthy eating habits. Sucrose can influence these systems via its sweet taste as well as via its postingestive effects. Indeed, it has previously been shown that the sweet taste and the postingestive effects of sucrose differentially influence its intake and associative learning processes. The aim of the current study was to investigate the contribution of sweet taste and postingestive effects to incentive motivation. Rats were trained to respond for saccharin (a non-caloric sweetener) or maltodextrin (a carbohydrate with similar postingestive effects as sucrose, but without the sweet taste) and motivation for these solutions was evaluated under a progressive ratio schedule of reinforcement. Initially, the animals responded more for saccharin than for maltodextrin. However, after a learning phase, in which the animals associated the taste of maltodextrin with its postingestive effects, the incentive value of maltodextrin increased compared to saccharin. Although initially, bodyweight was not associated with motivation for either solution, there was an association between body weight and motivation for maltodextrin after the incentive learning phase. Furthermore, maltodextrin, but not saccharin intake was strongly associated with body weight. The present results show that sweet taste and postingestive effects of food both support operant responding. Moreover, the postingestive effects of carbohydrates can increase the motivation for food through an incentive learning mechanism.

Introduction

Obesity and its comorbidities cardiovascular disease, type 2 diabetes and cancer are a major threat to public health and a serious socioeconomic burden (Flegal *et al*, 2010; Fry and Finley, 2005). There is an ongoing debate as to whether addiction-like processes directed at palatable food contribute to the obesity epidemic (Blundell and Finlayson, 2011; Gearhardt *et al*, 2011; Potenza, 2014; Volkow *et al*, 2012; Ziauddeen and Fletcher, 2012). Although the evidence for food addiction does not currently warrant categorization as a substance use disorder, uncontrolled eating can be viewed as a behavioral addiction, sharing similarities with gambling addiction and sex addiction (Hebebrand *et al*, 2014). An oft-cited contributing factor to the obesity epidemic is the omnipresence of easily available palatable, sucrose-rich foods. Indeed, several authors have claimed that sucrose, under certain conditions, may evoke behavior that is reminiscent of addiction (Avena and Gold, 2011; Corwin *et al*, 2011).

Sucrose has two reinforcing qualities. One is its sweet taste, the other are its postingestive effects. Both of these contribute to operant behavior for sucrose. Animals respond for sweet taste alone in the form of non-caloric sweeteners (Cason and Aston-Jones, 2013). Importantly, the postingestive effects of sucrose intake also mediate reinforcement, independent of sweet taste (Gottfried and de Araujo, 2011). Sucrose is rapidly broken down into glucose and fructose in the intestines, and gastric infusions of glucose are known to promote intake of liquid and support flavor conditioning (Myers *et al*, 2013; Zukerman *et al*, 2011; 2013). The mesolimbic dopamine system has been widely implicated in incentive learning and reinforcement (Schultz and Dickinson, 2000). Indeed, both the sweet taste as well as the postingestive effects of sucrose promote dopamine release. In sham-fed rats (that do not experience the post-ingestive effects of sucrose), sucrose evokes dopamine release in the nucleus accumbens (NAcc) (Hajnal *et al*, 2004) and the reinforcing qualities of sucrose in sham-fed rats depend on the dopamine system (Geary and Smith, 1985; Schneider, 1989). Furthermore, saccharin intake also promotes dopamine release in the NAcc (Mark *et al*, 1991; Scheggi *et al*, 2013). Independent of taste, glucose infusions into the hepatic vein have been shown to stimulate dopamine release in the NAcc (Oliveira-Maia *et al*, 2011; Steinberg *et al*, 2014). In fact, even in mice that lack essential parts of the sweet-taste signaling cascade and are thus unable to sense sweet taste, sucrose mediates dopamine release in the NAcc, likely facilitating sucrose preference in these animals (de Araujo *et al*, 2008). Thus, both the sweet taste

of sucrose as well as its postingestive effects are reinforcing, presumably by invoking dopamine release in the NAcc (Domingos *et al*, 2011).

Given the omnipresence of sugar-rich food and the present obesity epidemic, it is of great importance to investigate how sugar promotes incentive motivation. Here, we therefore sought to determine to what extent sweet taste and postingestive effects contribute to the incentive value of food. We did this by comparing the motivation that animals express in order to obtain a sweet (non-caloric) saccharin solution to a (not very palatable, but calorie-rich) maltodextrin solution. Maltodextrin is a polysaccharide that is not tasteless, but it does not taste sweet. It is digested and absorbed into the body as glucose. In humans, oral maltodextrin has been shown to facilitate taste conditioning, presumably via conditioned neuronal activity in the NAcc (de Araujo *et al*, 2013). This research extends previous work that compared motivation for saccharin to sucrose (Scheggi *et al*, 2013). Of note, whereas sweet taste is an immediately apparent quality of food, we reasoned that its postingestive effects require learning in order to influence motivation. We therefore compared the motivation of animals for either maltodextrin or saccharin both before and after a period of free intake during which the animals had the opportunity to experience the post-ingestive effects of the solutions. We hypothesized that both sweet taste and caloric contents contribute to the motivation for food, but that motivation for the maltodextrin solution is influenced by experience with its postingestive effects.

Methods

Experimental timeline

The experiment was divided into three phases preceded by a training phase. During phase 1, 20 rats were tested for their motivation for saccharin and maltodextrin according to a counterbalanced design. One rat was excluded because it failed to acquire stable operant responding. During phase 2, the rats were given the opportunity to consume the solutions in their homecage for 1 h per day. During phase 3, we again assessed the motivation for either saccharin or maltodextrin (Fig. 1A). The reasoning behind this approach is that rats receive only a minimal amount of the solutions when their motivation is assessed (phase 1 and 3, see Fig. 1), thus almost all experience with the postingestive effects of the solutions is gained during phase 2.

Animals

Six-week old male Wistar rats (Charles River, Sulzfeld, Germany) weighing 150-200g at the beginning of the experiment were pair housed in macrolon cages (37.8 x 21.7 x 18.0 cm) under a reversed 12:12 light/dark cycle (lights on at 19:00h). They had ad-libitum access to chow and water and home cage enrichment was provided in the form of a wooden block. All animal experiments were approved by the Animal Ethics Committee of Utrecht University and were conducted in agreement with Dutch laws (Wet op de Dierproeven, 1996) and European regulations (Guideline 86/609/EEC).

Solutions

The different solutions were prepared by dissolving sucrose (Merck, Darmstadt, Germany), maltodextrin (AppliChem, Darmstadt, Germany) or saccharin (Sigma-Aldrich, Hamburg, Germany) in tap water. All solutions were prepared fresh daily.

Operant conditioning apparatus

Rats were trained in operant conditioning chambers (30.5 x 24.1 x 21.0 cm; Med Associates Inc., St. Albans VT. USA). The chambers were placed in light- and sound-attenuating cubicles equipped with a ventilation fan. Each chamber was equipped with two 4.8cm wide retractable levers, placed 11.7cm apart and 6.0cm from the grid floor. The assignment of the left and right lever as active and inactive lever was counterbalanced across rats. A cue light (28 V, 100 mA) was present above each lever and a house light (28 V, 100 mA) was located on the opposite wall. A liquid dipper was placed outside the chamber in such a way that it could present the solutions to a reward receptacle situated in between the two levers. The dipper remained submerged in a container containing the solutions until the animals met the response requirement on the active lever, in which case the dipper moved up and down for 6 times in quick succession to allow the animal to sample the solution. The dipper holds 5µl of fluid so the maximum amount of fluid consumed per reward is 30µl.

Self-administration procedure

Animals were trained to respond for food as described before (la Fleur *et al*, 2007; Veeneman *et al*, 2012) during the training phase, animals received one week of fixed ratio (FR) training to self-administer a 10% sucrose solution until they acquired stable responding under an FR5 schedule of reinforcement, meaning that they were required to make 5 lever presses in order to obtain one sucrose reward. After this, the animals were trained to respond for 0.3%

saccharin and 10% maltodextrin under an FR5 schedule of reinforcement on alternate days to accustom them to these solutions. Next, the animals were tested under the progressive ratio (PR) schedule of reinforcement, in which the response requirement increased progressively after each reward (1, 2, 4, 6, 9, 12, 15, 20, 25, etc. (Richardson and Roberts, 1996)). A PR session ended when the animals failed to obtain a reward for 30 min. Animals received 21 PR sessions in phase 1 and 9 PR sessions in phase 3 of the experiment. All PR sessions were counterbalanced such that for each PR session half of the animals was working for saccharin and the other half responded for maltodextrin. All animals received all solutions (within-subject design) whereby 0.3% saccharin was compared to 10% maltodextrin and 0.6% saccharin to 20% maltodextrin. The outcome measure of the PR schedule was the average active lever presses of the last two sessions except for the sessions after food restriction, which were only measured once. Self-administration sessions were carried out once daily, between 1PM-6 PM, for 5-7 days a week.

Free intake procedure

To measure free intake of the different solutions, rats were presented with a bottle of the solution in their homecage while their standard water bottle was removed. All free intake measurements lasted 1h, from 3:30PM till 4:30PM. Intake was measured by weighing the sample bottles before and after the session. For comparison, we performed a water measurement in which the homecage water bottle was removed and replaced by a test bottle with also contained tap water. In this case, the average intake in 1h was 9.2 ± 0.9ml. The animals were pair-housed, thus for the home cage measurements of intake and the bodyweight correlations (see Fig. 3c and d) we took the average of the two animals in one cage. All free intake measurements were counterbalanced, such that half of the animals received maltodextrin (10% or 20%) and the other half received saccharin (0.3% or 0.6%). The outcome measure is the average of 2 measurements, except when we assessed intake after food restriction (Fig. 4), which was only measured once.

Food restriction procedure

Animals were limited to 45% of their food normal intake in the 24h before a test session. After the test session they received ad libitum chow for at least 48h.

Statistics

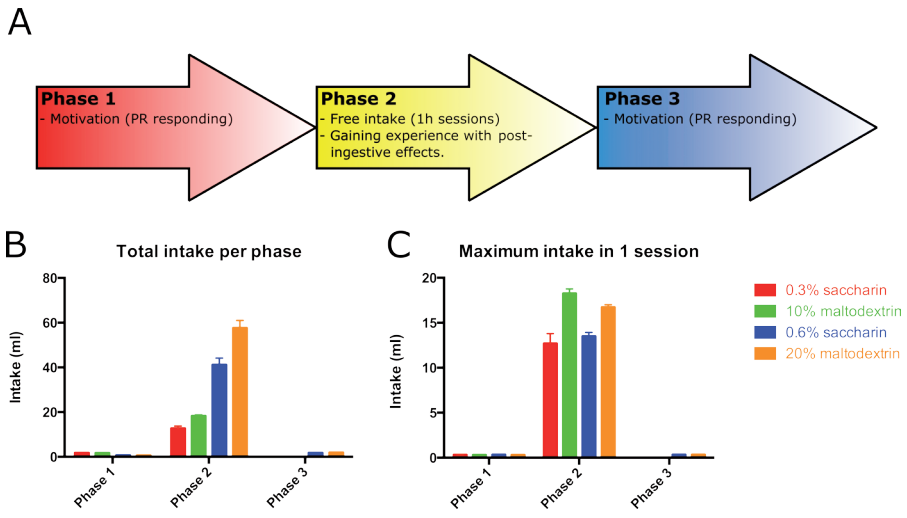
All data was analyzed using GraphPad Prism 6 (GraphPad Software). In the case of a comparison between two groups, a paired Student's T test was used. 0.3% saccharin was always compared to 10% maltodextrin and 0.6% saccharin to 20% maltodextrin. The experiments described in Fig. 4 and Fig. 5a were analyzed using a two-way repeated measures ANOVA with food restriction and solution (maltodextrin or saccharin) as within-subjects variables. Significant effects from the ANOVAs were followed up with the Holm-Sidak post-hoc test.

Results

Total intake during the different phases of the experiment

Animals were first trained on the operant task using sucrose as a reward. They then started self-administering the different caloric or sweet solutions under a PR schedule of reinforcement (phase 1). Because of the steep increase in the response requirement for each subsequent reward, the animals obtained on average only 9 rewards (approximately 0.3 ml) per session (which lasted approximately 40 min). This corresponds to 0.113 kCal and 0.226 kCal per session for 10% and 20% maltodextrin, respectively. The total amount of solution and

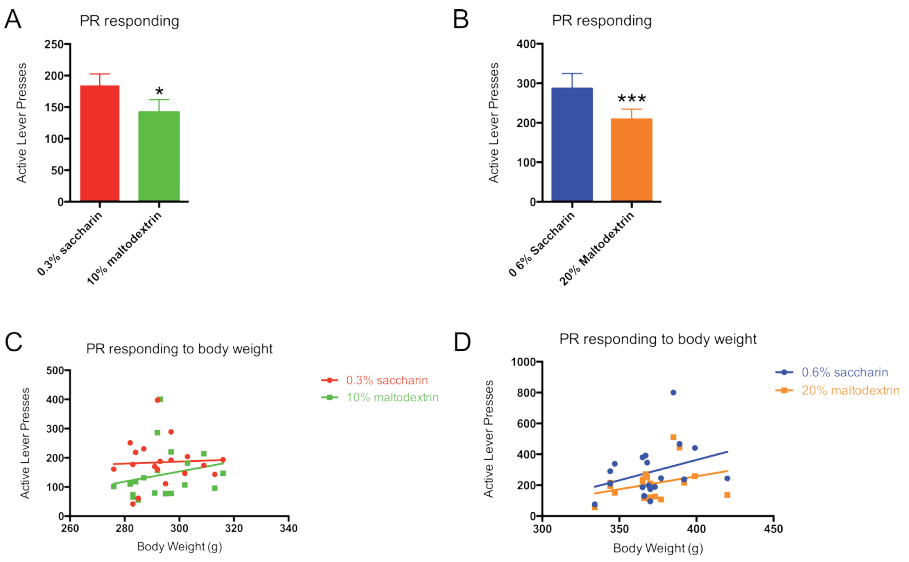
Figure 1



Saccharin and maltodextrin intake during the different phases of the experiment. A. Overview of the experiment. B. Total intake (ml) per phase. C. Maximum intake in one session, which lasted approx. 40 min in the case of a PR session (phase 1 and 3) and 1h in a free intake session (phase 2). Data represents mean ±S.E.M.

calories consumed during PR sessions is therefore low. In contrast, during the free feeding phase of the experiment (phase 2) the animals drank approximately 13.3 ml (4.99 kCal and 9.98 kCal for 10% and 20% maltodextrin, respectively) of solution. Thus, most experience with the post-ingestive effects of the solutions was gained during phase 2 of the experiment (Fig. 1), both in terms of total intake during the entire phase (Fig. 1b) and the maximum intake in one session (Fig. 1c).

Figure 2



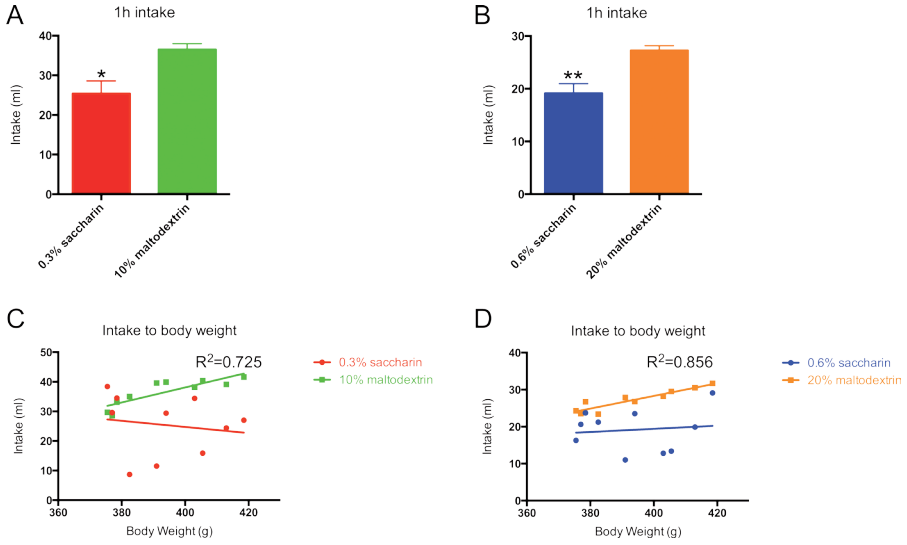
Rats expressed higher motivation for saccharin than for maltodextrin and motivation for neither solution correlates with body weight. A. PR responding for 0.3% saccharin and 10% maltodextrin. B. PR responding for 0.6% saccharin and 20% maltodextrin. C. Correlations between bodyweight and PR responding for 0.3% saccharin or 10% maltodextrin. D. Correlations between bodyweight and responding for 0.6% saccharin or 20% maltodextrin. Data represents mean ±S.E.M. * $p<0.05$, ***$p<0.001$.

Phase 1

Animals subjected to a PR schedule of reinforcement for either 0.3% saccharin or 10% maltodextrin responded significantly more for 0.3% saccharin than for 10% maltodextrin ($t=2.112$, $df=18$, $p=0.049$) (Fig. 2a). In order to enhance the contrast between the two groups, the concentration of saccharin and maltodextrin was doubled to 0.6% saccharin and 20% maltodextrin, respectively. The rats responded significantly more for 0.6% saccharin than for 20% maltodextrin ($t=3.923$, $df=18$, $p=0.0010$) (Fig. 2b). Interestingly, in both cases motivation was

not correlated with body weight ($F_{(1,17)\ 0.3\%\ saccharin}$=0.041, p=0.84, R2=0.002; $F_{(1,17)\ 10\%\ maltodextrin}$=0.845, p=0.37, R2=0.047; $F_{(1,17)\ 0.6\%\ saccharin}$=2.027, p=0.17, R2=0.107; $F_{(1,17)\ 20\%\ maltodextrin}$=1.785, p=0.20, R2=0.095)(fig. 2c and 2d).

Figure 3



When given free access, rats consume more maltodextrin than saccharin and maltodextrin intake correlates with body weight. A. Rats consumed significantly more 10% maltodextrin than 0.3% saccharin in a 1 h period. The same holds true for intake of 0.6% saccharin and 20% maltodextrin (B). Intake of 10% (C) and 20% maltodextrin (D) correlated with body weight, but saccharin intake did not. Data represents mean ±S.E.M (A and B). *p<0.05, **p<0.01.

Phase 2

During the home-cage free feeding phase, the animals were exposed to different saccharin and maltodextrin solutions in their home cage for 1 h. Again, we compared 0.3% saccharin to 10% maltodextrin and 0.6% saccharin to 20% maltodextrin. Under both conditions, the animals consumed more maltodextrin than saccharin ($T_{0.3\%\ saccharin\ vs\ 10\%\ maltodextrin}$=2.753, df=9, p=0.022; $T_{0.6\%\ saccharin\ vs\ 20\%\ maltodextrin}$=4.154, df=9, p=0.002)(Fig. 3a-b). Interestingly, from the first session onwards, maltodextrin intake strongly correlated with body weight, whereas this was not the case for saccharin intake ($F_{(1,8)\ 0.3\%\ saccharin}$=0.208, p=0.66, R2=0.025; $F_{(1,8)\ 10\%\ maltodextrin}$=21.08, p=0.002, R2=0.725; $F_{(1,8)\ 0.6\%\ saccharin}$=0.110, p=0.75, R2=0.0135; $F_{(1,8)\ 20\%\ maltodextrin}$=47.65, p=0.0001, R2=0.856)(fig. 3c and 3d).

During the second part of phase 2, we investigated whether food restriction influenced free intake of the solutions. We found that food restriction increases 20% maltodextrin intake whereas it reduced 0.6% saccharin intake ($F_{(1,9)\ solution\ x\ food\ restriction}$=7.558, p=0.023; $F_{(1,9)\ solution}$=62.92, p<0.0001) (Fig. 4).

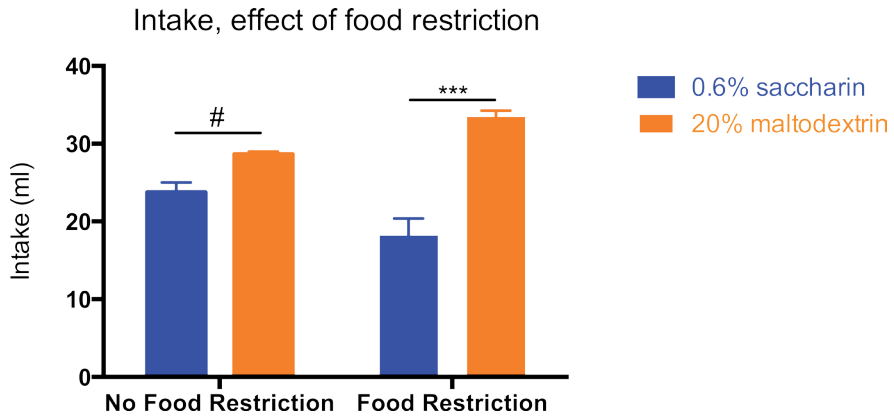
Phase 3

During phase 3 of the experiment, we assessed if the two preceding weeks of experience with free intake influenced the motivation for 0.6% saccharin or 20% maltodextrin. Animals were again tested under the PR schedule of reinforcement. In this phase, we also investigated the influence of food restriction on responding. There was an interaction between food restriction and solution ($F_{(1,19)\ solution\ x\ FR}$=6.044, p=0.024) (Fig. 5a). When the animals had access to ad-libitum chow prior to the session, there was no difference between responding for maltodextrin or for saccharin ($T_{sac\ vs\ malto}$=0.1506, df=19, p=0.882) (Fig. 5a). This is in contrast with what we observed in phase 1 (Fig. 2). Furthermore, after food restriction, the animals were significantly more motivated for maltodextrin than for saccharin ($T_{sac\ vs.\ malto}$=3.326, df=19, p=0.007). There was a modest correlation between the motivation for 20% maltodextrin and body weight ($F_{(1,17)}$=5.241, p=0.035, R^2=0.236) (Fig. 5b). Body weight did not correlate with responding for saccharin ($F_{(1,\ 17)}$=0.129, p=0.724, R2=0.008).

Discussion

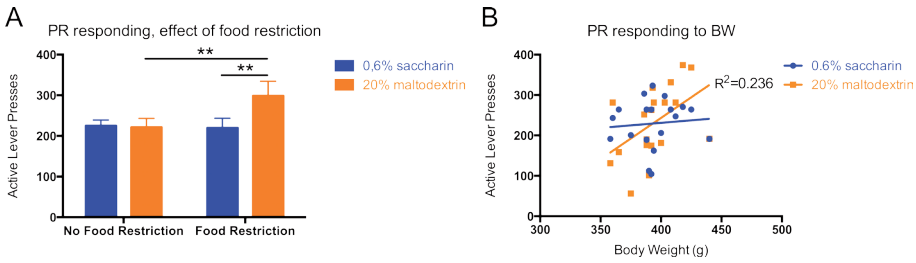
Here, we compared the motivation for a sweet non-caloric reward (saccharin) versus a caloric non-sweet reward (maltodextrin) both before and after exposure to the post-ingestive effects of these solutions. To this aim, we divided the experiment into three phases whereby intake of the solutions in phase 1 and 3 was low, in terms of both total intake and maximum intake per measurement, as a result of the task structure. For this reason, we expected that that the animals only learned about the post-ingestive effects of the solutions during phase 2 of the experiment. We show that initially, animals show more motivation to obtain 0.3% saccharin than 10% maltodextrin and likewise, they are more motivated for 0.6% saccharin than for 20% maltodextrin. During phase 2, which consisted of two weeks of daily 1 h access to the solutions, we observed that animals consumed more maltodextrin than saccharin and that body weight strongly correlated with maltodextrin but not saccharin intake. During the third,

Figure 4



Saccharin and maltodextrin intake are oppositely modulated by food restriction. One h free intake of the solutions in the home cage under ad lib or food restriction conditions. There was a significant interaction between food restriction and solution. Data represents mean ±S.E.M. #p=0.10, ***p<0.001

Figure 5



Motivation for 0.6% saccharin or 20% maltodextrin after previous experience with the postingestive effects of these solutions. A. PR responding for the two solutions. B. Correlation between PR responding for the two solutions and bodyweight. Data represents mean ±S.E.M. **p<0.01.

last phase of the experiment, the animals were again tested under a PR schedule of reinforcement. In this phase, the animals were no longer more motivated for 0.6% saccharin than for 20% maltodextrin. In addition, the motivation for 20% maltodextrin correlated with body weight. Furthermore, we observed that food restriction prior to the PR session increased motivation for 20% maltodextrin but not for 0.6% saccharin. Thus, extensive experience with the post-ingestive effects of an otherwise unpalatable foodstuff that is rich in calories, increases its incentive motivational value, as compared to an noncaloric, sweet solution. This indicates that both taste and caloric value contribute to the incentive value of food, but that the influence of the latter depends on experience with its post-ingestive effects.

Others have previously shown that animals are motivated to obtain a sweet non-caloric reward (Cason and Aston-Jones, 2013) but when given a choice, they will prefer a sucrose reward, a choice that is influenced by dopamine signaling (Domingos *et al*, 2011; 2013). This effect is thought to be due to the postingestive effects, more specifically the increase in blood glucose, that follows sucrose intake (Gottfried and de Araujo, 2011). Indeed, when post-oral glucose or maltodextrin (which is quickly digested into glucose in the intestines) infusions are paired to licking of a fluid dispenser, they rapidly promote an increase in intake (Ackroff and Sclafani, 2014; Elizalde and Sclafani, 1990). In the same way, the consumption of maltodextrin may have promoted additional intake in the current study. The fact that maltodextrin intake strongly correlated with average body weight and was increased after food restriction, strongly hints at the influence of homeostatic factors such as a satiety as leaner rats (with a smaller energy need) consumed less. Conversely, saccharin intake was not correlated with bodyweight and was not increased after food restriction, indicating a lack of homeostatic control over saccharin intake.

During phase 2, rats gained experience with the postingestive effects of saccharin and maltodextrin. Maltodextrin was previously shown to have reinforcing qualities that depend on its postingestive properties (Dwyer and Quirk, 2008; Elizalde and Sclafani, 1988). A probable explanation of our results in phase 3 is that the taste of maltodextrin acquired additional incentive value because the rats experienced the postingestive effects of maltodextrin intake in phase 2 (Balleine and Dickinson, 1998). Incentive learning theory predicts that once rats have learned about the nutritional value of maltodextrin, their motivation to obtain a maltodextrin reward will be influenced by their motivational state (i.e. hungry or sated) (Balleine and

Dickinson, 1998). Indeed, in this study, food restriction increased responding for maltodextrin (in phase 3), which is reminiscent of similar work comparing motivation for sucrose to saccharin (Scheggi *et al*, 2013). In that study, animals showed a comparable motivation for a sucrose or a saccharin solution under ad libitum conditions, but an 18h fast selectively increased motivation for sucrose. Interestingly, in human participants performing a similar task, food restricting increased motivation without changing the subjective evaluation ('liking') of the food (Epstein *et al*, 2003).

There is an extensive literature about how glucose-containing polysaccharides serve as powerful stimuli that mediate associative learning (Elizalde and Sclafani, 1990; Gottfried and de Araujo, 2011). Conditioned cues, such as taste, appearance or smell, but likely also food-related (environmental) stimuli such as a certain context (restaurant) or the packaging of food, can promote food intake beyond metabolic need and these cues are likely to play an important role in the current obesity epidemic (Bouton, 2011; Johnson, 2013; Meye and Adan, 2014; Petrovich, 2013; Petrovich *et al*, 2007). Palatable taste (such as the saccharin solution in this work) is, of course, a primary reinforcer, whereas neutral or even bitter tastants may serve as conditioned stimuli after becoming associated with glucose (Myers and Sclafani, 2003). A conditioned taste (such as the taste of maltodextrin after association with its postingestive effects in phase 2) can increase the incentive value of a solution (Sclafani and Ackroff, 2006). These effects of polysaccharide consumption have been likened to how drugs of abuse influence the brain (Avena *et al*, 2008; Colantuoni *et al*, 2001). There are however notable differences between food and drug conditioning, such as the exact site of dopamine release following presentation of food or drug related cues (Bassareo *et al*, 2007), the effect on long term plasticity (LTP) after multiple conditioning trials (Chen *et al*, 2008) and the extent to which they evoke compulsive seeking behavior (Limpens *et al*, 2014; Pelloux *et al*, 2007; Vanderschuren and Everitt, 2004).

In sum, the present study shows that maltodextrin, likely via its post-ingestive effects, promotes food intake. Furthermore, after experience with the post-ingestive qualities of maltodextrin, the incentive motivational value of this solution increases as compared to a non-caloric sweet solution in a situation of negative energy balance.

Acknowledgements

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6

GENERAL DISCUSSION: WHAT MAKES US OVEREAT?

FROM CALORIC NEED TO UNCONTROLLED FOOD INTAKE

Introduction

Here, I would like to discuss the question I asked at the beginning of this thesis. Can one be addicted to food? I also want to come back to the topic I introduced in the introduction, namely how cues in our environment influence our behavior and, specifically, how they might provoke us to seek and consume food. I will start out by discussing how hedonic control over eating drives overeating and how environmental cues promote food intake. Then I will discuss how the brain 'learns' to prefer foods (and food-associated stimuli) that contain energy in the form of sucrose and I will discuss the role of the neurotransmitter dopamine in this process. I will integrate the contributions from the work described in this thesis into this. Finally I would like to compare how our brain deals with food and food-related stimuli to how our brain is influenced by drugs of abuse and drug-related stimuli and I will discuss arguments in favor and against the existence of food addiction.

The question of why we eat seems very straightforward on first sight. We eat because we need energy. Indeed, when humans or rodents are exposed to a period of food deprivation, they will compensate for the decrease in caloric intake the next time they have the opportunity to eat (Telch and Agras, 1996). One of the first studies in laboratory animals on this topic investigated what happened to food intake when this was diluted by indigestible (but edible, see figure 1) cellulose (Adolph, 1947). In this case Adolph observed that rats would adjust their total daily food intake to meet a preset amount of calories. The same is true when rats self-administer a liquid diet via an gastric tube directly into the stomach (thus without tasting or smelling the food) (Epstein and Teitelbaum, 1962). This led early researchers to conclude that food intake is mainly driven by caloric need. Obviously, energy intake and expenditure is tightly regulated in all organisms from the smallest bacteria to complex mammals such as humans. When energy reserves fall, signals from the periphery such as leptin (from adipose tissue) and ghrelin (from the stomach and intestines) let the brain know that action needs to be undertaken to find food. But, as can be learned from the recent obesity epidemic, this homeostatic drive to eat is not the whole story. External factors strongly influence food intake. A recognizable example for instance is that we are hungry and continue to eat while satiated during mealtime simply because we are used to eating a certain number of meals at fixed times during the day. Blood levels of ghrelin (also known as the 'hunger hormone') are entrained to our daily eating pattern and mediate our anticipation to a meal (Blum

et al, 2009; Cummings *et al*, 2001; LeSauter *et al*, 2009; Merkestein *et al*, 2012). Furthermore, eating plays an important role in human social interaction and last but not least, we eat because we *like* food. This last part is especially important because this 'hedonic' eating is not necessarily related to a homeostatic need and may actually promote eating in excess of what is needed (Berridge *et al*, 2010; Johnson, 2013a; Zheng and Berthoud, 2007).

Environmental cues promote food intake

Probably the oldest and most famous example of an environmental cue associated with food intake comes the experiments for which Ivan Pavlov was awarded the Nobel Prize in 1904. In these experiments, Pavlov's dogs learned to associate a meaningless sound (a metronome) with the delivery of food. After conditioning, the dogs started to salivate when exposed to the sound alone (Pavlov, 2010). This classical form of conditioning is hence called Pavlovian conditioning. Pavlov came up with the terminology to describe this behavior. The food, that does not require any learning, is an unconditioned stimulus. The learned cue (the metronome, although it can be any arbitrary stimulus) is a conditioned stimulus, because it requires learning to become associated with the unconditioned stimulus. A very well-known example of a food-associated conditioned stimulus is the famous golden arches that most people have been conditioned (by one of the most well-funded marketing machines in the world) to associate with energy-rich food. This is an example of how environmental stimuli can heavily influence food intake by promoting 'wanting' and even craving of food.

Conditioned cues can directly potentiate feeding (cue-potentiated feeding). For instance, rats that have previously learned to associate a certain tone with the delivery of a meal will initiate consumption of that meal when it is freely available in response to this tone, even if they are sated (Weingarten, 1983). This works in humans from a very early age on (Birch *et al*, 1989). A specific food-related environment may promote excess food intake, such as a restaurant or even a certain behavior such as watching television while consuming a snack (Bouton, 2011; Braude and Stevenson, 2014). It is interesting to note that this cue-potentiated feeding involves ghrelin signaling (Walker *et al*, 2012). Cue-potentiated feeding depends upon a broad network of brain regions including the amygdala, lateral hypothalamus, prefrontal cortex and hippocampus (for a review

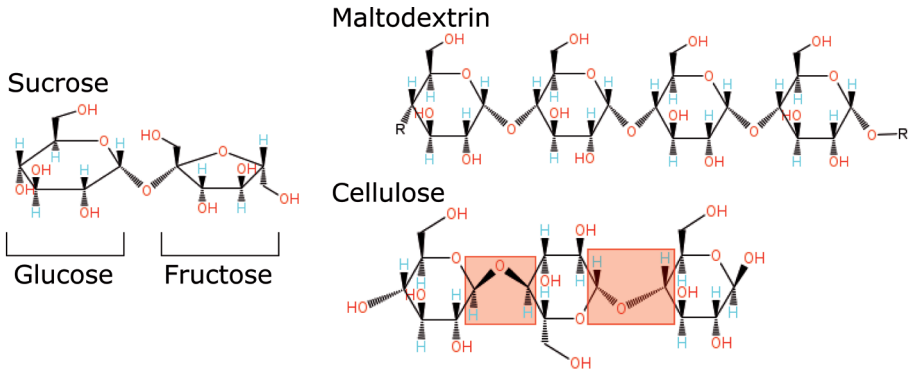
see: (Johnson, 2013b)). Apart from directly stimulating food intake, environmental cues may trigger motivated behavior to obtain food. After conditioning, certain stimuli may acquire 'incentive salience'. Incentive salience relates to how signals (presented in the environment or internally generated) can trigger motivated behavior such as 'wanting' or craving of an (un)conditioned stimulus (Robinson and Berridge, 1993). A picture of a chocolate cake, for instance, may trigger you to 'want' chocolate cake. The chocolate cake itself is a stimulus that has even more emotional value and will probably trigger more 'wanting' and increase the chance that you will buy the cake and eat it (Veilleux and Skinner, 2015). This is exactly why restaurant chains with good marketing departments put pictures of food on their menu and why Starbucks puts their chocolate cake on display rather than just describing it on the chalkboard next to the coffee selection. A clear example of incentive salience in laboratory animals comes from the data discussed in chapter 4. In this case rats learned to associate several cues (a cue-light and retraction of the operant levers) with the delivery of a sucrose reward. We then made the animals go through extinction, which means that they no longer received any sucrose when they pressed a lever and as a consequence were no longer willing to work (i.e. to press a lever in order to obtain sucrose). They had, however, not forgotten about the cues that were previously associated with sucrose delivery, because when we re-exposed them to these cues (cue-induced reinstatement) they started pressing the lever again. Importantly, they still did not receive any sucrose during this reinstatement session, thus they were pressing for the cues alone. In a similar way, people have suggested that cues, such as the Golden Arches, or the packaging of food, or food-related pictures on a menu or proximal cues such as the smell of palatable food may drive us to seek and consume food (Bouton, 2011). They will do so, even in humans that try to restrict their food intake (dieters) and importantly, cues can stimulate food seeking even in the sated state, when there is no homestatic need, thus promoting overeating (Fedoroff *et al*, 1997; Watson *et al*, 2014).

What does this have to do with obesity? In the next paragraph I'll make the case that especially energy-rich, sugar-containing foods, as opposed to tasty, but sugar-lacking foods, are good at conditioning the brain and thus promote the formation of conditioned stimuli and trigger incentive salience. Thus, unhealthy food is better at teaching us new behaviors than healthy, energy-poor, foods. This made sense in prehistoric times when food was scarce, but is highly problematic in modern times when high-caloric foods are omnipresent (Ulijaszek, 2002). Here, I will also discuss the findings described in chapter 5.

The reinforcing qualities of sucrose and glucose

Sucrose is a molecule consisting of fructose and glucose linked together (see figure 1). Sucrose has a sweet taste because it binds to the sweet taste receptors (T1R2 and T1R3) on our tongue. It is absorbed in our intestines and broken down into glucose and fructose. Fructose is then converted in the liver into glucose, which is an important source of energy for our internal organs. A sufficiently high blood glucose concentration is necessary for proper functioning of our brain (which uses glucose as its primary energy source) and this is therefore tightly regulated. Another important source of glucose is starch (from which the maltodextrin used in chapter 5 is derived), which consists of long chains of linked glucose molecules that are broken down into glucose in our intestines.

Figure 1



Molecule structures of Sucrose, Glucose, Fructose, Maltodextrin and Cellulose. Note that sucrose can be hydrolyzed in our intestines into fructose and glucose, and maltodextrin into glucose. Cellulose, however, although highly similar to maltodextrin, cannot be broken down by humans because they are unable to hydrolyze the β-linkages (red shading), which connect the individual glucose units. This is why cellulose has no nutritional value and very weak reinforcing qualities. Interestingly, rats will work for sweetened cellulose, but the amount of effort they are willing to exert in order to obtain cellulose rewards is negligible compared to the effort they will exert to obtain a sucrose reward (de Jong et al. unpublished data).

In chapter 5, we discussed how rats will consume more of a solution that contains maltodextrin (a high-caloric carb hydrate) than a sweet (non caloric) saccharin solution. Importantly, initially they expressed more motivation to obtain the sweet (non caloric) solution than the plain (caloric) maltodextrin solution. However, after several weeks of free consumption of both solutions, the rats learned

about the postingestive effect of both solutions (i.e. maltodextrin is a high caloric carbohydrate whereas saccharin holds no nutritional value) and showed equal motivation for both solutions and increased motivation for the maltodextrin solution when they are hungry. It is important to note that the animals could only consume a very small amount of the solutions during the progressive ratio (PR) sessions o when their motivation to obtain the reward was tested. This means that they will only experience the postingestive effects of these solutions to a very small extent during these sessions. In fact, the only thing different between a PR session for maltodextrin and a PR session for saccharin is the taste of the first few rewards (although maltodextrin is not sweet, it does have a characteristic taste). This *taste* had previously (during the free feeding phase) become associated with the postingestive effects of maltodextrin. This is an example of a (proximal) conditioned reinforcer.

On initial thought one might be tempted to think that sucrose is reinforcing because it tastes nice (sweet), but this, as it turns out, is not the complete story.[1] It is true that sweet taste is reinforcing: animals are willing to work for non-caloric sweeteners and will consume them when given the opportunity ((Cason and Aston-Jones, 2013), Chapter 5). However, when given a choice, mice and rats overwhelmingly prefer sucrose over sucralose (which recruits the same taste receptors but does not contain any calories) (Domingos *et al*, 2011; 2013). Interestingly, mice that lack the sweet receptors T1R3, T1R2 or the ion channel trpm5 (necessary for sweet taste signaling) no longer prefer sucralose over water, but are perfectly capable to learn to prefer sucrose over water (Damak *et al*, 2003; de Araujo *et al*, 2008). This shows that other, presumably post-ingestive, qualities of sucrose are sufficient for conditioning. In fact, the post-ingestive effects of sucrose not only support conditioning, they are actually much more potent reinforcers than sweet taste alone. That is, in a two-bottle conditioning paradigm where animals are given a choice between a water bottle on one side and a sucrose bottle on the other side, animals learn to prefer the sucrose side over the water side, even when they are presented with water bottles on both sides (de Araujo *et al*, 2008). Sucralose is not able to induce a side bias in this way (de Araujo *et al*, 2008). Furthermore, intragastric infusions of glucose (one of

[1] With sweet taste I mean to say that something activates the sweet taste receptors on the tongue. Naturally, there is a case to be made that nothing tastes sweet in itself, but that we are evolved to perceive sugar-containing foods as sweet. The case I make here is that even so, activation of the sweet taste.

the two components of sucrose, see figure 1) have strong reinforcing qualities (Ackroff and Sclafani, 2014; Sclafani *et al*, 1993; Zukerman *et al*, 2013). When intragastric infusions of 16% glucose are coupled to consumption of a specific tastant, rats will learn to prefer this tastant over a control solution, even when the test solution was perceived as aversive (i.e. bitter-tasting) initially (Myers and Sclafani, 2003; Pérez *et al*, 1998). Similarly, maltodextrin infusions into the stomach coupled to consumption of a certain tastant are sufficient to condition rats to prefer this taste over a tastant coupled to water infusions (Elizalde and Sclafani, 1990). Conversely, when rats consume maltodextin solutions, but do not experience its postingestive effects, because the solution is drained from their stomach via a gastric fistula, maltodextrin loses its capability to condition a specific taste (although this appears to be a concentration-dependent effect) (Sclafani and Ackroff, 2004; Sclafani *et al*, 1994).

Summarizing the last paragraph, glucose has strong reinforcing qualities that do not depend on its sweet taste. It is therefore much more potent to promote conditioning than artificial sweeteners.

The role of dopamine

In the last paragraph I discussed evidence for strong conditioning qualities of sucrose and glucose that are predominantly mediated via their postingestive effects. Here, I would like to discuss the role that the neurotransmitter dopamine plays in this process.

Dopamine is predominantly synthesized in the midbrain, specifically in the ventral tegmental area (VTA) and substantia nigra (SN). Especially the mesolimbic dopamine projection (from the VTA to the nucleus accumbens (NAcc)) plays an important role in incentive motivation (Kelley and Berridge, 2002; Salamone and Correa, 2012). This goes well with what we showed in chapter 4, where activation of the VTA to NAcc pathway resulted in significantly increased motivation to obtain a sucrose reward. Additionally, in chapter 3 we showed that removal of the dopamine D2 receptor (D2R) from dopamine neurons in the VTA (where it normally has a function in negative feedback) makes rats more motivated to obtain a sucrose or a cocaine reward, but does not affect their normal intake of these substances. Fast scan cyclic voltametry (FSCV) and *in-vivo* electrophysiology experiments have shown that dopamine neurons projecting from the VTA to the

NAcc process cues especially when they have a high incentive value (Flagel *et al*, 2011; Yun *et al*, 2004). Furthermore, dopamine plays an important role in reinforcement learning and the formation of incentive cues during conditioning (Cohen *et al*, 2012; Fenu *et al*, 2001; Schultz *et al*, 1997).

Interestingly, sucrose, or more precisely, the increase in blood glucose caused by sucrose is very potent at raising dopamine levels in the nucleus accumbens. Rodent studies have shown that intragastric infusions of glucose raise dopamine levels in the ventral striatum (e.i. NAcc and olfactory tubercle) (Ren *et al*, 2010). Infusions of glucose directly into the circulation, especially in the portal vein, are sufficient for conditioning and they raise dopamine levels in the NAcc (Oliveira-Maia *et al*, 2011).[2] Furthermore, cues that have previously been associated with sucrose evoke more dopamine release in the NAcc than saccharin-paired cues in rats (McCutcheon *et al*, 2012). Infusions of the dopamine D1 receptor antagonist SCH23390 into the NAcc shell block the acquisition of glucose conditioning (Touzani *et al*, 2008). Conversely, when optogenetic activation of dopamine neurons is paired with licking on a sipper of sucralose, mice will come to prefer this sipper over a sipper that dispenses sucrose when satiated (Domingos *et al*, 2011).

Similar observations come from research in human subjects. Sucrose intake evokes more neuronal activation in dopaminergic brain regions compared to sucralose intake (Frank *et al*, 2008). In an experiment that is to a large extent the human equivalent of what we did in chapter 5, Araujo *et al*. showed that humans can be conditioned to show increased brain activity in the NAcc following the presentation of a taste that had previously been paired with increased blood glucose (by addition of maltodextrin) (de Araujo *et al*, 2013). All of this shows that sucrose, presumably by increasing blood glucose, is very efficient at raising dopamine levels in the NAcc, which is arguably why it has such strong conditioning qualities compared to natural or artificial sweeteners that do not increase blood glucose.

[2] This is not to say that the sweet taste of sucrose does not stimulate dopamine release, in fact in sham fed animals with no history of sucrose consumption (without experience of its postingestive effects), oral sucrose does promote dopamine release (Hajnal et al, 2004; Schneider, 1989). It does so, however, to a considerable lesser extent than real feeding of the same amount of sucrose (Hajnal and Norgren, 2001).

So how does an increase in blood glucose (caused by sucrose or starch intake) influence dopamine signaling? It is possible that dopamine cells are sensitive to blood glucose concentration. There is evidence for glucose sensing in the brain, especially in the hypothalamus (Ashford *et al*, 1990). A similar system might be in effect on dopamine cells in the VTA (Schiemann *et al*, 2012). Alternatively, neurons in the hypothalamus, sensitive to glucose, might project to the VTA. Although possible, it does not seem likely. Contrary to other organs, the brain is almost completely dependent on glucose and the glucose concentration in the brain is therefore tightly regulated and influenced by active transport of glucose over the blood-brain barrier. Furthermore, infusions of glucose in the portal vein are more potent at raising NAcc dopamine levels than glucose infusions in the jugular vein (Oliveira-Maia *et al*, 2011). A postprandial increase in blood glucose is quickly detected in pancreatic β cells, which in response release insulin. It seems unlikely that information about the blood glucose concentration is at that point transmitted to the brain via the vagal nerve, since vagotomized rats can still be conditioned by maltodextrin infusions into the stomach (Sclafani and Lucas, 1996). Alternatively, insulin itself is a possible mediator of glucose conditioning. Insulin injections increase striatal dopamine (Potter *et al*, 1999). In fact, insulin is suggested to have a role in dopamine signaling even independent of food-related behavior, for instance in drug addiction (Daws *et al*, 2011; Schoffelmeer *et al*, 2011). That said, diabetic rats, in which insulin-producing cells have been lesioned by streptozotocin treatment, are still sensitive to gastric infusions of glucose (Ackroff *et al*, 1997).

In conclusion, food that contains sucrose is able to influence dopamine levels and condition food-associated stimuli such as taste, smell and more distal stimuli such as a food-related environment (e.g. restaurant) or food packaging in a way that tasty, but calorie-poor food, does not. It is important to realize that in the present western environment, with its omnipresence of high caloric palatable foods, we are constantly training our brain to focus on stimuli that are related to sugar-rich foods. A piece of cake, containing sugar (fructose and glucose) and starch (broken down into glucose) will be more attractive to the brain than a carrot, which consists for a large part of water and cellulose (neither of which contributes any calories). Cellulose is highly similar to starch, but humans are unable to break it down into glucose (see figure 1). For this reason, carrots are not very strong reinforcers and we are not strongly inclined to prefer them. It is quite possible that if one were able to break down cellulose into glucose it would take a fair amount of self-control not to eat this thesis.

Can one be addicted to food?

It has been proposed that addiction-like behavior may contribute to the current obesity epidemic. Indeed, there are several behavioral similarities between drug addiction and eating disorders such as binge eating disorder (BED) and bulimia nervosa (BN) (de Jong *et al*, 2012; Gearhardt *et al*, 2011b; Meule *et al*, 2014b; Volkow and O'Brien, 2007). The Yale food addiction score (YFAS) is a proposed questionnaire-based instrument to quantify food addiction (Gearhardt *et al*, 2009). Interestingly, although food addiction-like behavior (such as BED, BN or a high YFAS score) is likely to contribute to obesity, they are definitely separate constructs in that not all 'food-addicts' become obese and not all obese individuals meet criteria to be called 'food addicted' (Gearhardt *et al*, 2014; Kessler *et al*, 2013). Although there are obvious behavioral similarities between food and drug addiction, the scientific community is divided on this issue (Avena *et al*, 2012; Corsica and Pelchat, 2010; Hebebrand *et al*, 2014; Ziauddeen and Fletcher, 2012).

In chapter 1, we discussed whether 'food addiction' is a valid concept and we proposed an animal model to assess control over food intake. In chapter 2, we investigated whether a 'binge diet' evoked uncontrolled eating. It did not. There is a reasonable amount of literature that suggests otherwise. Several authors have observed 'addiction-like behavior' in animals after exposure to 'binge diets', that usually consist of alternating periods of food deprivation/restriction and periods of access to palatable food (Corwin *et al*, 2011; Gold and Avena, 2013; Hagan *et al*, 2002; Iemolo *et al*, 2012). These diets, which mimic so-called 'yo-yo diets', may evoke behavior that is reminiscent of food binging or they may evoke withdrawal-like behavior (Colantuoni *et al*, 2002; Cottone *et al*, 2009; Iemolo *et al*, 2012; Oswald *et al*, 2011). It is, however, all but clear whether they evoke real substance addiction in the same sense that some drugs of abuse do. In fact, a major problem with the food addiction hypothesis, is that 'food-addicted' individuals (humans or rodents) do not become addicted to a specific type of food as a consequence of consuming that food in excess (Ahmed and Koob, 1997; Buczek *et al*, 1999; de Jong *et al*, 2013; Deroche-Gamonet *et al*, 2004; Vanderschuren and Everitt, 2004)(But see: (Johnson and Kenny, 2010)). Conversely, 'food addiction' seems to be a more wide-ranging problem of disinhibition of eating in general, a behavioral problem that is probably more similar to gambling addiction or sex addiction than to substance dependence in a behavioral and neurobiological sense (Albayrak *et al*, 2012; Hebebrand *et al*,

2014). For that reason, the term 'eating addiction' might be more appropriate to refer to uncontrolled food intake (Hebebrand *et al*, 2014).

An important distinction to make is that it is very well possible to be motivated to obtain food, and be sensitive to food-related stimuli, without showing compulsive intake as is apparent in substance dependence. Disinhibiting of the dopamine system for instance, as we showed in chapter 3, robustly increases motivation for sucrose and cocaine, but does not promote compulsive cocaine seeking. Neither does DREADD activation of the VTA to NAcc pathway promote compulsive sucrose seeking although it does increase motivation for food (chapter 4). Indeed, although motivation for food and drugs (triggered by salient cues) involves similar (dopaminergic) mechanisms, the development of substance dependence involves further neurobiological changes, which have not convincingly been shown for sucrose or other foods (Chen *et al*, 2008; 2013; Limpens *et al*, 2014; Pelloux *et al*, 2007; Porrino *et al*, 2004; Vanderschuren and Everitt, 2004; Willuhn *et al*, 2012).

Regardless of the debate surrounding this topic, in light of this discussion, it is interesting to note that the way a healthy brain deals with food- or drug-related cues is similar. Functional magnetic resonance imaging (fMRI) studies have shown that drug-related cues in drug users and food-related cues in others recruit comparable brain regions (Pelchat *et al*, 2004; Rolls and McCabe, 2007; Tang *et al*, 2012; Tomasi *et al*, 2014)[3]. There is also data to show that obese individuals or persons with a high YFAS score may be differently affected by food-cues than healthy controls, just like drug addicts are differently affected by drug-related cues (Gearhardt *et al*, 2011c; Martin *et al*, 2010; Rothemund *et al*, 2007). (Although there are definitely conflicting results (Ziauddeen *et al*, 2012).) Obese children may be more sensitive to food related cues than normal weight children (Jansen *et al*, 2003). The concept that environmental cues evoke aberrant incentive salience (perhaps leading to craving) is a key concept in the incentive salience theory of drug abuse and some have suggested that a similar process may underlie obesity (Berridge, 2009; Robinson and Berridge, 1993). The idea is that food-related cues can promote excessive 'wanting' for food and

[3] Microdialysis studies in rats, which have a higher spatial resolution than fMRI studies in humans have shown notable differences however, whereby drug related cues promote dopamine release in the NAcc shell and food related cues in the NAcc core (Bassareo et al, 2007; Di Chiara and Bassareo, 2007).

that self-control is required to inhibit food seeking. Related to this, it is interesting to note that individuals who have less control over their impulses (highly impulsive people) are more likely to act in response to food cues and are more prone to obesity (Claes *et al*, 2006; Jansen *et al*, 2003; Meule *et al*, 2014a; Nederkoorn *et al*, 2007).

The question is, of course, is it possible to *un*condition the brain and remove the incentive value from food cues? The short answer appears to be 'no'. Cues that have been associated with palatable food will retain their incentive salience just like drug-related cues will always have an increased emotional value for a recovering drug addict. It is possible to extinguish the value of a conditioned cue, but extinction is a process that is highly context dependent, thus when food-related cues are extinguished in one setting (perhaps an obesity clinic), they will still evoke craving in another (the home environment) (Bouton, 2011). Furthermore, extinction does not entail the removal of the association between a certain cue and the expected reward but rather the formation of a new memory, which competes with the original cue-reward association that induces craving. Thus the 'old' association is still present and can be reinstated by the presentation of powerful cues, brief exposure to the reward itself or stress (caused by environmental stimuli or pharmacologically induced) ((Ghitza *et al*, 2005; Shaham *et al*, 2003), Chapter 2, 3 & 4). That said, cue exposure therapy, which aims to eliminate the link between cues and reward might be applied to obese individuals or patients with BED (Havermans and Jansen, 2003; Jansen, 2010; Pla-Sanjuanelo *et al*, 2014). Alternatively, a memory retrieval-extinction procedure has been shown to inhibit cue-induced drug craving in rodents and humans and this may be used to treat food craving (Xue *et al*, 2012). A popular and often suggested prevention strategy is that parents should promote 'healthy eating patterns' in children and thus prevent strong associations between food cues and teach self-control at the same time. It is, however, not completely clear what strategies parents should adopt to do this (Clark *et al*, 2007). Nonetheless, shielding children from advertising campaigns that are specifically designed to form strong associations between certain stimuli (colorful packaging, toys, games) and energy-rich food is probably an effective prevention measure (Gearhardt *et al*, 2011a). Another thing of note is that the speed at which glucose is absorbed from food might influence how the brain responds to food. A meal with a high glycemic index (meaning that it produces a fast, but short lasting increase in blood glucose) compared to an isocaloric meal with a low glycemic index, produces more activity in the NAcc (Lennerz *et al*, 2013). This

is reminiscent of the generalization that drugs of abuse that act faster have more addictive potential (Samaha and Robinson, 2005; Volkow and Swanson, 2003). Indeed authors in favor of the term 'food addiction' often place emphasis on the presence of 'ultraprocessed' refined carbohydrates in our food, which have a high glycemic index and should be replaced by 'slow' carbohydrates (Curtis and Davis, 2014; Davis, 2014; Ifland *et al*, 2009). Furthermore, parents should take care to prevent that unhealthy food becomes synonymous to 'having a good time' by associating caloric foods with happy events in a child's life, such as birthday parties, holidays and achievements such as obtaining a diploma or having finished homework. Such measures have been shown to be successful in the past to prevent teenage smoking. But just like in the case of tobacco addiction, unfortunately there is vast and influential industry dependent on achieving exactly the opposite (Brownell and Warner, 2009). In general, more research on how to prevent associations between sugar-rich foods and environmental stimuli will be beneficial to combat the obesity epidemic.

Contributions and limitations of this work

Here I have tried to give an idea of how food-related cues promote eating and I compared this to how drug-related stimuli promote substance use and (in some cases) substance abuse. I think that although there are similarities in how drug and food taking are initially mediated, 'eating addiction' and substance use disorder are probably different in terms of their underlying neurobiology. I am very well aware that this discussion only gives a very brief overview of this topic. Furthermore, a very serious limitation is that I focus heavily on the role of glucose and carbohydrates, whereas there is of course an important part for lipids, adipose tissue and leptin. Leptin arguably has an influence on the dopamine system and influences motivation for food and cue-driven behavior (Hommel *et al*, 2006; Rada *et al*, 2012; Thanos *et al*, 2012). Fat solutions are self-administered by rats, regardless of orosensory stimulation (Tellez *et al*, 2013). For more information on the interplay between peripheral hormones and the brain and how they control fat intake and appetite, I refer to the work of my colleague Rahul Pandit whose PhD thesis will appear soon.

As discussed in the introduction and in chapter 1, there is only limited evidence for food addiction in laboratory animals. In chapters 1 and 2, we stress that this research should not necessarily focus on compulsive food intake, since evidence

for compulsive food intake is only available in highly specific circumstances (i.e. after stringent diets, dependent on periods on forced food deprivation) (Corwin *et al*, 2011). Instead, uncontrolled food intake, sharing similarities with uncontrolled drug intake (such as an association with habitual behavior), should be further explored. In chapter 2, we show that animals with decreased control over food intake have a higher propensity to reinstate chocolate seeking after exposure to a priming dose of chocolate. Future research should continue to use multiple-criteria models to explore control over food intake.

In chapters 3 and 4, we explore the role of dopamine, in particular the mesolimbic pathway, to control over food and drug intake. Interestingly, disinhibition of dopamine neurons (via local knockdown of the somatodendritic D2 autoreceptor) increased motivation for sucrose and cocaine, but did not promote compulsive cocaine intake. Similarly, in chapter 4 we showed that activation of the mesolimbic pathway promoted incentive motivation (as well as general activity), but did not increase habitual sucrose seeking (i.e. responding after devaluation of the sucrose reward) or compulsive sucrose seeking (i.e. responding in the presence of a conditioned aversive stimulus). Both findings point to a selective role for dopamine in the ventral striatum in promoting incentive value of food, without *directly* promoting compulsive food intake.

Although we observed notable differences in control over food intake in chapter 2, and strongly influenced incentive motivation for food in chapter 3 and 4, in none of these situations did we observe compulsive food seeking such as has been shown for cocaine (Deroche-Gamonet *et al*, 2004; Limpens *et al*, 2014; Pelloux *et al*, 2007; Vanderschuren and Everitt, 2004). In chapter 3 and 4, we did, however, not assess whether an extensive history of sucrose consumption in combination with knockdown of the D2 autoreceptor (chapter 3) or activation of the mesolimbic pathway (chapter 4) promotes addiction-like behavior *over time.* Compulsive cocaine seeking arises in a subgroup of animals after an extensive history of cocaine use and is mediated, among other neural changes, by a transfer of involvement of the ventral striatum, to the dorsal striatum (Belin-Rauscent *et al*, 2012; Deroche-Gamonet *et al*, 2004; Everitt and Robbins, 2005; Pelloux *et al*, 2007; Vanderschuren and Everitt, 2004). It is not unlikely that our manipulations could promote this shift to habitual food seeking mediated by the dorsal striatum and there might thus be a role for ventral striatal dopamine signaling in the development of addiction-like behavior after an extensive history of sucrose self-administration.

Another limitation in chapter 4 is that, although our manipulation was projection-specific, it lacked cell-type specificity and thus we can therefore not confirm that our effects were mediated by dopamine or a different neurotransmitter. This is especially relevant for the mesocortical pathway since this pathway is known to contain a large percentage of GABAergic neurons (Carr and Sesack, 2000; Swanson, 1982). Future experiments using CRE-dependent Cav2-flp in combination with flp-dependent DREADD or optogenetic vectors will make simultaneous projection and cell-type specific activation or inhibition possible. Alternatively, CRE-dependent vectors might be expressed behind cell-type specific promoters, which limit expression to a specific cell type (Ferguson *et al*, 2013).

A future experiment could provide the 'missing link' between chapters 4 and 5. A FSCV or *in-vivo* electrophysiology experiment could measure dopamine release in the ventral striatum (FSCV) or neuronal activity of VTA dopamine neurons (in-vivo electrophysiology) following saccharin or maltodextrin intake. Both the direct response (after orosensory stimulation) and the prolonged response (caused by the post-ingestive increase in blood glucose) should be measured. My hypothesis is that initially the taste of saccharin will cause more dopamine release (Hajnal *et al*, 2004), while the postingestive effects of maltodextrin will promote dopamine activity (Ren *et al*, 2010). After conditioning, however, when maltodextrin acquires incentive value, the taste of maltodextrin is likely to promote dopamine release (Schultz, 1997). Further experiments could also investigate the role of food deprivation on this effect, since maltodextrin might promote more dopamine release in a hungry state (Balleine and Dickinson, 1998). This experiment would connect the role of dopamine in flavor conditioning and incentive learning.

Concluding remarks

In the present food-rich environment we are constantly teaching our brain about stimuli that predict the availability of unhealthy energy-rich food. After conditioning (in which mono- and polysaccharides play an important role), these stimuli gain a certain emotional value (for examples see figure 1 in the introduction). Inhibitory control is then required to suppress urges triggered by these stimuli. Although this process shares similarities with how drug cravings are caused by drug-related stimuli, 'eating addiction' is definitely distinct from drug addiction.

Future research should explore in more detail how conditioned food cues are formed and how they influence food choice. In chapter 5 we described that after prolonged access, a carbohydrate solution gains increased incentive value. It would be interesting to employ the technique described in chapter 4 to investigate how signaling of specific neuronal pathways mediates this effect. Integration of fundamental research using optogenetics and DREADD technology to identify the neurobiology of food choice and how environmental cues influence food intake is important to combat the obesity epidemic. This research should be integrated with psychological research to inform effective treatment and prevention strategies for obesity. Prevention strategies will no doubt include the discouragement of food-marketing directed at children and the stimulation of healthy eating habits (including 'slow' as opposed to 'fast' carbohydrates) among children and their parents.

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7

ALGEMENE DISCUSSIE: WAAROM ETEN WE TE VEEL?

VAN CALORISCHE BEHOEFTE NAAR ONGECONTROLEERDE VOEDSELINNAME

Introductie

Hier wil ik graag de vraag die ik stelde aan het begin van dit proefschrift bespreken. Kan men verslaafd zijn aan voedsel? Ik wil ook terugkomen op het onderwerp dat ik in de inleiding aansneed, namelijk hoe signalen uit onze omgeving ons gedrag beïnvloeden en in het bijzonder, hoe deze signalen ons kunnen provoceren om voedsel te zoeken en te consumeren. Eerst zal ik bespreken hoe hedonistische controle over eten kan leiden tot overeten en hoe omgevingsfactoren voedselinname bevorderen. Dan zal ik bespreken hoe het brein 'leert' om voorkeur te geven aan voedingsmiddelen (en voedsel-gerelateerde prikkels) die energie bevatten in de vorm van sucrose en ik zal de rol van de neurotransmitter dopamine bespreken in dit proces. De bijdragen van de in dit proefschrift beschreven experimenten zal ik in deze discussie integreren. Tot slot zou ik willen vergelijken hoe ons brein om gaat met voedsel en voedsel-gerelateerde prikkels en hoe onze hersenen wordt beïnvloed door gebruik van drugs en druggerelateerde prikkels en ik zal argumenten voor en tegen het bestaan van voedselverslaving bespreken.

De vraag waarom we eten lijkt op het eerste gezicht zeer eenvoudig. We eten omdat we energie nodig hebben. Inderdaad is het zo, dat als mensen of knaagdieren worden blootgesteld aan een periode van voedselonthouding, zij compenseren voor de misgelopen calorie-inname bij de volgende gelegenheid om te eten (Telch en Agras, 1996). Eén van de eerste studies in proefdieren over dit onderwerp onderzocht wat er met voedselinname gebeurde toen dit werd verdund met onverteerbaar (maar eetbaar, zie figuur 1) cellulose (Adolph, 1947). In dit geval werd door Adolph waargenomen dat ratten hun totale dagelijkse voedselinname aanpassen om aan een vooraf ingestelde hoeveelheid calorieën te voldoen. Hetzelfde geldt wanneer ratten de gelegenheid krijgen om zichzelf een vloeibare voeding via een maagsonde rechtstreekst in de maag toe te dienen (dus zonder dat zij het voedsel kunnen proeven of ruiken) (Epstein en Teitelbaum, 1962). Dit leidde vroege onderzoekers er toe te concluderen dat voedselinname voornamelijk gedreven wordt door calorische behoefte. Uiteraard is energieopname en uitgaven zeer gecontroleerd in alle organismen van de kleinste bacteriën tot aan complexe zoogdieren zoals mensen. Wanneer de energiereserves vallen, laten signalen uit de periferie, zoals leptine (uit vetweefsel) en ghreline (uit de maag en darmen) de hersenen weten dat actie moet worden ondernomen om voedsel te vinden. Maar, zoals kan worden geconcludeerd uit de recente obesitas-epidemie, is deze homeostatische aansturing van het

eetgedrag niet het hele verhaal. Externe factoren zijn sterk van invloed op voedselinname. Een herkenbaar voorbeeld is bijvoorbeeld dat we honger krijgen en blijven eten terwijl we verzadigd zijn op specifieke momenten gedurende de dag omdat we zijn gewend aan het eten van een aantal maaltijden op vaste tijden. Bloedspiegels van ghreline (ook bekend als het “honger hormoon”) worden door ons lichaam afgesteld op ons dagelijkse eetpatroon en bereiden ons lichaam voor op een maaltijd (Blum *et al*, 2009; Cummings *et al*, 2001; LeSauter *et al*, 2009; Merkestein *et al* , 2012). Bovendien speelt eten een belangrijke rol in de menselijke sociale interactie en, last but not least, we eten, omdat we het lekker vinden om te eten. Dit laatste deel is vooral belangrijk omdat deze ‘hedonistische’ aansturing van het eetgedrag niet noodzakelijkerwijs verband houdt met een homeostatische behoefte en in feite eetgedrag bevorderd dat niet nodig is (Berridge *et al*, 2010; Johnson, 2013a; Zheng en Berthoud, 2007).

Omgevingsfactoren bevorderen voedselinname

Waarschijnlijk het oudste en meest bekende voorbeeld van een omgevingsfactor geassocieerd met voedselinname komt uit de experimenten waarvoor Ivan Pavlov werd bekroond met de Nobelprijs in 1904. In deze experimenten, werd aan Pavlov’s honden geleerd om een betekenisloos geluid (een metronoom) te associëren met het verkrijgen van voedsel. Na deze conditionering begonnen de honden te kwijlen als zij alleen aan het geluid werden blootgesteld (Pavlov, 2010). Pavlov kwam met de terminologie om dit gedrag te beschrijven en we noemen deze klassieke vorm van conditionering daarom nog steeds Pavlov-conditionering. In dit voorbeeld is het eten, dat geen leren vereist, een ongeconditioneerde stimulus. De aangeleerde prikkel (de metronoom, maar het kan een willekeurige stimulans zijn) is een geconditioneerde prikkel, want het vereist dat het dier aanleert deze met de ongeconditioneerde stimulus te associëren. Een zeer bekend voorbeeld van een voedsel-geassocieerde geconditioneerde stimulus is de beroemde ‘Golden Arches’ die de meeste mensen (dankzij conditionering door één van de best gefinancierde marketingmachines in de wereld) associëren met energierijk voedsel. Dit is een voorbeeld van hoe een prikkel uit de omgeving de voedselinname sterk kan beïnvloeden en zelfs het ‘willen’ en ‘verlangen’ van voedsel kan bevorderen.

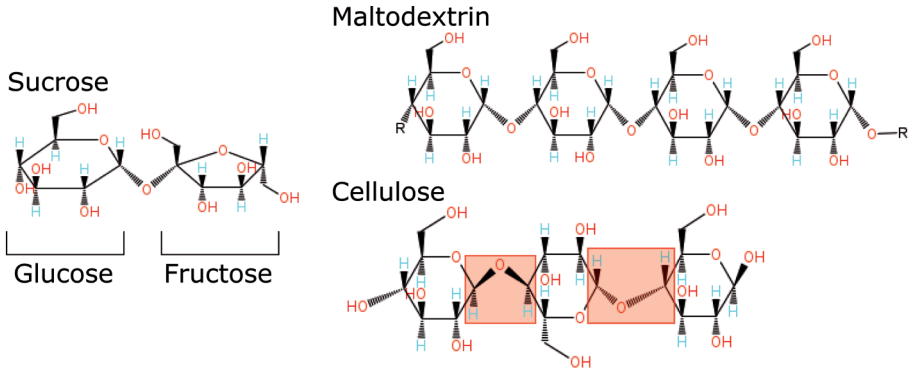
Geconditioneerde signalen kunnen direct eetgedrag stimuleren (cue-induced feeding). Een voorbeeld is dat ratten, die eerder geleerd hebben om een bepaalde

toon te associëren met het verkrijgen van een maaltijd, meer zullen eten van deze maaltijd als ze later worden blootgesteld aan deze toon, dan als ze alleen aan de maaltijd worden blootgesteld, zelfs als zij verzadigd zijn (Weingarten, 1983). Dit werkt bij mensen al vanaf zeer jonge leeftijd (Birch *et al*, 1989). Een specifiek voedsel-gerelateerde omgeving kan overmatige inname van voedsel bevorderen, zoals een restaurant of zelfs een bepaald gedrag, zoals televisie kijken tijdens het eten van een snack (Bouton, 2011; Braude en Stevenson, 2014). Het is interessant om op te merken dat dit gedrag voor een belangrijk deel onder de controle van ghrelin signalering staat (Walker *et al*, 2012). Cue-induced feeding hangt af van een breed netwerk van hersengebieden waaronder de amygdala, laterale hypothalamus, prefrontale cortex en de hippocampus (voor een overzicht zie: (Johnson, 2013b)). Naast de directe stimulatie van voedselinname, kunnen signalen uit de omgeving ook gemotiveerd gedrag uitlokken om voedsel te *verkrijgen*. Na het conditioneren, kunnen bepaalde prikkels 'incentive salience' verwerven. Incentive salience heeft betrekking op hoe signalen (gepresenteerd in de omgeving of intern gegenereerd) gemotiveerd gedrag sturen, zoals 'willen' of hunkering ('craving') van een (on) geconditioneerde stimulus (Robinson en Berridge, 1993). Een foto van een chocoladetaart, bijvoorbeeld , kan een gevoel van 'willen' uitlokken. De chocoladetaart zelf is een prikkel die nog meer emotionele waarde heeft en zal waarschijnlijk leiden tot meer 'willen' en vergroot de kans dat u de taart zal kopen en eten (Veilleux en Skinner, 2015). Dit is precies waarom restaurantketens met een goede marketingafdeling foto's van voedsel op hun menu plaatsen en waarom Starbucks hun chocoladetaart in een vitrine presenteert in plaats van deze alleen te beschrijven op het bord naast de koffie selectie. Een duidelijk voorbeeld van incentive salience in proefdieren is afkomstig van de experimenten besproken in hoofdstuk 4. In dat geval hadden ratten geleerd om verschillende signalen (een lampje en de terugtrekking van de operante hendels) met de levering van een sucrose beloning te associëren. We lieten deze dieren vervolgens een periode van extinctie (uitdoving) doormaken, wat betekent dat ze geen sucrose meer ontvingen wanneer ze een hefboom indrukte en als gevolg daarvan niet meer bereid waren om te werken (dat wil zeggen, ze hielden op met drukken op de hefboom). De ratten hadden echter niet vergeten welke signalen eerder werden geassocieerd met sucrose levering, want als ze opnieuw werden blootgesteld aan deze signalen (signaal-geïnduceerde reinstatement) begonnen ze opnieuw op de hendel te drukken. Belangrijk is dat ze nog steeds geen sucrose ontvingen tijdens deze reinstatement sessie, dus ze drukte alleen voor de signalen die eerder met sucrose in verband waren gebracht. Op soortgelijke wijze verondersteld men dat signalen, zoals de Golden Arches

of verpakkingen van levensmiddelen of voedsel-verwante afbeeldingen op een menu of proximale signalen zoals de geur van smakelijk eten ons dwingen tot het zoeken en consumeren van voedsel (Bouton, 2011). Deze prikkels uit de omgeving zullen dit doen, zelfs bij mensen die proberen hun voedselinname te beperken (diëten) en belangrijker, prikkels uit de omgeving kunnen eetgedrag stimuleren terwijl het organisme verzadigd is en er dus geen homeostatische behoefde is om te eten (Fedoroff *et al*, 1997; Watson *et al*, 2014).

Wat heeft dit te maken met obesitas? In de volgende paragraaf stel ik dat in het bijzonder energierijk, suikerhoudend eten, in tegenstelling tot smakelijk, maar suikervrij eten, goed is in het stimuleren van de hersenen en aldus de vorming van geconditioneerde stimuli en incentive salience kan bevorderen. Ongezond eten is dus beter in het ons aanleren van nieuw gedrag dan gezond, energiearm, eten. Dit was een voordeel in de prehistorie toen voedsel schaars was, maar is zeer problematisch in de moderne tijd waarin hoogcalorisch voedsel overal aanwezig is (Ulijaszek, 2002). Hier zal ik ook ingaan op de in hoofdstuk 5 beschreven bevindingen.

Figuur 1



Molecuulstructuren van sucrose, glucose, fructose, maltodextrine en Cellulose. Merk op dat sucrose kan worden gehydrolyseerd in darmen in fructose en glucose en maltodextrine in glucose. Cellulose daarentegen, hoewel vrijwel gelijkwaardig aan maltodextrine, kan niet worden afgebroken door mensen omdat zij niet de β-bindingen (rode arcering), waarmee de individuele glucose-eenheden zijn verbonden kunnen hydrolyseren. Daarom heeft cellulose geen voedingswaarde en zeer weinig belonende waarde. Interessant is dat ratten bereid zijn te werken voor gezoete cellulose, maar de hoeveelheid moeite die zij willen uitoefenen om een cellulose beloningen te verkrijgen is verwaarloosbaar in vergelijking met de inspanningen die zij doen om een beloning van sucrose te verkrijgen (de Jong et al. Ongepubliceerde data).

De belonende eigenschappen van sucrose en glucose

Sucrose is een molecuul bestaande uit fructose en glucose aan elkaar gekoppeld (zie figuur 1). Sucrose heeft een zoete smaak, omdat het bindt aan de zoete smaak receptoren (T1R2 en T1R3) op onze tong. Het wordt opgenomen in darmen en afgebroken tot glucose en fructose. Fructose wordt vervolgens omgezet in de lever tot glucose, hetgeen een belangrijke energiebron is voor onze interne organen. Een voldoende hoge bloedglucoseconcentratie is noodzakelijk voor een goede werking van de hersenen (dat glucose gebruikt als primaire energiebron) en wordt om die rede dus zeer strak gereguleerd door het lichaam. Een andere belangrijke bron van glucose is zetmeel (waarvan de maltodextrine die in hoofdstuk 5 werd gebruikt, is afgeleid). Zetmeel bestaat uit lange ketens van gekoppelde glucose moleculen die worden afgebroken tot glucose in de darmen.

In hoofdstuk 5, hebben we besproken hoe ratten meer van een oplossing van maltodextrine (een hoogcalorische koolhydraat) dan een zoete (non-calorische) oplossing van sacharine drinken. Belangrijker nog, aanvankelijk toonde zij meer motivatie om de zoete (non-calorische) oplossing dan de 'vlakke' (hoogcalorische) oplossing te verkrijgen. Echter na verscheidene weken vrij gebruik van beide oplossingen, leerde de ratten over de metabolische waarde van beide oplossingen (maltodextrine is een hoogcalorisch koolhydraat terwijl sacharine geen voedingswaarde bevat) en toonde gelijke motivatie voor beide oplossingen en grotere motivatie voor de maltodextrine oplossing wanneer ze honger hadden. Het is belangrijk om op te merken dat de dieren slechts een zeer kleine hoeveelheid van de oplossingen konden verkrijgen tijdens de zogenaamde 'progressive ratio' (PR) sessies waar hun motivatie om de beloning te verkrijgen werd getest. Dit betekent dat zij slechts in zeer geringe mate de effecten van deze oplossingen ervoeren tijdens deze sessies. In feite is het enige verschil tussen een PR-sessie voor maltodextrine en een PR-sessie voor sacharine de smaak van de eerste paar beloningen (hoewel maltodextrine niet zoet is, heeft het wel een karakteristieke smaak). Deze *smaak* werd eerder (tijdens de vrije inname fase) geassocieerd met de metabole eigenschappen van maltodextrine. Dit is een voorbeeld van een (proximaal) geconditioneerde beloner.

Op het eerste gezicht zou men geneigd kunnen zijn te denken dat sucrose belonend is, omdat het lekker (zoet) smaakt, maar dit, zo blijkt, is niet het

complete verhaal[1]. Het is waar dat zoete smaak belonend is: dieren zijn bereid om te werken voor niet-calorische zoetstoffen en ze zullen deze tot zich nemen wanneer hen de gelegenheid gegeven wordt ((Cason en Aston-Jones, 2013), Hoofdstuk 5). Echter, wanneer muizen en ratten de keuze krijgen tussen sucrose of sucralose (dat dezelfde smaak receptoren bindt maar geen calorieën bevat), kiezen zij overweldigend voor sucrose (Domingos *et al*, 2011; 2013). Interessant en opvallend is dat muizen die de zoet-receptoren T1R3, T1R2 of het ionenkanaal TRPM5 (nodig voor zoete smaak signalering) missen niet langer de voorkeur aan sucralose over water geven, maar nog steeds perfect in staat zijn om te leren om sucrose te prefereren over water (Damak *et al*, 2003; de Araujo *et al*, 2008). Hieruit blijkt dat andere, smaak-onafhankelijke eigenschappen van sucrose voldoende zijn voor conditionering. In feite zijn de smaak-onafhankelijke effecten van sucrose niet alleen ondersteunend voor conditionering, zij zijn eigenlijk veel krachtigere beloners dan zoete smaak alleen. Dat wil zeggen, in een paradigma waarin dieren de keuze wordt gegeven tussen een fles water aan de ene kant en een sucrose fles aan de andere kant, leren ze om de sucrose kant te verkiezen boven de waterkant, zelfs wanneer ze worden geconfronteerd met waterflessen aan beide kanten (de Araujo *et al*, 2008). Sucralose is niet in staat een zelfde 'side-bias' te induceren (Araujo *et al*, 2008). Bovendien, maaginfusies van glucose (één van de twee componenten van sucrose, zie figuur 1) hebben sterk belonende eigenschappen (Ackroff en Sclafani, 2014; Sclafani *et al*, 1993; Zukerman *et al*,2013). Wanneer infusies van 16% glucose in de maag zijn gekoppeld aan het nemen van een oplossing met een bepaalde smaak zullen ratten leren deze smaak te verkiezen boven een controlevloeistof, zelfs als de testoplossing in eerste instantie als aversief (bitter) werd ervaren (Myers en Sclafani, 2003 ; Perez *et al*, 1998). Ook maltodextrine infusies in de maag gekoppeld aan het verbruik van een bepaalde smaak zijn voldoende om ratten te conditioneren om deze smaak over een andere smaak te prefereren (Elizalde en Sclafani, 1990). Omgekeerd, wanneer ratten een maltodextine oplossing consumeren, maar de metabolische eigenschappen van deze oplossing niet ervaren, omdat de oplossing wordt afgevoerd uit de maag via een maagfistel, verliest maltodextrine zijn vermogen om een specifieke smaak te conditioneren

[1] Met zoete smaak bedoel ik te zeggen dat iets aan de 'zoete smaak receptoren' op de tong bindt. Natuurlijk is het mogelijk om te stellen dat niets op zich zelf zoet smaakt, maar dat wij geëvolueerd zijn om suikerrijke voedingstoffen als zoet te ervaren. Evenzo stel ik dat de activering van de zoete smaak receptoren niet zo'n potente beloner is als de smaak-onafhankelijke eigenschappen van sucrose.

(dit lijkt echter wel een concentratie-afhankelijk effect te zijn) (Sclafani en Ackroff, 2004; Sclafani *et al*, 1994).

Een samenvatting van de laatste paragraaf, glucose heeft sterke belonende kwaliteiten die niet afhankelijk zijn van de zoete smaak. Het is daarom in staat tot veel krachtigere conditionering dan energiearme zoetstoffen.

De rol van dopamine

In de laatste paragraaf besprak ik aanwijzingen voor de sterke conditionerende kwaliteiten van sucrose en glucose die voornamelijk worden gemedieerd via hun smaak-onafhankelijke effecten. Hier wil ik graag de rol die de neurotransmitter dopamine speelt in dit proces te bespreken.

Dopamine wordt hoofdzakelijk gesynthetiseerd in de middenhersenen, in het bijzonder in het ventrale tegmentale gebied (VTA) en de substantia nigra (SN). Vooral de mesolimbische dopamine projectie (van het VTA naar de nucleus accumbens (NACC)) speelt een belangrijke rol bij motivatie (Kelley en Berridge, 2002, Salamone en Correa, 2012). Dit gaat goed samen met wat we in hoofdstuk 4 lieten zien. Hier lieten we zien dat de activering van de hersenverbinding tussen de VTA en de NACC resulteerde in aanzienlijk toegenomen motivatie om een sucrose beloning te verkrijgen. Bovendien, in hoofdstuk 3 toonden we aan dat verwijdering van de dopamine D2 receptor (D2R) van dopamine neuronen in de VTA (waar zij normaal een functie van negatieve feedback heeft) ratten meer gemotiveerd maakte om een sucrose of cocaïne beloning te verkrijgen. Dezelfde manipulatie had geen effect op inname van sucrose of cocaïne wanneer de ratten hier geen moeite voor hoefde te doen (het was dus een heel specifiek effect). Fast Scan Cyclic Voltametry (FSCV) en in-vivo elektrofysiologie experimenten hebben aangetoond dat dopamine neuronen die de VTA met de NACC verbinden, belangrijk zijn voor de verwerking van opvallende prikkels uit de omgeving, vooral wanneer deze prikkels een grote aantrekkingskracht (incentive salience) hebben (Flagel *et al*, 2011; Yun *et al*, 2004). Bovendien speelt dopamine een belangrijke rol bij leeralgoritmen en de vorming van stimulerende signalen tijdens het conditioneren (Cohen *et al*, 2012; Fenu *et al*, 2001; Schultz *et al*, 1997).

Het is interessant om op te merken dat, sucrose, of preciezer, de verhoogde glucosespiegel als gevolg van sucrose inname, zeer goed is in het verhogen van

dopamine niveaus in de nucleus accumbens. Knaagdierstudies hebben aangetoond dat maaginfusies van glucose dopamine niveaus verhogen in het ventrale striatum (dat wil zeggen NACC en olfactorische knobbel) (Ren *et al*, 2010). Infusies van glucose direct in de bloedsomloop, vooral in de poortader, zijn potente beloners (dat wil zeggen ze bevorderen conditionering) en zij zijn in staat dopamine te verhogen in de NACC (Oliveira-Maia *et al*,2011)[2]. Bovendien is het zo dat signalen die eerder zijn geassocieerd met sucrose meer dopamine afgifte in de NACC uitlokken dan signalen die eerder zijn gekoppeld aan de inname van sacharine in ratten (McCutcheonet *et al*, 2012). Infusies van de dopamine D1 receptor antagonist SCH23390 in de schil van de NACC blokkeren glucose conditionering (Touzani *et al*, 2008). Omgekeerd, wanneer optogenetische activering van dopamine neuronen is gepaard met likken van een sipper waaruit een sucralose oplossing komt, zullen muizen deze sipper verkiezen boven een sipper die sucrose uitdeelt wanneer zij verzadigd zijn (Domingos *et al*, 2011).

Vergelijkbare waarnemingen komen uit het onderzoek bij menselijke proefpersonen. Sucrose inname lokt meer neuronale activatie in dopaminerge gebieden van de hersenen uit in vergelijking met sucralose inname (Frank *et al*, 2008). In een experiment dat grotendeels het menselijke equivalent is van wat we in hoofdstuk 5 gedaan hebben, tonen Araujo *et al.* aan dat mensen kunnen worden geconditioneerd om verhoogde hersenactiviteit in de NACC te laten zien na de presentatie van een smaak die eerder was gekoppeld aan een verhoogde bloedglucoseconcentratie (door toevoeging van maltodextrine) (de Araujo *et al*, 2013). Dit alles toont aan dat sucrose, vermoedelijk door een verhoging van de bloedsuikerspiegel, zeer efficiënt in staat is tot het verhogen van dopamine niveaus in de NACC. Dit is waarschijnlijk de reden waarom het zulke sterke conditionerende kwaliteiten heeft in vergelijking met natuurlijke of kunstmatige zoetstoffen. Deze zoetstoffen verhogen de bloedsuikerspiegel immers niet.

Maar hoe leidt een verhoging van de bloedglucoseconcentratie (veroorzaakt door sucrose of zetmeel inname) tot dopamine signalering? Het is mogelijk dat dopaminecellen gevoelig zijn voor de bloedglucoseconcentratie. Er zijn aanwijzingen voor glucose-gevoeligheid in de hersenen, vooral in de

[2] Dit wil niet zeggen dat de zoete smaak van sucrose geen dopamine afgifte stimuleert. In dieren die dankzij een maagfistel alleen de smaak, maar niet de smaak-onafhankelijke eigenschapen van sucrose ervaren, levert orale sucrose toediening dopamine-afgifte op (Hajnal et al, 2004, Scheider, 1989). Dit doet het echter in aanzienlijk mindere mate dan als dieren alle eigenschappen van sucrose ervaren (Hajnal en Norgren, 2001).

hypothalamus (Ashford *et al*, 1990). Een soortgelijk systeem zou kunnen werken op dopamine cellen in de VTA (Schiemann *et al*, 2012). Een alternatief is dat neuronen in de hypothalamus die gevoelig zijn voor glucose verbinding maken met de VTA. Hoewel dat mogelijk is (deze neuronen bestaan), lijkt het niet waarschijnlijk dat de bloedsuikerspiegel in de hersenen gemeten wordt. In tegenstelling tot andere organen zijn de hersenen vrijwel volledig afhankelijk van glucose en de glucoseconcentratie in de hersenen is ook strak gereguleerd en beïnvloed door actief transport van glucose over de bloed-hersenbarrière. Bovendien, infusies van glucose in de poortader zijn krachtiger in het verhogen van de NACC dopamine niveaus dan glucose infusies in de halsader (Oliveira-Maia *et al*, 2011). Een postprandiale toename van de bloedglucose wordt snel gedetecteerd in de pancreas in β-cellen, die in reactie insuline afgeven. Het lijkt onwaarschijnlijk dat informatie over de bloedglucoseconcentratie op dat moment verzonden wordt naar de hersenen via de nervus vagus, aangezien ratten waarin deze zenuw is gelaedeerd nog steeds geconditioneerd kunnen worden door maltodextrine infusies in de maag (Sclafani en Lucas, 1996). Als alternatief is insuline zelf een mogelijke mediator van glucose conditionering. Insuline-injecties verhogen striataal dopamine (Potter *et al*, 1999). Insuline wordt zelfs voorgesteld voor een rol in dopamine signalering, onafhankelijk van voedsel gedrag. Bijvoorbeeld in drugsverslaving (Daws *et al*, 2011; Schoffelmeer *et al*, 2011). Dat gezegd hebbende, diabetische ratten, waarbij insuline producerende cellen werden gelaedeerd door streptozotocine behandeling blijven gevoelig voor gastrische infusies van glucose (Ackroff *et al*, 1997).

Concluderend: voedsel dat sucrose bevat kan, door middel van het beïnvloeden van het dopamine systeem, de vorming van voedsel-geassocieerde prikkels zoals smaak, geur en distale prikkels zoals een voedsel-gerelateerde omgeving (bijvoorbeeld een restaurant) of voedselverpakkingen stimuleren, op een wijze die lekker, maar caloriearm voedsel dat niet kan. Het is belangrijk om u te beseffen dat men in een westelijke milieu, waarin calorierijke voedingsmiddelen alom vertegenwoordigd zijn, voortdurend de hersenen traint zich te richten op prikkels die gerelateerd zijn aan suikerrijke voedingsmiddelen. Een stuk taart gemaakt met suiker (fructose en glucose) en zetmeel (afgebroken tot glucose) is, dankzij conditionering, aantrekkelijker voor de hersenen dan een wortel die voor een groot deel uit water en cellulose bestaat (geen van beide draagt enige calorieën). Cellulose is zeer vergelijkbaar met zetmeel, maar mensen zijn niet in staat om het te splitsen in glucose (zie figuur 1). Om deze reden, zijn wortels niet erg sterke beloners en hebben ze op ons (uitzonderingen daar gelaten)

geen sterke aantrekkingskracht. Het is heel goed mogelijk dat als mensen in staat waren cellulose af te breken in glucose het fatsoenlijke zelfbeheersing zou vereisen om dit proefschrift niet op te eten.

Kan men verslaafd zijn aan voedsel?

Men heeft voorgesteld dat verslavings-achtig gedrag kan bijdragen aan de huidige obesitasepidemie. Inderdaad, er zijn verschillende gedragsmatige gelijkenissen tussen drugsverslaving en eetstoornissen zoals binge eating disorder (BED) en boulimia nervosa (BN) (de Jong *et al*, 2012; Gearhardt *et al*, 2011b; Meule *et al*, 2014b; Volkow en O O'Brien, 2007). De Yale Food Addiction Score (YFAS) is een voorgesteld instrument om eetverslaving te kwantificeren (Gearhardt *et al*, 2009). Interessant is, dat hoewel voedselverslavings-achtig gedrag (zoals BED, BN of een hoge YFAS score) zeer waarschijnlijk zal bijdragen tot overgewicht, ze zeker aparte constructen zijn. Aangezien niet alle 'voedselverslaafden' te zwaar zijn en niet alle zwaarlijvige personen voldoen aan de voorgestelde criteria voor 'voedselverslaving' (Gearhardt *et al*, 2014; Kessler *et al*,2013). Hoewel er duidelijke gedragsmatige gelijkenissen tussen voedsel- en drugsverslaving zijn, is de wetenschappelijke gemeenschap verdeeld over dit onderwerp (Avena *et al*, 2012; Corsica en Pelchat, 2010; Hebebrand *et al*, 2014; Ziauddeen en Fletcher, 2013).

In hoofdstuk 1 hebben we besproken of 'voedselverslaving' een geldig begrip is en we hebben een diermodel voorgesteld om de controle over voedselinname te beoordelen. In hoofdstuk 2 hebben we onderzocht of er een 'binge dieet' ongecontroleerd eetgedrag uitlokte. Dat gebeurde niet. Er is een redelijke hoeveelheid literatuur die anders suggereert. Verschillende auteurs hebben 'verslavings-achtig gedrag' bij dieren na blootstelling aan 'binge diëten' waargenomen. Deze diëten bestaan meestal uit afwisselende periodes van voedsel ontbering / beperking en periodes van toegang tot verteerbaar voedsel (Corwin *et al*, 2011; Goud en Avena, 2013 ; Hagan *et al*, 2002; Iemolo *et al*, 2012). Deze diëten, die zogenaamde 'jojo-diëten' nabootsen, kunnen gedrag dat doet denken aan voedsel 'binging' uitlokken of ze kunnen gedrag oproepen dat (een beetje) doet denken aan compulsief gedrag of zelfs afkickverschijnselen (Colantuoni *et al*, 2002; Cottone *et al*, 2009; Iemolo *et al*, 2012 ; Oswald *et al*, 2011). Het is echter alles behalve duidelijk of ze echte verslaving veroorzaken in dezelfde zin dat sommige drugs dat doen. In feite is een groot probleem met

het voedselverslavingshypothese dat voedsel-verslaafde individuen (mensen of knaagdieren) niet verslaafd zijn aan een specifiek soort voedsel als gevolg van de consumptie van dat voedsel, zoals dat bij drugs het geval is (Ahmed en Koob, 1997 ; Buczek *et al*, 1999; de Jong *et al*, 2013; Deroche-Gamonet *et al*, 2004; Vanderschuren en Everitt, 2004) (Maar zie: (Johnson en Kenny, 2010)). 'Voedselverslaving' lijkt een breder probleem van ontremming van het eten in het algemeen, een gedragsprobleem dat waarschijnlijk beter vergelijkbaar is met gokverslaving of seksverslaving dan drugsverslaving in gedrags- en neurobiologische zin (Albayrak *et al* , 2012; Hebebrand *et al*, 2014). Om die reden zou de term 'eetverslaving' meer geschikt zijn om te verwijzen naar ongecontroleerde voedselinname (Hebebrand *et al*, 2014).

Een belangrijk onderscheid om te maken is dat het zeer wel mogelijk is om gemotiveerd te zijn om voedsel te verkrijgen, en gevoelig te zijn voor voedsel-gerelateerde prikkels, zonder dwangmatige inname te vertonen zoals voor komt bij drugsverslaving. Ontremming van het dopaminesysteem bijvoorbeeld, zoals we laten zien in hoofdstuk 3, verhoogt robuust de motivatie voor sucrose en cocaïne, maar is niet bevorderlijk voor dwangmatig cocaïne of sucrose zoekgedrag. Evenmin leid DREADD activering van de VTA naar NACC projectie tot enige vorm van compulsief zoekgedrag hoewel het de motivatie om sucrose te verkrijgen aanzienlijk vergroot (hoofdstuk 4). Hoewel motivatie voor voedsel en drugs (geactiveerd door uitlokkende prikkels) soortgelijke (dopaminerge) mechanismen behelst, omvat de ontwikkeling van drugsverslaving verdere neurobiologische veranderingen die niet overtuigend zijn aangetoond voor sucrose of andere voedingsmiddelen (Chen *et al*, 2008; 2013 ; Limpens *et al*, 2014; Pelloux *et al*, 2007; Porrino *et al*, 2004; Vanderschuren en Everitt, 2004; Willuhn *et al*, 2012).

Ongeacht het debat over dit onderwerp, in het licht van deze discussie, is het interessant om op te merken dat de manier waarop gezonde hersenen omgaat met voedsel- of drugs-gerelateerde signalen vergelijkbaar is. Functionele magnetische resonantie imaging (fMRI) studies hebben aangetoond dat drugs-gerelateerde signalen in drugsgebruikers en voedsel-gerelateerde signalen in anderen, vergelijkbare hersengebieden activeren (Pelchat *et al*, 2004; Rolls en McCabe, 2007; Tang *et al*, 2012; Tomasi *et al*, 2015)[3]. Er zijn ook aanwijzingen dat personen met zeer veel overgewicht of personen met een hoge YFAS-score anders worden beïnvloed door voedsel-gerelateerde signalen dan gezonde controles, net zoals drugsverslaafden anders worden beïnvloed door drugs-gerelateerde

prikkels (Gearhardt *et al*, 2011c; Martin ea , 2010; Rothemund *et al*, 2007). (Er zijn in dit veld echter absoluut tegenstrijdige resultaten, zie: Ziauddeen *et al*, 2012.) Kinderen met overgewicht zijn gevoeliger voor aan voeding gerelateerde signalen dan kinderen van gemiddeld gewicht (Jansen *et al*, 2003). Het concept dat signalen uit de omgeving afwijkende incentive salience (misschien zelfs 'cravings') oproepen is een sleutelbegrip in de 'incentive salience theory of addiction' en sommigen hebben gesuggereerd dat een soortgelijk proces ten grondslag ligt aan obesitas (Berridge, 2009; Robinson en Berridge, 1993). Het idee is dat voedsel-gerelateerde signalen excessieve 'willen' (hunkerring, 'craving') bevorderen en dat zelfbeheersing nodig is om voedsel-zoekgedrag te remmen. In dit verband is het interessant om op te merken dat mensen die minder controle over hun impulsen hebben (sterk impulsieve mensen) meer kans lopen om te handelen in reactie op voedselsignalen en meer vatbaar zijn voor overgewicht (Claes *et al*, 2006; Jansen *et al*, 2003; Meule *et al*, 2014a; Nederkoorn *et al*, 2007).

De vraag is natuurlijk: is het mogelijk om de hersenen te on-conditioneren en de waarde van voedsel-gerelateerde signalen uit de hersenen te verwijderen? Het korte antwoord lijkt te zijn 'nee'. Prikkels die zijn geassocieerd met smakelijk voedsel zullen hun incentive salience (uitlokkende opvallendheid, aantrekkingskracht, emotionele waarde) behouden net zoals drugs-gerelateerde signalen altijd een verhoogde emotionele waarde voor een herstellende drugsverslaafde zullen behouden. Het is mogelijk om de waarde van een geconditioneerde prikkel uit te doven, een proces dat psychologen 'extinctie' noemen. Maar extinctie is een proces dat zeer contextafhankelijk is waardoor voedsel-gerelateerde prikkels die zijn uitgedoofd in een zekere context (misschien een obesitas kliniek), nog steeds 'cravings' kunnen oproepen in andere omgeving (de woonomgeving bijvoorbeeld) (Bouton, 2011). Bovendien betekent extinctie niet de verwijdering van de associatie tussen een bepaalde prikkel en de verwachte beloning, maar de vorming van een nieuw geheugenpatroon, dat concurreert met de originele prikkel-beloning associatie die verlangen induceert. Zo is de 'oude' associatie nog steeds aanwezig en kan worden hersteld door de presentatie van krachtige signalen, korte blootstelling aan de beloning zelf of stress (veroorzaakt door

[3] Microdialyse studies in ratten, met een hogere ruimtelijke resolutie dan fMRI-studies bij de mens, hebben echter opmerkelijke verschillen aangetoond, waarbij drugs-gerelateerde signalen dopamine afgifte in de schill van de NACC stimuleren en voedsel-gerelateerde signalen in de kern van de NACC (Bassareo et al, 2007; Di Chiara en Bassare, 2007).

de omgeving of farmacologisch geïnduceerd in proefdieren) (Ghitza *et al*,2005; Shaham *et al*, 2003, hoofdstuk 2, 3 en 4). Dat gezegd hebbende, cue exposure therapie, een therapie die is gericht op het verbreken van het verband tussen prikkel en beloning, kan worden toegepast op zwaarlijvige individuen of patiënten met BED (Havermans en Jansen, 2003; Jansen, 2010; Pla-Sanjuanelo *et al*, 2014). Een alternatief is een 'memory-retrieval extinction procedure' waarvan is aangetoond dat het prikkel-geïnduceerde drug 'craving' bij knaagdieren en mensen kan remmen en wellicht ook gebruikt kan worden om voedsel cravings te behandelen (Xue *et al*,2012). Een populaire en vaak gesuggereerd preventiestrategie is dat ouders een gezond eetpatroon bij kinderen moeten bevorderen om daarmee te voorkomen dat sterke associaties tussen voedsel en voedselsignalen ontstaan en kinderen zelfbeheersing ontwikkelen op hetzelfde moment. Het is echter niet helemaal duidelijk welke strategieën ouders moeten ontplooien om dit te bewerkstelligen (Clark *et al*, 2007). Niettemin, afscherming van kinderen voor reclamecampagnes die specifiek zijn ontworpen om sterke associaties tussen bepaalde prikkels (kleurrijke verpakking, speelgoed, games) en energierijk voedsel te vormen is waarschijnlijk een effectieve preventie maatregel (Gearhardt *et al*, 2011a). Een andere belangrijke consideratie is dat de snelheid waarmee glucose wordt geabsorbeerd uit voedsel invloed kan hebben op hoe de hersenen reageren op voedsel. Een maaltijd met een hoge glycemische index (wat betekent dat het een snelle, maar korte toename van de bloedglucoseconcentratie veroorzaakt) produceert, in vergelijking met een isocalorisch maaltijd met een lage glycemische index, meer activiteit in de NACC (Lennerz *et al*, 2013). Dit doet denken aan de generalisatie dat drugs die sneller effect op de hersenen hebben (bijvoorbeeld geïnjecteerd cocaïne vergeleken met oraal of gesnoven cocaïne) meer verslavend potentieel hebben (Samaha en Robinson, 2005; Volkow en Swanson, 2003). Het is opvallend dat auteurs die voorvechter zijn van de term 'voedselverslaving' vaak de nadruk leggen op de aanwezigheid van zogenaamde 'ultraprocessed foods' waaronder geraffineerde koolhydraten, die een hoge glycemische index hebben en zouden moeten worden vervangen door 'langzame' koolhydraten (Curtis en Davis, 2014; Davis, 2014; Ifland *et al*, 2009). Bovendien moeten ouders proberen te voorkomen dat ongezond voedsel synoniem wordt aan 'het hebben van een leuke tijd' door calorierijk voedsel te associëren met vrolijke gebeurtenissen in het leven van een kind, zoals verjaardagen, feestdagen en prestaties, zoals het behalen van een diploma of met afgewerkte huiswerk. Dergelijke maatregelen zijn succesvol gebleken om in het verleden tieners van het roken te houden. Maar net als in het geval van tabaksverslaving, is

er helaas een grote en invloedrijke industrie afhankelijk van het bereiken van precies het tegenovergestelde (Brownell en Warner, 2009). In het algemeen zal meer onderzoek over hoe de verbinding tussen suikerrijke voedingsmiddelen en prikkels uit de omgeving verhinderd kan worden gunstig zijn voor de bestrijding van de obesitasepidemie.

Bijdragen en beperkingen van dit werk

Hier heb ik geprobeerd om een idee te schetsen over hoe voedsel-gerelateerde signalen eetgedrag bevorderen en ik heb dit vergeleken met hoe drugs-gerelateerde stimuli drugsgebruik bevorderen en (in sommige gevallen) drugsmisbruik. Ik denk dat, hoewel er overeenkomsten zijn in hoe onze hersenen in eerste instantie met drugs en voedsel om gaan, eetverslaving en drugsverslaving waarschijnlijk verschillen in hun onderliggende neurobiologie. Ik ben me er van bewust dat deze discussie slechts een zeer kort overzicht geeft van dit onderwerp. Bovendien is een zeer ernstige beperking van dit werk dat ik sterk focus op de rol van glucose en koolhydraten, terwijl er natuurlijk een belangrijke rol voor lipiden, vetweefsel en leptine is weggelegd. Leptine heeft aantoonbaar invloed op het dopaminesysteem en beïnvloedt motivatie voor voedsel en prikkel-gedreven gedrag (Hommel *et al*, 2006; Rada *et al*, 2012; Thanos *et al*, 2012). Muizen dienen zich zelf vetoplossingen toe, zelfs als zij niet de mogelijkheid krijgen deze te proeven (d.w.z. direct in de maag)(Tellez *et al*, 2013). Voor meer informatie over de wisselwerking tussen perifere hormonen, vetweefsel en de hersenen en hoe zij de controle over voedselinname en de eetlust beïnvloeden, verwijs ik naar het werk van mijn collega Rahul Pandit wiens proefschrift binnenkort zal verschijnen.

Zoals besproken in de inleiding en in hoofdstuk 1, is er slechts weinig bewijs voor voedselverslaving bij proefdieren. In hoofdstuk 1 en 2, benadrukken we dat dit onderzoek zich niet noodzakelijkerwijs moet richten op dwangmatig voedselinname, omdat bewijs voor dwangmatige inname van voedsel alleen beschikbaar is in zeer specifieke omstandigheden (dat wil zeggen na strenge diëten, afhankelijk van perioden van gedwongen voedsel ontbering) (Corwin *et al* , 2011). In plaats daarvan zou dit onderzoek zich moeten focussen op ongecontroleerde inname van voedsel, wat overeenkomsten vertoond met ongecontroleerde inname van het drugs (zoals het ontstaan van automatisch gedrag). In hoofdstuk 2 tonen we aan dat dieren met een verminderde controle

over voedselinname een sterkere neiging vertonen om chocolade te zoeken na blootstelling aan een kleine 'priming' dosis chocolade. Toekomstig onderzoek moet gebruik maken van dit soort 'multiple-criteria models' om de neurobiologie die in controle straat van de voedselinname te verkennen.

In de hoofdstukken 3 en 4, verkennen we de rol van dopamine, in het bijzonder de mesolimbische projectie, in de controle over voedselinname en de toediening van cocaïne. Interessant is, dat ontremming van dopamine neuronen (via lokale knock-down van de somatodendritische D2 autoreceptor) verhoogde motivatie voor sucrose en cocaïne oplevert, maar geen compulsieve cocaïne inname uitlokt. Ook in hoofdstuk 4 tonen we aan dat de activering van de mesolimbische hersenverbinding motivatie verhoogt (evenals algemene activiteit), maar geen effect heeft op sucrose inname uit gewoonte (d.w.z. de dieren reageren op devaluatie van de sucrosebeloning) of dwangmatig sucrose zoekgedrag (d.w.z. de ratten reageerden op de aanwezigheid van een geconditioneerde aversieve stimulus). Beide bevindingen wijzen op een selectieve rol voor dopamine in het ventrale striatum bij de organisatie van motivatie en incentive salience, zonder rechtstreeks dwangmatige voedselinname te stimuleren.

Hoewel we grote individuele verschillen hebben waargenomen in de controle over voedselinname in hoofdstuk 2, en sterk de motivatie voor sucrose konden beïnvloeden in hoofdstuk 3 en 4, hebben we in geen van deze experimenten compulsieve voedselinname waargenomen, zoals dit wel is aangetoond voor cocaïne (Deroche-Gamonet *et al* , 2004; Limpens *et al* , 2014; Pelloux *et al* , 2007; Vanderschuren en Everitt, 2004). In hoofdstuk 3 en 4, hebben we echter niet onderzocht of een *langdurige* blootstelling aan grote hoeveelheden sucrose *in combinatie* met knock-down van de D2 autoreceptor (hoofdstuk 3) of activering van de mesolimbische projectie (hoofdstuk 4) verslavings-achtig gedrag bevordert *naar verloop van tijd*. Compulsief cocaïne zoekgedrag ontstaat alleen in een subgroep van dieren na een uitgebreide geschiedenis van cocaïnegebruik en wordt gemedieerd door verschillende neurale veranderingen, waaronder verschuiving van betrokkenheid van het ventrale striatum, naar het dorsale striatum (Belin-Rauscent *et al* , 2012; Deroche- Gamonet *et al* , 2004; Everitt en Robbins, 2005; Pelloux *et al* , 2007; Vanderschuren en Everitt, 2004). Het is niet onwaarschijnlijk dat onze manipulaties deze verschuiving naar het dorsale striatum (dat is geassocieerd met automatisch gedrag, gekenmerkt door een zeer sterke relatie tussen prikkel en gedrag) kunnen bevorderen en er kan dus een rol zijn voor ventrale striatale dopamine signalering bij de

ontwikkeling van een verslavings-achtig gedrag na een uitgebreide geschiedenis van sucrose zelftoediening.
Een andere beperking in hoofdstuk 4 is dat, hoewel onze manipulatie projectie-specifiek was, celtype specificiteit ontbrak en bijgevolg kunnen we dus niet bevestigen dat onze effecten werden gemedieerd door dopamine of een andere neurotransmitter. Dit is met name relevant voor de mesocorticale projectie aangezien van deze route bekend is dat zij een groot percentage GABA neuronen bevat (Carr en Sesack, 2000; Swanson, 1982). Toekomstige experimenten met CRE-afhankelijke CAV2-FLP in combinatie met FLP-afhankelijke DREADD of optogenetische vectoren zullen in-vivo gelijktijdige projectie en celtype specifieke activatie of remming mogelijk maken. Een alternatief kan zijn om CRE-afhankelijke vectoren tot expressie te brengen achter promotor sequenties die geassocieerd zijn met specifieke cel populaties (Ferguson *et al* , 2013).

Een toekomstig experiment zou de 'missing link' tussen de hoofdstukken 4 en 5 kunnen vormen. Een FSCV of in-vivo elektrofysiologie experiment kan dopamine afgifte in het ventrale striatum (FSCV) of neuronale activiteit van VTA dopamine neuronen (in-vivo elektrofysiologie) na inname van saccharine of maltodextrine meten. Zowel de directe respons (na het waarnemen van de smaak) en de langdurige respons (veroorzaakt door toename van de bloedglucosespiegel) moet dan worden gemeten. Mijn hypothese is dat aanvankelijk de smaak van sacharine zal leiden tot meer dopamine afgifte (Hajnal *et al* , 2004), terwijl de smaak-onafhankelijke (metabolische) eigenschappen van maltodextrine dopamine activiteit zullen bevorderen (Ren *et al* , 2010). Na conditionering, waarbij de dieren leren dat de smaak van maltodextrine een verhoging van de bloedsuikerspiegel voorspelt, zal ook de smaak van maltodextrine dopamine afgifte uitlokken (Schultz, 1997). Verdere experimenten zouden ook moeten onderzoeken wat de rol van tijdelijke voedselrestrictie is op dit effect, omdat maltodextrine meer dopamine afgifte in een hongerige toestand zou kunnen uitlokken (Balleine en Dickinson, 1998). Dit experiment zou de rol van dopamine kunnen verbinden met smaak-conditionering en het aanleren van incentive salience.

Slotopmerkingen

In de huidige voedselrijke omgeving stellen wij onze hersenen voortdurend bloot aan prikkels die de beschikbaarheid van ongezond, energierijk eten voorspellen. Na conditionering (waarbij mono- en polysacchariden een belangrijke rol spelen), verkrijgen deze prikkels een zekere gevoelswaarde (zie bijvoorbeeld figuur 1 in de inleiding van deze thesis). Zelfcontrole is dan nodig om driften veroorzaakt door deze prikkels te onderdrukken. Hoewel dit proces overeenkomsten deelt met hoe drugs-gerelateerde signalen onbedwingbare trek veroorzaken in drugsverslaafden, is eetverslaving zeker neurobiologisch te onderscheiden van drugsverslaving.

Toekomstig onderzoek moet in meer detail verkennen hoe geconditioneerde voedselsignalen worden gevormd en hoe ze van invloed zijn op voedselkeuze. Integratie van fundamenteel onderzoek met optogenetica en DREADD technologie om de neurobiologie van voedselkeuze te beschrijven en te verkennen hoe omgevingsfactoren de voedselinname beïnvloeden is belangrijk om de obesitas-epidemie te bestrijden. Dit onderzoek moet worden geïntegreerd met psychologisch onderzoek om effectieve behandeling en preventiestrategieën te informeren. Preventiestrategieën zullen ongetwijfeld de ontmoediging van voedsel-marketing gericht op kinderen behelzen even als het stimuleren van gezonde eetgewoonten (inclusief het promoten van ‘langzame’ in tegenstelling tot de ‘snelle’ koolhydraten) bij kinderen en hun ouders.

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ADDENDUM

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Curriculum Vitae

Johannes Willem de Jong was born on November 18th 1986 in Rhenen, the Netherlands. In 2005, he graduated from high school in Doorn and commenced his undergraduate studies in biomedical sciences at Utrecht University. In 2008 he enrolled in the research master Neuroscience and Cognition at that same university. His first scientific internship was in the lab of Prof. Dr. Roger Adan in Utrecht. This project involved the exploration of the neural circuits involved in food-anticipatory behavior and the possible role for Ghrelin signaling. His second research project was in the group of Ralph DiLeone PhD at Yale University in New Haven, where he investigated diet-induced gene expression changes in specific brain regions. In 2010 he started his PhD work with Prof. Dr. Roger Adan, the results of which are presented in this thesis. He is currently employed as a postdoctoral scholar at University of California Berkeley in de group of Dr. Stephan Lammel. At Berkeley he investigates plasticity changes in the mesolimbic dopamine system following cocaine exposure.

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