THE PHARMACOLOGY OF VISUOSPATIAL ATTENTION AND INHIBITION

H.N. Alexander Logemann

Printed by GVO drukkers & vormgevers B.V. | Ponsen & Looijen, Ede
ISBN 9789064647048

THE PHARMACOLOGY OF VISUOSPATIAL ATTENTION AND INHIBITION

De farmacologie van visuospatiële aandacht en inhibitie
(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op maandag 28 oktober 2013 des middags te 12.45 uur

door

Herman Nico Alexander Logemann

geboren op 25 september 1979
te Leusden

Promotor: Prof. dr. J.L. Kenemans

Co-Promotor: Dr. K.B.E. Böcker

De projecten die in dit proefschrift zijn beschreven zijn gefinancierd door een open competitie MaGW subsidie van NWO (Dossiernr.: 400-07-130).

Table of Contents

CHAPTER 1

GENERAL INTRODUCTION

Inhibition and visuospatial attention are of importance in everyday functioning. Two components in visuospatial attention are bias and disengagement (Maurizio Corbetta & Shulman, 2002). Bias refers to the result of the directing of attention to a specific location in space in terms of facilitated processing of the stimulus or stimuli at that location. Disengagement refers to the decoupling of attention, which makes it possible to redirect attention. Corbetta & Shulman (2002) have outlined the neuroanatomical correlates of these two systems, which can be categorized as a dorsal and a ventral system. The dorsal system subserves bias and consists broadly of the frontal eye fields (FEF), and the Intra-Parietal Sulcus (IPS). The ventral system subserves disengagement and consists of the right Inferior Frontal Gyrus (IFG), and the right Temporal Parietal Junction (TPJ). Previous studies have implicated the cholinergic and noradrenergic neurotransmitter systems in bias and disengagement (i.e. Witte et al. (1997) and Clark (1989), respectively). The dominant theory posits that bias depends on cholinergic signaling, whereas disengagement depends on noradrenergic signaling (i.e. Corbetta et al. (2002)). However, previous literature pertaining to manipulations of these systems by pharmacological agents, paint a conflicting picture. Careful scrutiny of the literature suggests an alternative model, which states the opposite, namely that bias is subserved by noradrenergic signaling, and disengagement by cholinergic signaling.

Inhibition, as will be discussed in detail, is conceptually linked to disengagement and therefore the focus of the thesis is extended to the domain of inhibitory control. With respect to the neuroanatomical correlates underlying inhibitory functioning, a dorsal frontal and ventral frontal mechanism have been described in the literature (Kenemans & Kähkönen, 2011; Schmajuk, Liotti, Busse, & Woldorff, 2006). As with visuospatial attention, the neurotransmitter systems pertaining to inhibition are yet to be elucidated. This is an important issue, since problems in the domain of attention and inhibitory functioning are central to disorders such as Attention Deficit / Hyperactivity Disorder (ADHD) (Kenemans et al., 2005). As is well known, methylphenidate is the golden standard in the treatment of this psychopathology. Specifically, methylphenidate affects both the dopaminergic as well as the noradrenergic system (Zetterstrom, Sharp, Collin, & Ungerstedt, 1988) and methylphenidate affects both attention and inhibition with respect to ADHD pathology (Kenemans et al., 2005). The problem is that the role of these systems in attention or inhibition is unclear. This is an important issue, since the balance of side-effects and clinical effects is negative for a

substantial group of patients that do not benefit from this treatment (Barkley, 1998; Swanson et al., 1998).

The aim of the current thesis was to determine the role of the cholinergic, noradrenergic, and dopaminergic system neurotransmitter systems in visuospatial attention and in inhibition.

1.1. Assessing visuospatial attention and inhibition: combining specific computer tasks with EEG

In the projects presented in my thesis, two computer tasks were combined with EEG to provide brain activity indices of attention and inhibition. Previous studies have reported electrophysiological indices of bias, disengagement and inhibition and we have replicated these components mainly successfully. One of the advantages in using EEG indices compared to using solely performance measures is the reference to brain mechanisms that ultimately subserve behaviour. Especially for the present research questions, it is important to supplement performance measures with brain-activity measures, because the former mainly reflect the summed output of a combination of brain mechanisms that can be parcellated using the latter. This parcellation is greatly aided by the exquisite temporal resolution of EEG measures, which allows for a straightforward distinction between mechanisms of bias and of disengagement, respectively.

Briefly, the scalp-recorded EEG reflects the electrical component of neural activity, more specifically the graded waxing and waning of post-synaptic potentials throughout the cerebral cortex. While EEG reflects this electrical component with virtually no temporal delay, its spatial resolution is poor, i.e., it is hard to make firm statements about the anatomical location of intracranial generators of the scalp-recorded signal.

In addition to spontaneous activity, EEG may also encompass the response of the brain to specific events, termed Event-Related Potentials (ERPs). Deriving the relatively tiny ERPs from the on-going EEG is possible because ERPs are time-locked to discrete events, such as stimuli. Given a sufficient amount of repeated measures, the method of signal averaging is applied, based on the idea that the spontaneous EEG has no fixed temporal

relationship with the point in time at which the stimulus was presented, while the ERP has a much more constant time course relative to the stimulus (Kenemans & Kähkönen, 2011).

1.1.2. The Visual Spatial Cueing (VSC) task

The VSC task has been frequently used to assess visuospatial attention, or more specifically, bias and disengagement (Posner, Snyder, & Davidson, 1980). Broadly there are two variants that differ with respect cueing, exogenous or endogenous. The latter will be discussed in this thesis. The typical trial of this variant of the VSC task is depicted in figure 1. The trial generally starts with a centrally presented cue signaling the likely location of the subsequent target, which is presented in either the left or right visual hemifield. Usually, upon presentation of the target, a (choice) response is required. Trials can be either valid or invalid. Valid trials are trials in which the target is validly cued. In this case, the target is presented at the location as indicated by the cue. For invalid trials, targets are incongruently cued. In this case the target is presented at a location opposite to the location as indicated by the cue. The relevant behavioural output is the validity effect, which is the benefit in terms of reaction time to the target for valid trials as opposed to invalid trials. A reduction of the validity effect may implicate a reduction of bias and/or enhanced disengagement. An increase of the validity effect may implicate the opposite. Some variants of the VSC task incorporate neutral trials, which makes the decomposition of costs or benefits in terms of reaction time resulting from respectively invalid and valid cueing relative to a neutral condition possible. The ensuing common interpretation is that a modulation of the invalid minus neutral difference specifically indicates a modulation of disengagement. However, we did not implement such condition in the projects outlined in the current thesis. First, the task would become too lengthy and thus too demanding for participants. Second, the use of contrasts including neutral cues does not unequivocally yield unambiguous interpretations. For example, assume that bias involves independent mechanisms of amplification and attenuation. A state manipulation (e.g., a drug) that specifically affects the attenuation part would result in a specific modulation of the difference between invalid and neutral, not of valid minus neutral. Hence, a specific state-induced modulation of the invalid-neutral difference does not necessarily imply modulation of disengagement. In sum, with or without implementation of neutral cues, performance data

leave room for ambiguity. Hence, reference to brain activity measures of bias and disengagement is of crucial importance to tease apart effects on bias from effects on disengagement related processing.

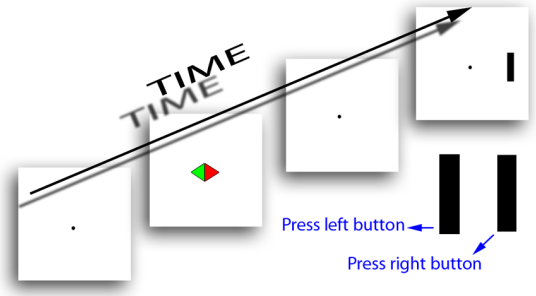

Figure 1. The visual spatial cueing task, a typical trial.

1.1.2.1. Electrophysiological correlates of (the onset of) bias and disengagement

Combining EEG with the VSC task has identified electrophysiological correlates of the onset of bias, the result of bias and of disengagement. Components primarily pertaining to this thesis will be discussed. With respect to the onset of bias, or in other words, the orienting of attention, the parietal cue response has been identified. More specifically, upon presentation of a cue necessitating the directing of attention, the Frontal Eye Fields (FEF) and Superior Parietal Cortex become active (M. Corbetta, Patel, & Shulman, 2008; Maurizio Corbetta & Shulman, 2002; van der Lubbe, Neggers, Verleger, & Kenemans, 2006). This activity is thought to be associated with the electrophysiological parietal cue response consisting of the Early Directing Attention Negativity (EDAN, associated with parietal activity), Anterior Directing Attention Negativity (ADAN, associated with FEF activity), and Late Directing Attention Negativity (LDAP, associated with parietal activity) (van der Lubbe et al., 2006). The result of bias can be assessed by the P1 and N1 modulations by cueing (Hopf & Mangun, 2000; Mangun & Hillyard, 1991). Visual targets in the VSC task result in a positive wave within a 100-136 ms window termed the P1, which is followed by a negative wave within the 140 – 188 ms window. Both ERPs are present at parietal/occipital leads and these target-locked

ERPs are modulated by cueing (valid versus invalid cueing of the target location). More specifically, the amplitudes of both components are enhanced for validly cued targets as opposed to invalidly cued targets. The P1/N1 complex is associated with visual processing and may be thought of as a gating mechanism in which the P1 modulation plausibly reflects suppression of unattended stimuli whereas the N1 modulation reflects enhanced processing of the stimulus within the locus of attention (Meinke, Thiel, & Fink, 2006).

Orienting related activity as reflected in the EDAN, ADAN and LDAP does not simply map onto target-locked ERP effects pertaining to bias; numerous independent variables may affect the latter but not the former or vice versa (P1/N1) (Hopf & Mangun, 2000). One possibility is that besides the EDAN, ADAN, and LDAP, other neuroanatomical correlates are also involved in orienting. In other words, these ERPs reflect part (but not all) of the orienting response. Another possibility is that these cue-elicited brain potentials reflect more than 'simply' biasing of visual cortex, the latter subsequently resulting in P1 modulation. For EDAN, this 'more' has been substantiated in that this component reflects not so much directing attention to a cued location (van Velzen & Eimer, 2003), but rather directing attention to the location of the relevant information (viz., the location of the cue itself). Another possibility is that a certain dependent variable affects only the biasing signals as they arrive in sensory cortex, but not to the initiation of these signals in more controlling areas of the brain.

Disengagement is most plausibly reflected in the Late Positive Deflection (LPD), a component which has been reported previously (Mangun & Hillyard, 1991). More specifically, the amplitude of the LPD within range 228 – 300 ms is larger for invalidly cued targets as opposed to validly cued targets, providing support for the link with disengagement. Meinke et al. (2006) reported a late positive deflection possibly reflecting the LPD as reported in Mangun et al. (1991). Assuming this deflection indeed represents the LPD, this component seems to be modulated specifically by invalidly cued targets providing further support for a link with disengagement related processing. One plausible candidate with respect to the generator of the LPD is the Temporal Parietal Junction that is thought to be important in the reorienting (i.e. disengagement) of attention (M. Corbetta et al., 2008; Maurizio Corbetta & Shulman, 2002). However, LPD-like phenomena have also been reported with a more central (vertex)

topography (Meinke et al., 2006), which might be indicative of a more anteriorly and dorsally based generator, such as in the Superior Frontal Gyrus (SFG, (Kenemans & Kähkönen, 2011).

1.1.3. The Stop Signal Task (SST)

The Stop Signal Task has frequently been used to assess inhibitory motor control (Logan, Cowan, & Davis, 1984). In the typical SST, go stimuli to which a (choice) response is required are followed in a minority of trials by stop stimuli signaling to withhold a response. Broadly, two variants have been reported in the literature, the visual variant (Schmajuk et al., 2006) and the auditory variant (Bekker, Kenemans, Hoeksma, Talsma, & Verbaten, 2005). In the visual variant, both the go stimuli as well as the stop stimuli are presented in the visual domain. In the auditory variant, the stop stimuli are presented in the auditory domain. Performance variables thought to reflect (some aspect of) attention are mean reaction time, response variability as reflected in the standard deviation of reaction time, and the omission rate (or percentage of failed responses to go stimuli) (Berwid et al., 2005; Johnson et al., 2008). The relevant behavioural output pertaining to inhibition for both SST variants is the Stop Signal Reaction Time (SSRT) (Logan et al., 1984). The SSRT cannot be measured directly, but can be estimated under certain assumptions, knowing the proportion of inhibitions (corrected for omissions) and reaction time distribution on go stimuli in go trials. In figure 2, a schematic overview is depicted. It must be noted that the SSRT depends on assumptions that are difficult to verify (such as the independence of go – stop processes) (Band, van der Molen, & Logan, 2003; Overtoom et al., 2002). Hence, reference to brain activity measures of inhibition is important.

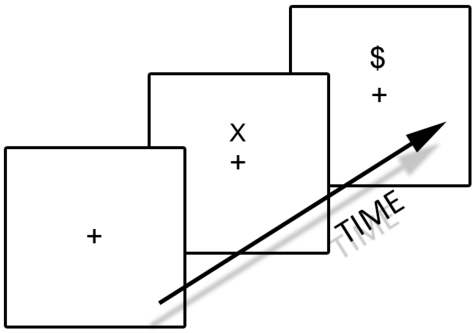

Figure 2. The stop signal task, a typical trial.

1.1.3.1. Electrophysiological correlates of inhibition

Several inhibition related ERPs have been reported in the SST that are specifically modulated (i.e. enhanced for successful stops) by stopping success, and therefore are addressed in this thesis as "stop" ERPs. The specific ERPs differ between stop signal variants. Stop signals in the auditory domain result in a stop N1 (80 – 120 ms), with a topographical distribution consistent with a generator in auditory cortex (Bekker et al., 2005). Stop signals in the visual variant do not typically result in a stop N1, but result in a later stop N2 172 – 192 ms) with a right frontal maximum (chapter 3 and 5). Stop signals of both variants result in a stop P3 with a central maximum, however the stop P3 has a shorter latency for the auditory variant as opposed to the visual variant (Bekker et al., 2005; Schmajuk et al., 2006). It is thought that the stop N1 reflects the potentiated link between sensory cortex and motor cortex that is under control by the right Inferior Frontal Gyrus (Overtoom et al., 2009). The stop N2 on the other hand, is thought to reflect stop-signal elicited activity in the right Inferior Frontal Gyrus (Schmajuk et al., 2006). Consistently, lesions in the right IFG are associated with disrupted inhibition as indexed by SSRT (Aron, Fletcher, Bullmore, Sahakian, & Robbins, 2003). Furthermore, methylphenidate significantly improves the stop N2 in ADHD patients (Pliszka et al., 2007). With respect to the stop P3, it is plausible that this component originates from the superior frontal gyrus (Floden & Stuss, 2006) The stop P3 has also been suggested to reflect error related negativity following a failed stop (Falkenstein, Hohnsbein, Hoormann, & Blanke, 1991; Gehring, Goss, Coles, Meyer, & Donchin, 1993). However, results of Lansbergen et al.

(2007) disprove this notion as stop P3 and ERN are differentially affected by specific independent variables.

As noted there is an obvious conceptual link between inhibition and disengagement. This notion also fits with the possible overlap between the LPD (from the VSC task) and stop P3. In addition, a combined right-anterior stop N2 and right posterior LPD may reflect the combination of right-hemisphere anterior and posterior mechanisms of disengagement.

In any case, it is likely that overlap, in terms of brain mechanisms, is sizeable with regard to mechanisms involved in stopping in the SST, and disengagement in the VSC task. Successful stopping is likely governed by TPJ and/or SFG activation as reflected in the LPD modulation and P3 modulation (SFG), and right inferior frontal gyrus activity as reflected in the N2 modulation.

1.1.4. Wrapping up: The VSC task and SST in combination with EEG as an excellent tool to assess brain activity related to bias, disengagement and inhibition

Using the VSC task and SST in combination with EEG makes it possible to assess brain activity indices specifically related to bias, disengagement and inhibition. Furthermore, it is possible to assess temporal effects since EEG has a very high temporal resolution. Combining the tasks with EEG, it is possible to measure brain activity related to the onset of bias (EDAN, ADAN, LDAP), the result of bias (P1/N1 effects) and the process of disengaging/inhibiting (LPD effect), along with activity within ventral (Stop N2) and dorsal (Stop P3) inhibition mechanisms.

1.2. The pharmacology of bias, disengagement and inhibition

The dominant theory, which arises from previous literature (i.e. Corbetta & Shulman (2002)), posits that bias and disengagement are subserved by cholinergic and noradrenergic neurotransmission, respectively. However, as will be discussed in the next section, an alternative model that posits the opposite may fit better with results in the previous literature. In addition, dopaminergic and noradrenergic mechanisms have been implicated in inhibition, and dopaminergic mechanisms have also been implicated in selective attention.

1.2.1. Noradrenaline

Some authors implicate a role of noradrenaline in bias (Coull, Nobre, & Frith, 2001), whereas other research groups suggest noradrenaline is involved in disengagement (Clark et al., 1989). With respect to performance, noradrenergic antagonism results in a decrease of the validity effect on reaction time in the VSC task (Clark et al., 1989; Coull et al., 2001). Such decrease may imply a reduction of bias, facilitated disengagement or a combination of both. Hence, reference to brain activity measures of bias and disengagement is necessary. One isolated fMRI study provided such reference and investigated the effect of noradrenergic antagonism by clonidine in the VSC task(Coull et al., 2001). Results showed a reduction of the validity effect on reaction time with respect to performance. Most importantly, it was shown that clonidine effectively reduced the parietal response (orienting response) to the cue. These results do support a bias account with respect to the noradrenergic system.

The exact balance between bias on the one hand and disengagement on the other may depend on the specific functioning of the Locus - Coeruleus - Norepinephrine (C-NE) system. Adaptive Gain Theory in essence posits that "exploitation" (conceptually similar to "bias") and "exploration" (conceptually similar to "disengagement") depend on the balance between tonic (baseline) activity and phasic responses of the LC-NE system (Aston-Jones & Cohen, 2005). For instance, a reduction of tonic LC - NE activity would result in a reduction of disengagement and facilitation of bias, in turn resulting from enhanced phasic activity in response to afferent inputs; and a reduction of phasic LC - NE activity would result in a reduction of bias.

Noradrenergic facilitation by atomoxetine in the SST decreases SSRT (reflecting speeded inhibition), and has been shown to enhance right IFG activity (Chamberlain et al., 2007; Chamberlain et al., 2009; Chamberlain et al., 2006). This suggests that noradrenergic attenuation has a detrimental effect on disengagement and inhibition. One complicating issue however is that atomoxetine does not solely affect noradrenaline, but also blocks the reuptake of dopamine (Bymaster et al., 2002; Pliszka, 2005), which is also implicated in inhibition as discussed in section 1.2.3. Furthermore, Graf et al. (2011) showed that a higher dose (80mg)of atomoxetine resulted in an increase in errors of commission in a flanker go/no-go task.

Although a slightly different paradigm was used, results seem to indicate that a higher dose may actually have a detrimental effect on inhibition. It is plausible that the dose-response follows an inverted u curve. It may be that a dosage of 60mg atomoxetine moves noradrenergic activity towards a more optimal level, whereas an 80mg dosage moves noradrenergic activity towards a suboptimal level.

1.2.2. Acetylcholine

Many studies have been performed using nicotine to augment cholinergic neurotransmission. Indeed, cholinergic enhancement using nicotine has been shown to enhance cognitive functioning (Heishman, Kleykamp, & Singleton, 2010; Newhouse, Potter, & Singh, 2004; Rezvani & Levin, 2001). Interestingly, it has been proposed that many ADHD patients smoke as a means to self-medicate, and thereby reduce ADHD symptomatology (Kollins, McClernon, & Fuemmeler, 2005; Potter, Newhouse, & Bucci, 2006). With respect to visuospatial attention, nicotine has the same effect as clonidine on the validity effect on reaction time in the VSC task (Meinke et al., 2006; Thiel & Fink, 2008; Vossel, Thiel, & Fink, 2008). Both pharmacological agents cause a reduction of the validity effect on reaction time. It is interesting to note that a stimulant like nicotine and sedators like clonidine can apparently have the same effect on behaviour. Not surprisingly, the reduction of the validity effect under nicotine (like clonidine) has been interpreted as reflecting attenuated bias (Meinke et al., 2006), or as reflecting enhanced disengagement (Witte et al., 1997). It must be noted though, that the effect of nicotine on the validity effect seems to be primarily a result of a reaction time reduction in invalid trials, suggesting an enhancing effect on disengagement (Meinke et al., 2006; Thiel & Fink, 2008; Witte et al., 1997).

Results of cholinergic manipulation by nicotine in the SST seem to mirror those in the VSC task. More specifically, at least for ADHD patients, nicotine reduces SSRTs in the SST suggesting an enhancing effect on inhibition (Potter, Bucci, & Newhouse, 2012; Potter & Newhouse, 2004, 2008). It must be noted here that the effect of cholinergic augmentation by nicotine on SSRT may possibly be restricted to samples with enough room for improvement, as these effects were reported in ADHD patients, but not in healthy controls (Potter et al., 2012). Importantly, attenuating cholinergic neurotransmission by mecamylamine does affect

SSRT in healthy controls (Potter et al., 2012). As can be expected, mecamylamine results in an increase in SSRTs (Potter 2012).

Some pharmacological studies have combined behavioural measurements with brain activity measures of visuospatial attention and inhibition. With respect to the visuospatial cueing task, nicotine has been shown to decrease activity in the inferior parietal cortex on invalid trials (Thiel & Fink, 2008; Thiel, Zilles, & Fink, 2005; Vossel et al., 2008). This has been interpreted by Yu et al. (2005) as reflecting reduced reliance on the cue, and in this way fits an explanation in terms of a reduction of bias following nicotine. Such reduced bias should be paralleled by reduced attentional modulation in occipital cortex. However, results of fMRI studies fail to show such nicotine induced reduction of modulation of activity in the occipital cortex (Thiel & Fink, 2008; Vossel et al., 2008), discounting an explanation of the performance effects in the VSC task in terms of a reduction of bias. Hence, it may be possible that the reduction of the validity effect may be explained in terms of enhanced disengagement. There is some support for this notion. Firstly, very recently it has been suggested that smokers present with anomalous inhibitory control (de Ruiter, Oosterlaan, Veltman, van den Brink, & Goudriaan, 2012; Luijten et al., 2012; Nestor, McCabe, Jones, Clancy, & Garavan, 2011), suggesting a role of cholinergic neurotransmission as smokers repeatedly self-administer nicotine. Secondly, as mentioned, for healthy controls, nicotine does not seem to affect bias, however, one isolated EEG study does suggest an effect on disengagement. Specifically, Meinke et al. (2006) failed to show a nicotine-induced modulation of the P1 and N1 effect (effect = contrast target-locked ERP for valid trials minus invalid trials). However, although analyzed in a post-hoc manner and described without too much detail, nicotine seemed to affect a frontocentral positive-deflection of later latency, specifically for invalid trials, which could fit the LPD description of Mangun (1991). In other words, it seems plausible that the performance effects in the VSC task may be described in terms of a facilitation of disengagement, and not in terms of a reduction of bias.

1.2.3. Dopamine

Methylphenidate has been shown to reduce problems of attention and inhibition which are central to ADHD(Kenemans et al., 2005). As mentioned, methylphenidate also positively affects the right-anterior stop N2 in ADHD patients, consistent with an effect on inferior frontal gyrus activity (Liotti et al., 2007). This is reminiscent of the effect of atomoxetine as outlined in section 1.2.1. However, like atomoxetine, methylphenidate affects both dopaminergic and noradrenergic neurotransmission (Zetterstrom et al., 1988), and the contribution or either system in visuospatial attention and inhibition is still unknown.. Clark et al. (1989), showed that attenuation of dopaminergic neurotransmission by droperidol (like attenuation of noradrenergic neurotransmission) results in a reduction of the validity effect which was interpreted again as resulting from enhanced disengagement. Indeed, Lijffijt et al. (2006), shows a correlation between SSRT and dopamine metabolites, providing some support for a link between dopaminergic neurotransmission and inhibition/disengagement. However, a recent fMRI study showed that the attenuation of dopaminergic neurotransmission by haloperidol reduces prefrontal brain activity related to inhibition in a go/no go task (Luijten et al., 2012). It must be noted though, that an increase in Temporal Parietal Junction activity was reported under haloperidol, possibly the result of a compensatory mechanism. If disengagement is negatively affected by attenuating dopaminergic neurotransmission, one would expect the validity effect on reaction time in the VSC task to increase, which is opposite to what has been reported (Clark et al., 1989). One possibility is that dopaminergic attenuation results in both a decrease of disengagement and a decrease of bias, but that the decrease of bias is more substantially reflected in performance.

1.3. Manipulating the systems, the current thesis

1.3.1. Noradrenergic challenge by clonidine

Clonidine is a partial α2-receptor agonist, and reduces Locus- Coeruleus - Norepinephrine (LC-NE) signaling (Svensson, Bunney, & Aghajanian, 1975). According to the Summary of Product Characteristics (Centrafarm Services B.V., Etten-Leur, The Netherlands), peak blood

plasma levels of clonidine are reached between 1 to 3 hours post-ingestion. Clonidine has a relatively long half-life of approximately 9 hours (SPC).

A questionnaire (Profile Of Mood States; POMS) was implemented to assess the subjective effects of clonidine, as clonidine has sedative properties (Kennedy, Gnam, Ralevski, & Brown, 1995). Furthermore, we assessed cardiovascular effects of clonidine, since clonidine has well known hypotensive effects (Turetsky & Fein, 2002). Cardiovascular assessment was part of the medical screening prior to inclusion of subjects. We excluded participants with relatively low blood pressure and a slow or fast heart rate. Furthermore, as responses to clonidine may vary between subjects, measuring cardiovascular responses to clonidine enabled us to use blood pressure as a proxy for individual differences in responsivity.

1.3.2. Cholinergic challenge by mecamylamine / nicotine

Mecamylamine is a nicotinic acetylcholine receptor (nAChR) antagonist (Varanda et al., 1985) and, according to the SPC (Targacept, Inc.), is relatively long-acting (6 to 12 hours or even longer). Another possibility is to facilitate cholinergic neurotransmission using nicotine. Two considerations made mecamylamine our first choice. Firstly, mecamylamine is long acting, which was important since our tasks were relatively lengthy. In other words, the pharmacological effect is likely maintained to the end of the experiment. The other reason is that in healthy subjects, the effect of attenuating cholinergic neurotransmission (using mecamylamine) may be more pronounced as opposed to enhancing cholinergic neurotransmission (using nicotine). This can be explained in terms of room for a drug effect. Support for this rationale comes from Potter et al. (2012). It was shown that nicotine (which facilitates cholinergic neurotransmission) does not affect inhibitory functioning (as indicated by behavioural output) in healthy participants (a plausibly ceiling effect). However, in ADHD patients who show detrimental inhibitory functioning, nicotine seems to improve inhibition. The opposite holds with respect to mecamylamine. Mecamylamine has a detrimental effect on inhibitory performance in healthy participants (Potter et al., 2012), but in the group of ADHD patients already showing poor inhibitory motor control, mecamylamine did not affect inhibition as indicated by performance measures (a possible floor effect).

However, in 2009 the production and distribution of Inversine (brand name of mecamylamine) was discontinued, and nicotine became inevitable as the means to manipulate the cholinergic system (given that previous studies exclusively addressed the nicotine and not the muscarine ACh receptor). Nicotine was administered via prolacrilex chewing gum (Nicorette Freshmint 2 mg). Maximum levels of nicotine in blood plasma are reached after chewing for approximately 30 minutes (according to the SPC). Vossel et al. (2008) report that chewing at a rate of one chew per three seconds for 25 minutes amounts to average nicotine blood levels of 3.57 ng/ml. Two issues must be noted here. Firstly, nicotine has a relatively short half-life of approximately two hours (SPC), meaning that a substantial amount of the drug is metabolized during the tasks. Secondly, as mentioned previously, there may be a ceiling effect with respect to the drug effect in healthy participants (at least with respect to performance variables). With respect to the first issue a higher dosage may be considered. However, one study suggested marked side effects in healthy participants following the administration of 4mg nicotine (G. Nyberg, Panfilov, Sivertsson, & Wilhelmsen, 1982), and it has been suggested that central effects are not significantly enhanced at this dosage relative to a lower dose (Thiel & Fink, 2007). As nicotine asserts clear cardiovascular effects (Fisher, Daniels, Jaworska, Knobelsdorf, & Knott, 2012; Wignall & de Wit, 2011), our solution was to use blood pressure as a proxy for individual responsivity to the drug (in a similar vein as in the clonidine study), under the assumption that a stronger peripheral effect corresponds to a stronger central effect. This information could then be used (if no effect is evident in the complete sample), to restrict analyses to a subsample in which the drug effect is most pronounced as indicated by the drug effect on peripheral variables. Pertaining to the latter, secondary analyses were performed on half of the sample in which the room for a possible drug effect on relevant performance variables was largest.

1.3.3. Manipulating the dopaminergic system: Haloperidol

Dopaminergic neurotransmission was weakened using 2mg haloperidol. Haloperidol is an antagonist with strong affinity for the D2 dopamine receptor (Kapur, Zipursky, Jones, Remington, & Houle, 2000). Two plasma peak levels of haloperidol have been identified (SPC haloperidol). The first occurs between 3 to 6 hours post-administration, the second occurs

between 12 to 20 hours post-ingestion. Haloperidol has a half-life of approximately 19 hours (SPC). Based on the scarce literature available, an initial dosage of 2 mg was chosen. The preliminary estimate was that 2 mg haloperidol would render a sample size of 19 participants sufficient in order to detect an effect (with 80 percent statistical power and alpha set at 0.05). However, since this was a rough estimate based on variables not precisely comparable to the variables in our study we implemented an interim analysis. The goal of the interim analysis was to assess the effect size of 2 mg haloperidol on the most relevant variables. If the effect size would imply a sample size exceeding 30 subjects, a higher dose of 3 mg would be considered. As described in chapter 7, 2mg haloperidol induced cardiovascular effects are negligible, especially compared to the clonidine study described in chapters 2 and 3.

One of the side effects of haloperidol pertains to motor activity (extrapyramidal side-effects, as described in SPC). Receptor occupancy at a fixed dose has been reported to vary markedly across participants (de Haan et al., 2003). Furthermore, at receptor occupancy exceeding 78 - 80 %, extrapyramidal effects may occur (Kapur et al., 2000; S. Nyberg, Nordstrom, Halldin, & Farde, 1995). In the haloperidol dose-finding pilot as described in chapter 7, we assessed the effect of 2 mg haloperidol on spontaneous motor activity and velocity scaling. The latter refers to the general tendency of normal healthy individuals to scale speed of movement depending on the distance of an object/target (Caligiuri, Lohr, & Ruck, 1998). Haloperidol induced dyskinesia was assessed by measuring spontaneous motor activity (using an Actigraph), and haloperidol induced bradykinesia is thought to be reflected in reduced velocity scaling (Caligiuri et al., 1998). Importantly, the drug-induced effect on these movement-parameters may be used as a proxy for individual responsivity in a similar vein as cardiovascular variables were used as a proxy for responsivity.

1.4. Elucidating the roles of noradrenaline, acetylcholine and dopamine in visuospatial attention and inhibition: outline of the current thesis

Summing up, the hypotheses to be tested are listed in Table 1.1

Table 1.1. Overview of hypotheses.

Manipulation	**Task**	**Output: behaviour**	**Output: EEG**
Clonidine	SST	SSRT ↑	Stop N2 ↓, Stop P3 ↓
	VSC	Validity effect on RT ↓	EDAN ↓, LDAP ↓; P1/N1 effect↓
Nicotine	SST	SSRT ↓	Stop N2 ↑, Stop P3 ↑
	VSC	Validity effect on RT ↓	LPD effect ↑
Baseline differences: nicotine abstinent smokers - non smokers	SST	SSRT ↑, more in smokers	
	VSC	Validity effect on RT ↑, more in smokers	
Nicotine effect in nicotine abstinent smokers - non smokers	SST	SSRT ↓ by nicotine, more in smokers	
	VSC	Validity effect on RT ↓ by nicotine, more in smokers	
Haloperidol	SST	SSRT ↑	
	VSC	Validity effect on RT ↓	

As described, the roles of noradrenaline, acetylcholine and dopamine remain to be elucidated with respect to visuospatial attention and inhibition. The aim of the current thesis was to specifically investigate the specific roles of these neurotransmitters with respect to bias, disengagement and inhibition. The VSC task and SST task combined are excellent tools to assess the net behavioural output of these mechanisms. However, to disentangle the relative contributions of these mechanisms, specific reference to brain activity measures of bias, disengagement and inhibition is warranted. As described, EEG provides an excellent tool in combination with the mentioned tasks to assess these mechanisms and tease out relative effects. Moreover, EEG has a high temporal resolution making it possible to separately assess the effects at different latencies. For example, as described, it is possible to investigate manipulations on the creation of bias (the orienting response), and the result of bias. For the current thesis the same combination of cognitive tasks was used for all pharmacological manipulations. Firstly, a modified version of the visual spatial cueing task of Van den Lubbe et al. (2006) was used. Although the orienting response was clearly reported and described in Van den Lubbe et al. (2006), there was no data reported on a target-locked P1/N1, or LPD response (as this was not the aim of that study). In a preliminary study, we found that the original targets as in Van den Lubbe et al. (2006) did not yield a reliable response in the visual cortex. The P1 or N1 (nor modulation) was not clearly evident. Therefore, we implemented

the targets as used in Mangun et al. (1991), as clear modulations of the P1 and N1 (and LPD) were evident.

With respect to the SST task, we chose to implement the visual variant similar to the one described in Schmajuk et al. (2006), since presumed activity of the right Inferior Frontal Gyrus, as reflected in the N2 modulation by stopping, can readily be detected. Besides the above-mentioned combination of computer tasks, we assessed cardiovascular functioning and subjective experience (using the Profile of Mood States Questionnaire) for all pharmacological studies.

In chapter 2 and chapter 3 we report, respectively, the effect of noradrenergic attenuation by 100 microgram clonidine specifically on visuospatial attention in the VSC task and on inhibition in the SST. Initially a dosage of 200 microgram was planned as this has been reported to induce acceptable levels of sedation (Kennedy et al., 1995). However, two initial participants experienced unexpectedly marked side effects, eventually leading to the implementation of a lower dosage. Cardiovascular variables were used as a proxy for individual central responsivity.

Chapters 4, 5, and 6 report on the nicotine study. Chapters 4 and 5 report the effects of nicotine on visuospatial attention in the VSC task and on inhibition in the SST, in healthy non-smokers. Chapter 6 reports on baseline differences between smokers and nonsmokers and differences between smokers and non-smokers with respect to the acute effect of nicotine on visuospatial attention and inhibition. In chapter 6, we did not include EEG variables. In these studies we also used cardiovascular variables as a proxy for individual responsivity since nicotine asserts significant effects on these variables. In chapter 7 we investigated the effect of dopaminergic antagonism by 2mg haloperidol on behavioural indices of bias, disengagement and inhibition. This report is part of a still ongoing study that also includes EEG parameters. For this study we also included measures of spontaneous motor activity (using an actigraph) and bradykinesia (using a device measuring velocity scaling). The effect of haloperidol on motor activity is one indication of receptor occupancy of haloperidol in the brain, and in this way may be used as a proxy for individual sensitivity to the drug (Kapur et al., 2000; S. Nyberg et al., 1995)

REFERENCES

Aron, A. R., Fletcher, P. C., Bullmore, E. T., Sahakian, B. J., & Robbins, T. W. (2003). Stop-signal inhibition disrupted by damage to right inferior frontal gyrus in humans. *Nat Neurosci, 6*(2), 115-116.

Aston-Jones, G., & Cohen, J. D. (2005). An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. *Annu Rev Neurosci, 28*, 403-450.

Band, G. P., van der Molen, M. W., & Logan, G. D. (2003). Horse-race model simulations of the stop-signal procedure. *Acta Psychol (Amst), 112*(2), 105-142.

Barkley, R. A. (1998). *Attention deficit hyperactivity disorder: A handbook for diagnosis and treatment* (2 ed.). New York: Guildford.

Bekker, E. M., Kenemans, J. L., Hoeksma, M. R., Talsma, D., & Verbaten, M. N. (2005). The pure electrophysiology of stopping. *Int J Psychophysiol, 55*(2), 191-198.

Berwid, O. G., Curko Kera, E. A., Marks, D. J., Santra, A., Bender, H. A., & Halperin, J. M. (2005). Sustained attention and response inhibition in young children at risk for Attention Deficit/Hyperactivity Disorder. *J Child Psychol Psychiatry, 46*(11), 1219-1229.

Bymaster, F. P., Katner, J. S., Nelson, D. L., Hemrick-Luecke, S. K., Threlkeld, P. G., Heiligenstein, J. H., et al. (2002). Atomoxetine increases extracellular levels of norepinephrine and dopamine in prefrontal cortex of rat: a potential mechanism for efficacy in attention deficit/hyperactivity disorder. *Neuropsychopharmacology, 27*(5), 699-711.

Caligiuri, M. P., Lohr, J. B., & Ruck, R. K. (1998). Scaling of movement velocity: a measure of neuromotor retardation in individuals with psychopathology. *Psychophysiology, 35*(4), 431-437.

Chamberlain, S. R., Del Campo, N., Dowson, J., Muller, U., Clark, L., Robbins, T. W., et al. (2007). Atomoxetine improved response inhibition in adults with attention deficit/hyperactivity disorder. *Biol Psychiatry, 62*(9), 977-984.

Chamberlain, S. R., Hampshire, A., Muller, U., Rubia, K., Del Campo, N., Craig, K., et al. (2009). Atomoxetine modulates right inferior frontal activation during inhibitory control: a pharmacological functional magnetic resonance imaging study. *Biol Psychiatry, 65*(7), 550-555.

Chamberlain, S. R., Muller, U., Blackwell, A. D., Clark, L., Robbins, T. W., & Sahakian, B. J. (2006). Neurochemical modulation of response inhibition and probabilistic learning in humans. *Science, 311*(5762), 861-863.

Clark, C. R., Geffen, G. M., & Geffen, L. B. (1989). Catecholamines and the covert orientation of attention in humans. *Neuropsychologia, 27*(2), 131-139.

Corbetta, M., Patel, G., & Shulman, G. L. (2008). The reorienting system of the human brain: from environment to theory of mind. *Neuron, 58*(3), 306-324.

Corbetta, M., & Shulman, G. L. (2002). Control of goal-directed and stimulus-driven attention in the brain. *Nature Reviews, 3*, 201 - 215.

Coull, J. T., Nobre, A. C., & Frith, C. D. (2001). The noradrenergic alpha2 agonist clonidine modulates behavioural and neuroanatomical correlates of human attentional orienting and alerting. *Cereb Cortex, 11*(1), 73-84.

de Haan, L., van Bruggen, M., Lavalaye, J., Booij, J., Dingemans, P. M., & Linszen, D. (2003). Subjective experience and D2 receptor occupancy in patients with recent-onset schizophrenia treated with low-dose olanzapine or haloperidol: a randomized, double-blind study. *Am J Psychiatry, 160*(2), 303-309.

de Ruiter, M. B., Oosterlaan, J., Veltman, D. J., van den Brink, W., & Goudriaan, A. E. (2012). Similar hyporesponsiveness of the dorsomedial prefrontal cortex in problem gamblers and heavy smokers during an inhibitory control task. *Drug Alcohol Depend, 121*(1-2), 81-89.

Falkenstein, M., Hohnsbein, J., Hoormann, J., & Blanke, L. (1991). Effects of crossmodal divided attention on late ERP components. II. Error processing in choice reaction tasks. *Electroencephalogr Clin Neurophysiol, 78*(6), 447-455.

Fisher, D. J., Daniels, R., Jaworska, N., Knobelsdorf, A., & Knott, V. J. (2012). Effects of acute nicotine administration on behavioral and neural (EEG) correlates of working memory in non-smokers. *Brain Res, 1429*, 72-81.

Floden, D., & Stuss, D. T. (2006). Inhibitory control is slowed in patients with right superior medial frontal damage. *J Cogn Neurosci, 18*(11), 1843-1849.

Gehring, W. J., Goss, B., Coles, M. G. H., Meyer, D. E., & Donchin, E. (1993). A Neural System for Error Detection and Compensation. *Psychological Science, 4*(6), 385-390.

Graf, H., Abler, B., Freudenmann, R., Beschoner, P., Schaeffeler, E., Spitzer, M., et al. (2011). Neural correlates of error monitoring modulated by atomoxetine in healthy volunteers. *Biol Psychiatry, 69*(9), 890-897.

Heishman, S. J., Kleykamp, B. A., & Singleton, E. G. (2010). Meta-analysis of the acute effects of nicotine and smoking on human performance. *Psychopharmacology (Berl), 210*(4), 453-469.

Hopf, J. M., & Mangun, G. R. (2000). Shifting visual attention in space: an electrophysiological analysis using high spatial resolution mapping. *Clin Neurophysiol, 111*(7), 1241-1257.

Johnson, K. A., Barry, E., Bellgrove, M. A., Cox, M., Kelly, S. P., Daibhis, A., et al. (2008). Dissociation in response to methylphenidate on response variability in a group of medication naive children with ADHD. *Neuropsychologia, 46*(5), 1532-1541.

Kapur, S., Zipursky, R., Jones, C., Remington, G., & Houle, S. (2000). Relationship between dopamine D(2) occupancy, clinical response, and side effects: a double-blind PET study of first-episode schizophrenia. *Am J Psychiatry, 157*(4), 514-520.

Kenemans, J. L., Bekker, E. M., Lijffijt, M., Overtoom, C. C., Jonkman, L. M., & Verbaten, M. N. (2005). Attention deficit and impulsivity: selecting, shifting, and stopping. *Int J Psychophysiol, 58*(1), 59-70.

Kenemans, J. L., & Kähkönen, S. (2011). How human electrophysiology informs psychopharmacology: from bottom-up driven processing to top-down control. *Neuropsychopharmacology, 36*(1), 26-51.

Kennedy, S. H., Gnam, W., Ralevski, E., & Brown, G. M. (1995). Melatonin responses to clonidine and yohimbine challenges. *J Psychiatry Neurosci, 20*(4), 297-304.

Kollins, S. H., McClernon, F. J., & Fuemmeler, B. F. (2005). Association between smoking and attention-deficit/hyperactivity disorder symptoms in a population-based sample of young adults. *Arch Gen Psychiatry, 62*(10), 1142-1147.

Lansbergen, M. M., Bocker, K. B., Bekker, E. M., & Kenemans, J. L. (2007). Neural correlates of stopping and self-reported impulsivity. *Clin Neurophysiol, 118*(9), 2089-2103.

Lijffijt, M., Kenemans, J. L., ter Wal, A., Quik, E. H., Kemner, C., Westenberg, H., et al. (2006). Dose-related effect of methylphenidate on stopping and changing in children with attention-deficit/hyperactivity disorder. *Eur Psychiatry, 21*(8), 544-547.

Liotti, M., Pliszka, S. R., Perez, R., 3rd, Luus, B., Glahn, D., & Semrud-Clikeman, M. (2007). Electrophysiological correlates of response inhibition in children and adolescents with ADHD: influence of gender, age, and previous treatment history. *Psychophysiology, 44*(6), 936-948.

Logan, G. D., Cowan, W. B., & Davis, K. A. (1984). On the ability to inhibit simple and choice reaction time responses: a model and a method. *J Exp Psychol Hum Percept Perform, 10*(2), 276-291.

Luijten, M., Veltman, D. J., Hester, R., Smits, M., Nijs, I. M., Pepplinkhuizen, L., et al. (2012). The role of dopamine in inhibitory control in smokers and non-smokers: A pharmacological fMRI study. *Eur Neuropsychopharmacol.*

Mangun, G. R., & Hillyard, S. A. (1991). Modulations of sensory-evoked brain potentials indicate changes in perceptual processing during visual-spatial priming. *J Exp Psychol Hum Percept Perform, 17*(4), 1057-1074.

Meinke, A., Thiel, C. M., & Fink, G. R. (2006). Effects of nicotine on visuo-spatial selective attention as indexed by event-related potentials. *Neuroscience, 141*(1), 201-212.

Nestor, L., McCabe, E., Jones, J., Clancy, L., & Garavan, H. (2011). Differences in "bottom-up" and "top-down" neural activity in current and former cigarette smokers: Evidence for neural substrates which may promote nicotine abstinence through increased cognitive control. *Neuroimage, 56*(4), 2258-2275.

Newhouse, P. A., Potter, A., & Singh, A. (2004). Effects of nicotinic stimulation on cognitive performance. *Curr Opin Pharmacol, 4*(1), 36-46.

Nyberg, G., Panfilov, V., Sivertsson, R., & Wilhelmsen, L. (1982). Cardiovascular effect of nicotine chewing gum in healthy non-smokers. *Eur J Clin Pharmacol, 23*(4), 303-307.

Nyberg, S., Nordstrom, A. L., Halldin, C., & Farde, L. (1995). Positron emission tomography studies on D2 dopamine receptor occupancy and plasma antipsychotic drug levels in man. *Int Clin Psychopharmacol, 10 Suppl 3*, 81-85.

Overtoom, C. C., Bekker, E. M., van der Molen, M. W., Verbaten, M. N., Kooij, J. J., Buitelaar, J. K., et al. (2009). Methylphenidate restores link between stop-signal sensory impact and successful stopping in adults with attention-deficit/hyperactivity disorder. *Biol Psychiatry, 65*(7), 614-619.

Overtoom, C. C., Kenemans, J. L., Verbaten, M. N., Kemner, C., van der Molen, M. W., van Engeland, H., et al. (2002). Inhibition in children with attention-deficit/hyperactivity disorder: a psychophysiological study of the stop task. *Biol Psychiatry, 51*(8), 668-676.

Pliszka, S. R. (2005). The neuropsychopharmacology of attention-deficit/hyperactivity disorder. *Biol Psychiatry, 57*(11), 1385-1390.

Pliszka, S. R., Liotti, M., Bailey, B. Y., Perez, R., 3rd, Glahn, D., & Semrud-Clikeman, M. (2007). Electrophysiological effects of stimulant treatment on inhibitory control in children with attention-deficit/hyperactivity disorder. *J Child Adolesc Psychopharmacol, 17*(3), 356-366.

Posner, M. I., Snyder, C. R. R., & Davidson, B. J. (1980). Attention and the detection of signals. *Journal of Experimental Psychology: General, 109*(2), 160 - 174.

Potter, A. S., Bucci, D. J., & Newhouse, P. A. (2012). Manipulation of nicotinic acetylcholine receptors differentially affects behavioral inhibition in human subjects with and without disordered baseline impulsivity. *Psychopharmacology (Berl), 220*(2), 331-340.

Potter, A. S., & Newhouse, P. A. (2004). Effects of acute nicotine administration on behavioral inhibition in adolescents with attention-deficit/hyperactivity disorder. *Psychopharmacology (Berl), 176*(2), 182-194.

Potter, A. S., & Newhouse, P. A. (2008). Acute nicotine improves cognitive deficits in young adults with attention-deficit/hyperactivity disorder. *Pharmacol Biochem Behav, 88*(4), 407-417.

Potter, A. S., Newhouse, P. A., & Bucci, D. J. (2006). Central nicotinic cholinergic systems: a role in the cognitive dysfunction in attention-deficit/hyperactivity disorder? *Behav Brain Res, 175*(2), 201-211.

Rezvani, A. H., & Levin, E. D. (2001). Cognitive effects of nicotine. *Biol Psychiatry, 49*(3), 258-267.

Schmajuk, M., Liotti, M., Busse, L., & Woldorff, M. G. (2006). Electrophysiological activity underlying inhibitory control processes in normal adults. *Neuropsychologia, 44*(3), 384-395.

Svensson, T. H., Bunney, B. S., & Aghajanian, G. K. (1975). Inhibition of both noradrenergic and serotonergic neurons in brain by the alpha-adrenergic agonist clonidine. *Brain Res, 92*(2), 291-306.

Swanson, J. M., Sergeant, J. A., Taylor, E., Sonuga-Barke, E. J. S., Jensen, P. S., & Cantwell, D. P. (1998). Attention-deficit hyperactivity disorder and hyperkinetic disorder. *The Lancet, 351*(9100), 429-433.

Thiel, C. M., & Fink, G. R. (2007). Visual and auditory alertness: modality-specific and supramodal neural mechanisms and their modulation by nicotine. *J Neurophysiol, 97*(4), 2758-2768.

Thiel, C. M., & Fink, G. R. (2008). Effects of the cholinergic agonist nicotine on reorienting of visual spatial attention and top-down attentional control. *Neuroscience, 152*(2), 381-390.

Thiel, C. M., Zilles, K., & Fink, G. R. (2005). Nicotine modulates reorienting of visuospatial attention and neural activity in human parietal cortex. *Neuropsychopharmacology, 30*(4), 810-820.

Turetsky, B. I., & Fein, G. (2002). Alpha2-noradrenergic effects on ERP and behavioral indices of auditory information processing. *Psychophysiology, 39*(2), 147-157.

van der Lubbe, R. H. J., Neggers, S. F. W., Verleger, R., & Kenemans, J. L. (2006). Spatiotemporal overlap between brain activation related to saccade preparation and attentional orienting. *Brain Research, 1072*(1), 133-152.

van Velzen, J., & Eimer, M. (2003). Early posterior ERP components do not reflect the control of attentional shifts toward expected peripheral events. *Psychophysiology, 40*(5), 827-831.

Varanda, W. A., Aracava, Y., Sherby, S. M., VanMeter, W. G., Eldefrawi, M. E., & Albuquerque, E. X. (1985). The acetylcholine receptor of the neuromuscular junction recognizes mecamylamine as a noncompetitive antagonist. *Mol Pharmacol, 28*(2), 128-137.

Vossel, S., Thiel, C. M., & Fink, G. R. (2008). Behavioral and neural effects of nicotine on visuospatial attentional reorienting in non-smoking subjects. *Neuropsychopharmacology, 33*(4), 731-738.

Wignall, N. D., & de Wit, H. (2011). Effects of nicotine on attention and inhibitory control in healthy nonsmokers. *Exp Clin Psychopharmacol, 19*(3), 183-191.

Witte, E. A., Davidson, M. C., & Marrocco, R. T. (1997). Effects of altering brain cholinergic activity on covert orienting of attention: comparison of monkey and human performance. *Psychopharmacology (Berl), 132*(4), 324-334.

Yu, A. J., & Dayan, P. (2005). Uncertainty, neuromodulation, and attention. *Neuron, 46*(4), 681-692.

Zetterstrom, T., Sharp, T., Collin, A. K., & Ungerstedt, U. (1988). In vivo measurement of extracellular dopamine and DOPAC in rat striatum after various dopamine-releasing drugs; implications for the origin of extracellular DOPAC. *Eur J Pharmacol, 148*(3), 327-334.

CHAPTER 2

THE EFFECT OF ATTENUATING NORADRENERGIC NEUROTRANSMISSION BY CLONIDINE ON BRAIN ACTIVITY MEASURES OF VISUOSPATIAL ATTENTION

Logemann, H.N.A., Böcker, K.B.E., Deschamps, P.K.H., Kemner, C., Kenemans, J.L.

Submitted

ABSTRACT

In the current study we investigated the role of noradrenaline in directing (bias) and disengagement of visuospatial attention. We assessed the effect of clonidine on event-related brain potential (ERP) reflections of bias and disengagement in a double-blind placebo-controlled crossover design. An initial dose of 200 microgram clonidine was replaced by 100 microgram because of marked side effects. Twenty-one healthy male participants performed the Visual Spatial Cueing task while EEG was recorded. The behavioural output is the validity effect (benefit of cueing in terms of reaction time to targets). ERP indices for bias were the cue-related Early Directing Attention Negativity and Late Directing Attention Positivity, and the target-elicited P1 and N1 modulation by validity ('validity effect'). The ERP index for disengagement was the target-elicited 'late positive deflection' modulation by validity. Behavioural analyses were performed on 16 participants, electrophysiological analyses on a subset (n=9). Clonidine attenuated the N1 effect; however neither cue-elicited ERPs nor the behavioral validity effect were affected. Clonidine induced blood-pressure reduction was correlated with the reduction of the LPD effect under clonidine. In conclusion, clonidine attenuated the result of bias and may have a modulating effect on disengagement.

INTRODUCTION

Considerable research has been conducted to elucidate the role of the noradrenergic (NE, norepinephrine) system in visuospatial attention. This is of crucial importance since abnormal functioning of the NE system is implicated in problems of attention and pathology such as seen in Attention Deficit / Hyperactivity Disorder (Beane & Marrocco, 2004; Brennan & Arnsten, 2008). However, to date the exact role of this system remains to be clarified.

Two mechanisms are key to visuospatial attention. A top down mechanism is responsible for creating attentional bias at a specific location, and a stimulus driven mechanism is important for circuit breaking, also called disengagement (M. Corbetta, Patel, & Shulman, 2008; Maurizio Corbetta & Shulman, 2002). Bias refers to augmented sensory processing due to the allocation of attention. It should be stressed that this bias is realized before the target for behavioral responding has actually been presented, resulting in increased processing of a target at the attended location. Disengagement refers to the decoupling of attention that makes it possible to reorient attention to the relevant stimulus.

One task frequently utilized to study visuospatial attention is the Visual Spatial Cueing (VSC) task (Posner, Snyder, & Davidson, 1980). In the VSC task a central cue signals the likely location of a target subsequently presented in either the right or left hemifield. In a minority of the trials, the target location is cued invalidly. Upon presentation of the target, a behavioral response has to be made in relation to a specific target feature (e.g., discrimination of its size). The most important behavioral outcome is the difference in reaction time for a validly cued target versus an invalidly cued target. This is termed the validity effect and is thought to reflect both elements of bias and disengagement (Clark, Geffen, & Geffen, 1989)

The noradrenergic system has been implicated in mechanisms of visual-spatial attention, either by modulating disengagement (M. Corbetta et al., 2008; Maurizio Corbetta & Shulman, 2002) or bias mechanisms (Kenemans et al., 2005; Kenemans & Kähkönen, 2011). Several studies have investigated the effect of a noradrenergic challenge on bias and disengagement, assessed by performance in the VSC task, using clonidine (Clark et al., 1989; Coull, Nobre, & Frith, 2001; Witte, Davidson, & Marrocco, 1997). Clonidine is an α2-receptor agonist and is thought to reduce Locus Coeruleus – Norepinephrine (LC-NE) signaling (Svensson, Bunney, & Aghajanian, 1975). Clark *et al.* (1989) and Coull *et al.* (2001) reported a

reduction of the validity effect in the VSC task following clonidine. Clark *et al.* (1989) further reported that clonidine specifically decreased the cost in terms of reaction time on invalidly cued targets. Thus, it was concluded that clonidine facilitates disengagement.

One issue is that a reduced validity effect may imply either enhanced disengagement or attenuated bias, or both. Behavioral output reflects the combined contribution of both bias and disengagement mechanisms. One way to disentangle these mechanisms is to implement measures of brain activity. A potentially useful clue in this respect is the evidence that bias initiation involves primarily dorsal parietal and frontal regions, whereas disengagement would be implemented in primarily ventral parietal-temporal and frontal areas (M. Corbetta et al., 2008; Maurizio Corbetta & Shulman, 2002). Functional MRI results from Coull *et al.* (2001) have indicated that directing attention in response to spatial cues elicits activation in dorsal parietal cortex, and that this attention-related activity in dorsal parietal cortex is reduced by clonidine. This is consistent with the hypothesis that it is primarily the dorsal bias system that is sensitive to clonidine. However, a more definite conclusion about these biochemical-function relations necessitates a clearer separation between bias and disengagement, as for example by separating cue-elicited and target-elicited responses.

Such a separation of bias and disengagement in time is perhaps most readily achieved using event-related brain potentials (ERPs) of the EEG. Several studies reported brain activity indices of bias and disengagement using the VSC task in combination with EEG (Hopf & Mangun, 2000; Mangun & Hillyard, 1991; van der Lubbe, Neggers, Verleger, & Kenemans, 2006). Importantly, using EEG, brain activity related to the onset of bias (cue related activity) can be distinguished from brain activity related to the result of this bias (target related activity). The onset of bias has been characterized by the parietal response to the cue. This response consists of the Early Directing Attention Negativity (EDAN) and Late Directing Attention Positivity (LDAP) Event Related Potential (ERP) (van der Lubbe *et al.*, 2006). Both components are thought to reflect activity in the parietal cortex. The result of bias has been identified as the modulation of the target elicited occipital/parietal P1/N1 ERP peaks by validity (Mangun & Hillyard, 1991). More specifically, the P1 and N1 are enhanced for validly cued targets as opposed to invalidly cued targets (Mangun & Hillyard, 1991). Here, this enhancement is referred to as the "P1 *effect"* and "N1 *effect"*. Thus, the onset and result of bias are reflected by different ERPs.

Likewise, disengagement is thought to be reflected in the modulation of the Late Positive Deflection (LPD) ERP by validity (LPD *effect*) (M. Corbetta et al., 2008; Mangun & Hillyard, 1991; Meinke, Thiel, & Fink, 2006). More specifically, the LPD is larger in response to invalidly as opposed to validly cued targets. The source of the LPD is still unclear, however, it most likely reflects processing in areas related to spatial reorienting such as the right inferior frontal gyrus and temporal-parietal junction (M. Corbetta et al., 2008).

In the current study the aim was to investigate the effect of noradrenergic attenuation by clonidine on the onset of bias, on the result of bias, and on disengagement. A double-blind placebo controlled crossover design was used. Participants performed in the VSC task, while EEG was recorded. It was hypothesized that clonidine would decrease all bias related parameters and have no facilitating effect on disengagement. Specifically, with respect to behavior, it was expected that clonidine would decrease the validity effect (reflecting both bias and disengagement related processing). With respect to brain activity indices of bias and disengagement, it was expected that clonidine would reduce the EDAN and LDAP (related to the onset of bias) as well as the P1 and N1 effect (related to the result of bias). No facilitating effect of clonidine was expected on the LPD modulation by validity (reflecting disengagement).

METHODS

Participants

The total sample consisted of 21 healthy non-smoking male participants (age, M: 22 SD: 3), from the student population from Utrecht University, The Netherlands. We only included non-smokers as nicotine has been shown to significantly affect measures of visuospatial attention (Thiel & Fink, 2008; Thiel, Zilles, & Fink, 2005; Vossel, Thiel, & Fink, 2008). It was required that participants pass the medical screening consisting of a medical interview and blood pressure measurement. With respect to the medical interview, participants were asked whether they met any of the following exclusion criteria: (a history of) fainting easily, psychopathology, and or any contraindications listed in the Summary of Product Characteristics (SPC). With respect to the blood pressure measurement, subjects with a relatively low blood pressure (systolic below 100 mmHg / diastolic below 70 mmHg) and or

heart rate below 60 or above 100 bpm were excluded. Participants were requested not to drink substances containing caffeine within 2 hours prior to the experiment. All participants had normal or corrected to normal vision. The study was approved by the medical ethics committee of the University Medical Center (UMC) Utrecht, and conducted in accordance with the declaration of Helsinki. All participants signed the informed consent prior to participation.

VSC task parameters

The VSC task was modeled after van der Lubbe *et al.* (2006) and Mangun and Hillyard (1991). Trials started with a fixation dot, which was presented for 600 ms. Subsequently the dot was replaced by a cue consisting of a diamond (width 1.3°, height 0.7°). The diamond consisted of either a red arrow pointing to the left and a green one pointing to the right, or vice versa, and was presented for 400 ms. Afterwards, the fixation dot was presented again for 600 ms. Subsequently, a target, a vertical short (width 0.8°, height 2°) or long bar (width 0.8°, height 2.4°) was presented for 100 ms in either the left or right visual field (6.4° relative to the center of the screen). Lastly, the fixation dot was presented for 1400 ms. The total duration of one single trial was 3100 ms. 75 percent of trials were valid trials, these are defined as trials in which the location of the target is congruent with the location the cue is pointing at.

Trials were organized in semi-random fashion in a number of separate blocks. Each block started with an instruction. The specific cue (green or red half of the diamond) as well as the response required upon presentation of the short or long bar (left or right button press, or vice versa) was specified in the instruction. A first practice block consisted of 32 trials (8 invalid), and subsequent experimental blocks consisted of 256 trials (192 valid). The VSC task was administered two times per session, before (pretest) and after (posttest) the administration of the capsule(s). The pretest consisted of one practice block and one experimental block. The pretest was only used for performance data; EEG was not recorded during these blocks. The posttest consisted of four experimental blocks that differed with respect to cue-color and response-target assignment (counterbalancing). The posttest started with an experimental block equal to the experimental block in the pretest with respect to response-target and cue-color assignment.

Drug treatment

Clonidine is a partial α-adrenergic agonist, but in effect reduces noradrenergic signaling. Initially a dose of 200 microgram was implemented based on Coull *et al.* (2001) and Clark *et al.* (1989) in which the effect of clonidine on performance measures was reported.

Procedures

Participants performed in two experimental sessions, and sessions were separated by at least one week. Sessions differed with respect to the capsule administered (placebo or clonidine). Participants were requested to fill out the Profile Of Mood States (POMS) questionnaire for assessing the subjective effect of clonidine (results described elsewhere). In addition to the VSC task, another task (Stop Signal Task, similar to Schmajuk *et al.* (2006)) was administered, which will be reported elsewhere. The order of the computer tasks and block order within tasks was counterbalanced across participants. Within participants, block order and task order remained the same across sessions (drug/placebo).

The pretest versions of the two tasks lasted approximately 30 minutes. After the pretest blood pressure and heart rate were assessed. Ten minutes after blood pressure and heart rate assessment, two capsules containing either clonidine (acquired from Teva Nederland B.V.) or placebo, were administered (t=0.00h). After about 40 minutes the EEG cap and EOG was placed and at t=1.40h (relative to capsule ingestion) the POMS was administered. At t=1.50h blood pressure and heart rate were assessed and at t=2.00h, participants performed the posttest version of the two tasks. In total the tasks lasted approximately 140 minutes with a 20 minute break dividing the two tasks.

Participants initially received either 200 μg clonidine (two capsules, each containing 100 μg clonidine) or placebo. However, the first participant became unwell after clonidine intake, most likely due to the large drop in systolic and diastolic blood pressure. The second participant also became unwell and likely experienced a vasovagal collapse after clonidine intake. Both participants were excluded and due to medical ethical concerns, the clonidine dose was lowered to 100 μg. During the remainder of the experiment, three additional participants were excluded. One participant vomited after clonidine administration, another participant experienced slow pulse and cardiac arrhythmia after the pretest (before capsule

administration) during the second session. Lastly, one participant was excluded due to excessive eye movements in excess of 60 μV (see below for a description of the criteria).

Data acquisition

Data acquisition was performed using the ActiveTwo system, Biosemi Inc., Amsterdam (Metting van Rijn, Peper, & Grimbergen, 1990). EEG was recorded from 64 sintered Ag-AgCl active electrodes positioned in a cap (10/20 labeling system). Horizontal eye movements were recorded from active electrodes located at the outer canthi of the eyes, vertical eye movements were recorded from electrodes located infra- and supraorbital of the eye. Online data sampling was at 2048 Hz, with a bandwidth of DC to 400 Hz.

EEG analyses

Brain Vision Analyzer was used for EEG analyses. All EEG was re-referenced to the right mastoid and down sampled to 250 Hz. A high pass filter of 0.1592 Hz (24 dB/oct), low pass filter of 30 Hz (12 dB/oct) and notch filter of 50 Hz were used. EEG was segmented to cue-locked epochs ranging from -100ms to 2300. Subsequently, artifacts in the epochs related to blinks were corrected (Gratton, Coles, & Donchin, 1983). In line with Van der Lubbe *et al.*(2006), epochs in which horizontal eye movements were present in excess of 60 μV were removed. Artifact rejection was used with a maximal difference criterion of 100 μV. Epochs were separated for synchronization to cue onset and to target onset and baseline-corrected for the average amplitude in the -100 to 0 ms latency window.

For each subject, epochs were averaged separately to validly cued left (visual field), validly cued right, invalidly cued left and invalidly cued right targets. Based on Mangun *et al.* (1991), and after visual inspection of grand average waveforms, the P1 and N1 were analyzed at electrode PO7 and PO8 in time windows 92 – 132 ms and 140 – 188 ms respectively. The LPD was analyzed at electrode Cz in time window 228 – 300 ms (Mangun & Hillyard, 1991).

Cue-locked difference waves were calculated in accordance with Van der Lubbe *et al.* (2006). For each subject activity at ipsilateral electrodes was subtracted from that at contralateral electrodes relative to cue direction. Subsequently, the difference waves for cues pointing to the left were averaged with the difference waves for cues pointing to the right. In

accordance with Van der Lubbe *et al.* (2006) the EDAN and LDAP were analyzed at electrode PO7/PO8 in time windows 240 – 280 ms and 560 – 640 ms respectively.

Statistical analyses and sample selection

ANOVAs were performed for all analyses with alpha set at 0.05. Performance data were analyzed using a design with factors time (pre- vs. post-treatment), drug, and validity. Initial analyses were performed on the total sample (N=16). To account for individual differences in drug response, blood pressure data were used as a proxy for individual responsivity. Relative blood pressure change in response to the drug manipulation was defined as the post- minus pre-measurement change after clonidine minus the post minus pre change after placebo. Correlations were evaluated between the neurocognitive variables of interest (Drug-induced modulation of RT validity effect, LDAP, P1, N1, and LPD validity effect) on the one hand, and the Time x Drug interaction effects for diastolic and systolic blood pressure values on the other. Thirdly, additional analyses were performed on a sub-sample (N=9, "EEG limited sample") in which the effect size (Cohen's d) per participant, pertaining to the overall validity effect in the pretests, was larger than .10. This ensured enough room for a possible drug effect, as not all participants showed a clear attentional modulation (validity effect) during the pretest. Cohen's d as opposed to the mere validity effect in RT was used in order to account for individual differences in variability of responding.

RESULTS

Performance

Within the Prepost (2) x Drug (2) x Validity (2) design a main effect of validity was observed ($F(1,15) = 10.9$, $p = .005$): Average RTs were 19.4 ms shorter for valid trials. The Drug x Validity x Prepost interaction did not nearly reach significance, nor did the Drug x Prepost effect ($p = .11$ and $p = .35$, respectively). As can be seen in figure 1, post-test RTs were clearly different for clonidine versus placebo, while pretest RTs appeared to be heterogeneous across the 4 conditions, suggesting that inclusion of the pretest data in the ANOVA would result in increased, rather than reduced, variance with respect to the drug response across individuals. Separate analysis for the post period revealed longer RTs (43 ms) under clonidine than under

placebo ($F(1,15) = 4.57$, $p < .05$), as well as a validity main effect (12.6 ms, $F(1,15) = 8.7$, $p < .05$), but no interaction. These effects are illustrated in the right panel of figure 1.

An analogous analysis of the proportion of errors made on invalidly and validly cued targets (choice error rates) did not yield any effect in the Prepost x Drug x Validity design; however, in the Drug x Validity analysis separately for post-treatment, more errors were emitted under clonidine compared to under placebo (20 versus 14 %, $F(1,15) = 9.7$, $p < .01$)

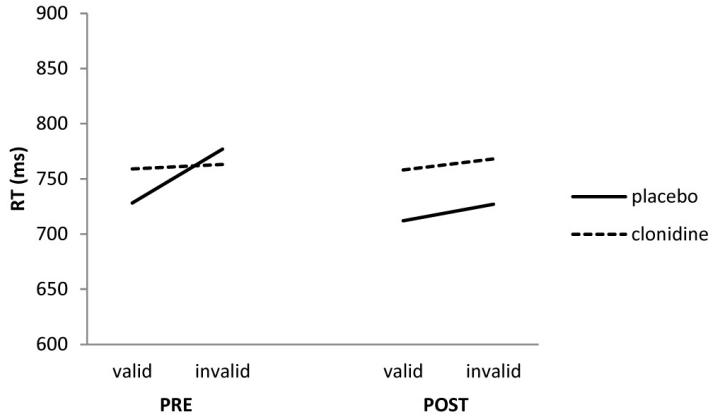

Figure 1. Reaction time on validly and invalidly cued targets before (PRE, left panel) and after (POST, right panel) administration of placebo or clonidine.

EEG: complete sample analysis

There were no significant drug-related effects on any measure. Data is available from the authors upon request.

Cardiovascular data

Both diastolic and systolic blood pressure decreased in response to clonidine as compared to placebo, respectively $F(1,16) = 58.259$, $p < 0.001$, and $F(1,16) = 12.245$, $p < 0.01$. Heart rate was not affected by clonidine ($F(1,16) < 1$).

Individual differences

Figure 2 shows blood-pressure values as a function of time (pre- and posttreatment) and drug, separated for individuals with high versus low reductions of systolic and diastolic levels.

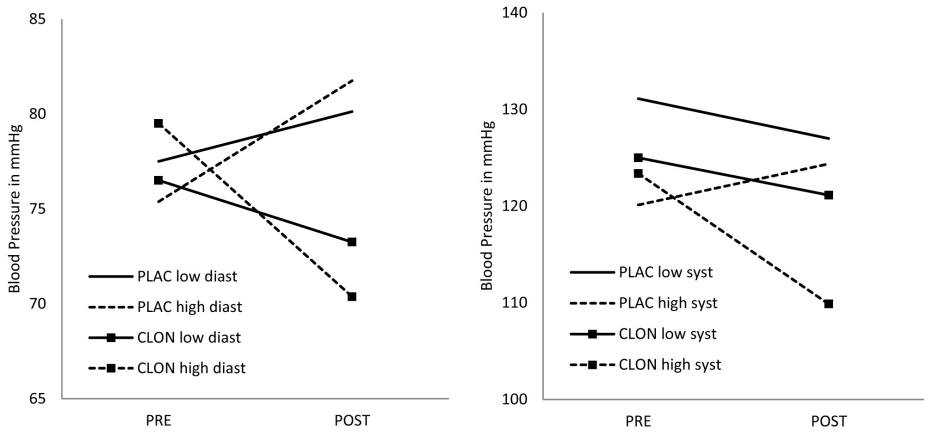

Figure 2. For both conditions (clonidine, CLON; placebo,PLAC) and for pre- and post measurement, diastolic (diast) reduction (left panel) and systolic (syst) reduction (right panel) for high vs. low relative reducers.

Analysis of the correlations between on the one hand drug-induced modulation of the validity effect on RT, LDAP, and P1, N1 and LPD validity effects, and on the other drug-induced reduction in systolic and diastolic blood pressure, revealed significance only for systolic response and LPD validity effects ($r = -0.618$, $p < .05$) As shown in figure 3 and figure 4, those subjects with a larger blood-pressure reduction in response to clonidine, exhibited a relatively enhanced validity effect on the LPD. More precisely, more negative values for the systole-contrast clonidine (post minus pre) minus placebo (post minus pre) were associated with more positive values for the LPD-contrast clonidine (invalid minus valid) minus placebo (invalid minus valid).

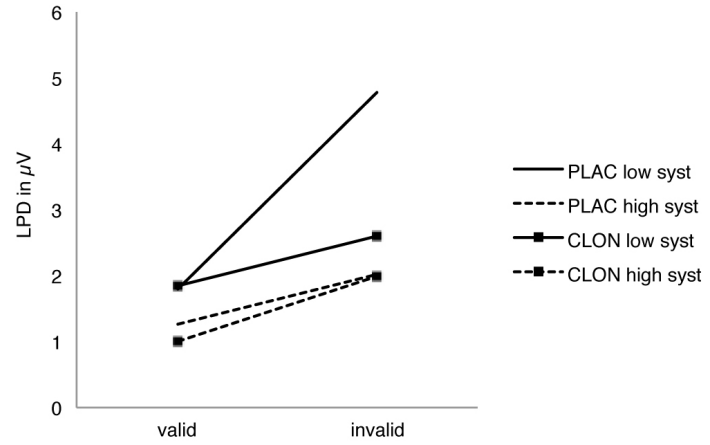

Figure 3. Split half for high systolic reducers (high syst) versus low systolic reducers (low syst) in relation to the LPD for valid versus invalid, under clonidine (CLON) versus placebo (PLAC).

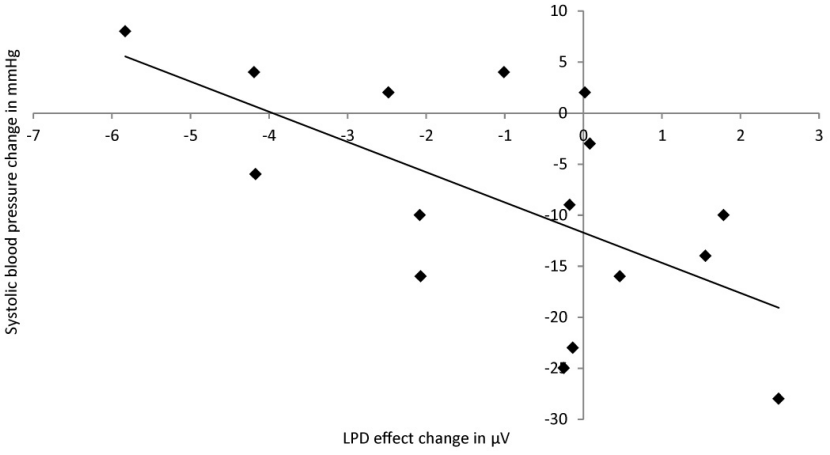

Figure 4. Correlation between the relative effect of clonidine on systolic blood pressure and the relative effect of clonidine on the LPD modulation by validity. For both variables, a lower value denotes a larger reduction.

EEG Limited sample (N=9)

Cue-locked ERPs

The EDAN and LDAP are depicted in figure 5. Only the LDAP differed significantly from baseline (LDAP: $F(1,8) = 28.521$, $p = 0.001$; EDAN: $(F1,8) < 1$). Both the EDAN and LDAP were not affected by clonidine (EDAN: $F(1,8) < 1$; LDAP: $F(1,8) < 1$).

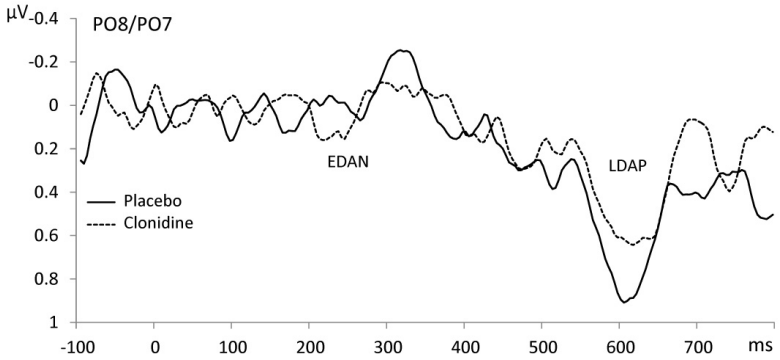

Figure 5. The EDAN and LDAP at electrode pair PO8/PO7 after administration of placebo and clonidine.

Target-locked ERPs

The modulation of the P1 by validity (P1 effect, depicted in figure 6) was not significant (main effect of validity: $F(1,8) < 1$). Both the N1 effect and LPD effect (depicted in figures 6 and 8, respectively) were significant with respectively $F(1,8) = 11.448$, $p = 0.01$, and $F(1,8) = 8.75$, $p = 0.018$. Clonidine did not affect the P1 effect nor the LPD effect (for both $F(1,8) < 1$). With respect to the N1, the drug x hemisphere x validity interaction was significant indicating that the effect of clonidine on the modulation of the N1 by validity depends on the hemisphere of recording ($F(1,8) = 6.982$, $p = 0.030$). As depicted in figure 7, clonidine significantly attenuated the N1 effect at electrode PO8 irrespective of visual field ($F(1,8) = 8.541$, $p = 0.019$). The N1 effect was not affected by clonidine at electrode PO7.

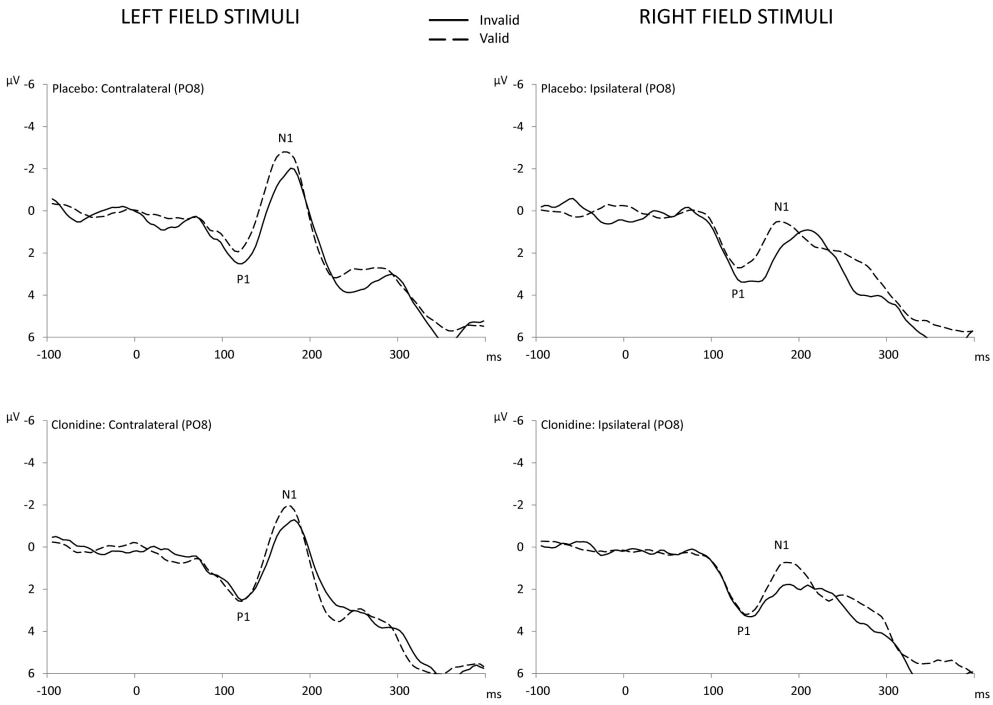

Figure 6. The P1 and N1 modulation by validity for clonidine and placebo at electrode PO8 for targets presented in the left and right hemifield.

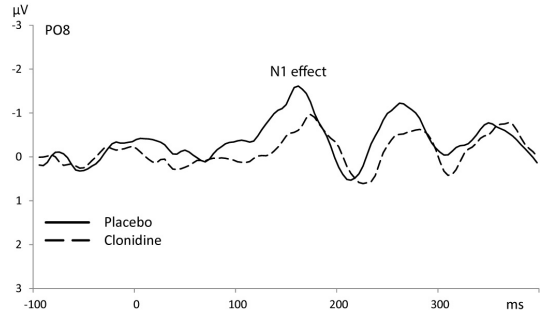

Figure 7. The N1 effect (N1 modulation by validity) for electrode PO8, separately for placebo and clonidine, collapsed over visual field.

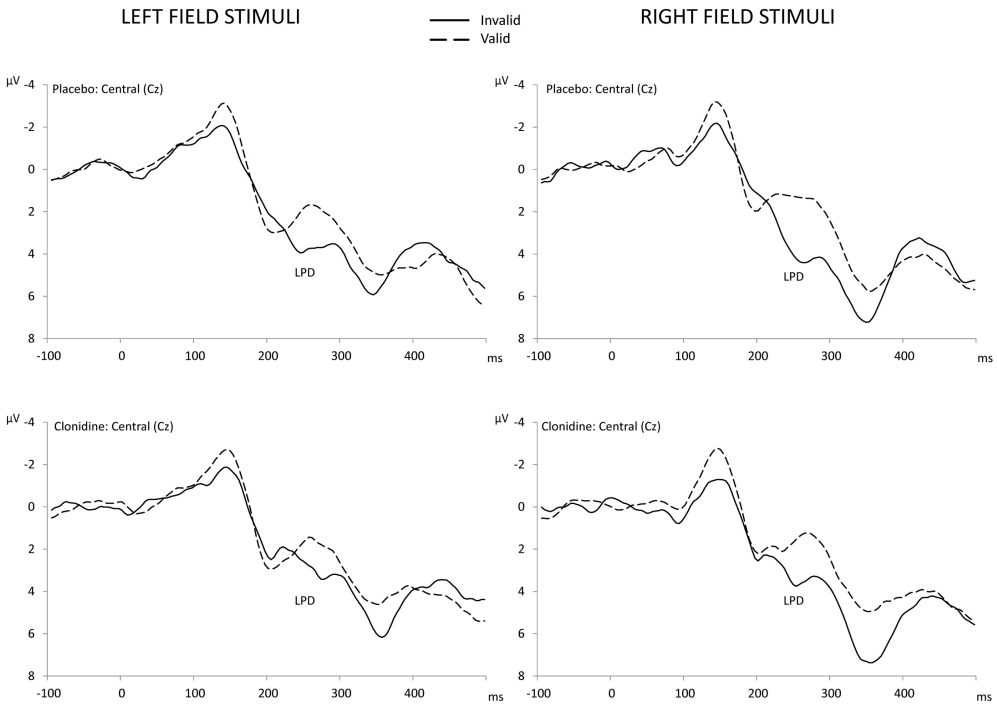

Figure 8. The LPD modulation by validity for clonidine and placebo at electrode cz for targets presented in the left and right hemifield.

DISCUSSION

In this study we investigated the effect of reducing noradrenergic signaling using clonidine on brain activity indices of the onset of bias (EDAN, LDAP), the result of bias (P1, N1), and disengagement (LPD). In line with our hypotheses, results show that clonidine negatively affected the result of bias. More specifically, clonidine attenuated the N1 modulation by validity. Additional correlations analysis suggested that clonidine also affects disengagement, depending on individual drug responsivity. In contrast to our hypothesis, clonidine did not affect the behavioral validity effect.

The components related to (initiation of) bias, EDAN and LDAP were not affected by clonidine. It may seem paradoxical that clonidine affected the N1 effect related to the result of bias but not the components related to the initiation of bias. It has been argued, however, that the EDAN and LDAP amplitudes do not fully predict the P1 and related N1 modulation by valid cueing (Hopf & Mangun, 2000). This suggests that other mechanisms important in spatial orienting may have been more sensitive to the effect of clonidine. More specifically, the biasing signals from parietal or even more anterior cortices to the visual cortex may be unaffected by clonidine, but the result of these biasing signals in visual cortex are in fact affected. Although the N1 effect was modulated by clonidine, the P1 effect was not. As reported in Mangun *et al.* (1991), the effect size for the modulation the P1 by validity is much smaller than that for the N1. The relatively small P1 modulation by validity then, leaves less room for a drug effect than the N1 modulation. Together with the small sample size this could account for the lack of visible modulation of the P1 effect.

It has been suggested that clonidine affects selective attention (as evident, for example in clonidine-induced modulation of the validity effect) through a more non-specific alerting mechanism (Nieuwenhuis, van Nieuwpoort, Veltman, & Drent, 2007). Briefly, stimulus processing in general is weakened by clonidine. This also holds for visual-spatial cues (as well as for targets), and any process that is contingent on basic cue or target processing, such as biasing signals or a disengagement response. The current pattern of effects in which the result of bias, as well as disengagement, but not the biasing signal itself were affected by clonidine, could be taken as counter-evidence for the non-specificity notion. However, this

argument rests on accepting the null-hypothesis (with respect to EDAN/ LDAP) and needs stronger support from future studies specifically targeting this dissociation.

The additional correlations suggest that clonidine affects disengagement. In the current study, a larger systolic blood pressure reduction due to clonidine was associated with an increase of the modulatory effect of validity on the LPD. In other words, a stronger peripheral effect of clonidine is associated with facilitated disengagement. However, alternatively, a stronger peripheral effect may result in a decreased central effect due to reduced central bioavailability of clonidine. Hence, the direction of the effect remains to be elucidated. However, the latter interpretation seems more in line with our behavioural data. If both bias and disengagement are reduced, a netto nill effect on the modulation of RT by validity can be expected. Moreover, a mere slowing of responses following clonidine may be expected, which is what was evident in the post-treatment RT data.

Several limitations should be noted. Several participants did not show an effect of cueing on behavior (validity effect on pretests). If attention is not effectively modulated by cueing, a drug effect cannot be measured due to a likely floor effect. To further address this issue, we performed additional analyses on a subsample. More specifically, participants with an effect size smaller than 0.10 (Cohen's d), with respect to the pretest validity effect, were excluded from the sample. Although this ensures room for a possible drug effect, one issue inherent to this selection is reduced overall statistical power. Another possible issue is the relatively low dose of clonidine in the current study. In contrast to the intensity of side effects as reported before (i.e. Smith *et al.* (2003); Kennedy *et al.*, (1995)), the first two participants experienced markedly strong side effects following 200 microgram clonidine. Because of the ensuing medical-ethical concerns, it was necessary to lower the dose to 100 microgram. Although this dose is lower than that used in previous studies, we did observe the expected effects on blood pressure as well as reaction times during the post-treatment phase.

In the current study clonidine did not affect the behavioural validity effect in the total sample, nor in the selected sample, which is in marked contrast to previous studies. As mentioned, this may be related to a possible detrimental effect on the LPD effect by clonidine, but other possibilities remain. The VSC task was modeled after Van der Lubbe *et al.* (2006) and Mangun *et al.* (1991), since EEG indices of bias and disengagement have been reported in these paradigms. However, it may be that the modulation by validity on RT was not large

enough in our task to assess a drug effect on this modulation. There is some support for this idea. Cohen's d with respect to the (behavioural) validity effect is somewhat larger in Coull *et al.* (2001), which used a slightly different VSC task variant, as opposed to Mangun *et al.* (1991) from which we implemented the specific target parameters (respectively d = 1.9 and d=1.6). Importantly, a drug effect on the modulation by validity on RT was found in Coull *et al.* (2001). In view of the failed replication of the effect of clonidine on behaviour, it is interesting that the N1 effect was affected by clonidine. It is plausible that in this case, EEG is a more sensitive measure than behavioural output.

With respect to the LC-NE system, it is thought that bias and disengagement depend on the specific functioning of this system (M. Corbetta et al., 2008; Maurizio Corbetta & Shulman, 2002). This system has been hypothesized to function in two modes (Aston-Jones & Cohen, 2005). The first one is the phasic mode, in which tonic noradrenergic activity is low but phasic responses to relevant stimuli are high; this mode is thought to support 'exploitation', the maintenance of a relatively rigid focus on a relatively specific attentional set. The second mode is the tonic mode, in which tonic noradrenergic activity is high but phasic responses to relevant stimuli are low. This mode is thought to support 'exploration', the flexible orientation towards new, possibly relevant stimuli. Conceptually, in the present analysis 'bias' corresponds to exploitation and 'disengagement' to exploration. Clonidine may have complex pre-synaptic and post synaptic effects and mixed results have been reported due to these competing effects (Ramos & Arnsten, 2007), and the question remains whether clonidine and other NE-modulators affect tonic levels, phasic responses or both (Pliszka, McCracken, & Maas, 1996). The present results could be taken as suggesting that reducing noradrenergic signaling weakens both bias and disengagement (see also Corbetta *et al.*, 2008). Hence, clonidine, as a net NE antagonist must reduce both tonic levels of NE and phasic NE responses. It should additionally be noted that NE enhancers, such has the reuptake inhibitor atomoxetine, have been reported to promote inhibitory control over on-going response tendencies (Chamberlain *et al.*, 2009). Conceptually, such inhibitory control is tightly linked to disengagement. It is yet unknown whether and if so how NE enhancers affects bias, or more general 'exploitation' processes. Furthermore, atomoxetine also affects dopamine in the frontal cortex associated with inhibition (Sauer, Ring, & Witcher, 2005), and, in high dosages, has also been reported to weaken, rather than promote, inhibitory control (Graf *et al.*, 2011).

The results from the current study show that noradrenergic attenuation by clonidine has a weakening effect on brain activity related to bias, and possibly on disengagement. Future research should focus on how other manipulations of tonic and phasic LC-NE activity translate into differential effects with respect to mechanisms of bias and disengagement.

ACKNOWLEDGMENTS

This study was financially supported by the open MAGW grant from NWO (Dutch organization for scientific research), NWO: 400-07-130

REFERENCES

Aston-Jones, G., & Cohen, J. D. (2005). An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. *Annu Rev Neurosci, 28*, 403-450.

Beane, M., & Marrocco, R. T. (2004). Norepinephrine and acetylcholine mediation of the components of reflexive attention: implications for attention deficit disorders. *Prog Neurobiol, 74*(3), 167-181.

Brennan, A. R., & Arnsten, A. F. (2008). Neuronal mechanisms underlying attention deficit hyperactivity disorder: the influence of arousal on prefrontal cortical function. *Ann N Y Acad Sci, 1129*, 236-245.

Chamberlain, S. R., Hampshire, A., Muller, U., Rubia, K., Del Campo, N., Craig, K., et al. (2009). Atomoxetine modulates right inferior frontal activation during inhibitory control: a pharmacological functional magnetic resonance imaging study. *Biol Psychiatry, 65*(7), 550-555.

Clark, C. R., Geffen, G. M., & Geffen, L. B. (1989). Catecholamines and the covert orientation of attention in humans. *Neuropsychologia, 27*(2), 131-139.

Corbetta, M., Patel, G., & Shulman, G. L. (2008). The reorienting system of the human brain: from environment to theory of mind. *Neuron, 58*(3), 306-324.

Corbetta, M., & Shulman, G. L. (2002). Control of goal-directed and stimulus-driven attention in the brain. *Nature Reviews, 3*, 201 - 215.

Coull, J. T., Nobre, A. C., & Frith, C. D. (2001). The noradrenergic alpha2 agonist clonidine modulates behavioural and neuroanatomical correlates of human attentional orienting and alerting. *Cereb Cortex, 11*(1), 73-84.

Graf, H., Abler, B., Freudenmann, R., Beschoner, P., Schaeffeler, E., Spitzer, M., et al. (2011). Neural correlates of error monitoring modulated by atomoxetine in healthy volunteers. *Biol Psychiatry, 69*(9), 890-897.

Gratton, G., Coles, M. G., & Donchin, E. (1983). A new method for off-line removal of ocular artifact. *Electroencephalogr Clin Neurophysiol, 55*(4), 468-484.

Hopf, J. M., & Mangun, G. R. (2000). Shifting visual attention in space: an electrophysiological analysis using high spatial resolution mapping. *Clin Neurophysiol, 111*(7), 1241-1257.

Kenemans, J. L., Bekker, E. M., Lijffijt, M., Overtoom, C. C., Jonkman, L. M., & Verbaten, M. N. (2005). Attention deficit and impulsivity: selecting, shifting, and stopping. *Int J Psychophysiol, 58*(1), 59-70.

Kenemans, J. L., & Kähkönen, S. (2011). How human electrophysiology informs psychopharmacology: from bottom-up driven processing to top-down control. *Neuropsychopharmacology, 36*(1), 26-51.

Kennedy, S. H., Gnam, W., Ralevski, E., & Brown, G. M. (1995). Melatonin responses to clonidine and yohimbine challenges. *J Psychiatry Neurosci, 20*(4), 297-304.

Mangun, G. R., & Hillyard, S. A. (1991). Modulations of sensory-evoked brain potentials indicate changes in perceptual processing during visual-spatial priming. *J Exp Psychol Hum Percept Perform, 17*(4), 1057-1074.

Meinke, A., Thiel, C. M., & Fink, G. R. (2006). Effects of nicotine on visuo-spatial selective attention as indexed by event-related potentials. *Neuroscience, 141*(1), 201-212.

Metting van Rijn, A. C., Peper, A., & Grimbergen, C. A. (1990). High-quality recording of bioelectric events. Part 1. Interference reduction, theory and practice. *Med Biol Eng Comput, 28*(5), 389-397.

Nieuwenhuis, S., van Nieuwpoort, I. C., Veltman, D. J., & Drent, M. L. (2007). Effects of the noradrenergic agonist clonidine on temporal and spatial attention. *Psychopharmacology (Berl), 193*(2), 261-269.

Pliszka, S. R., McCracken, J. T., & Maas, J. W. (1996). Catecholamines in attention-deficit hyperactivity disorder: current perspectives. *J Am Acad Child Adolesc Psychiatry, 35*(3), 264-272.

Posner, M. I., Snyder, C. R., & Davidson, B. J. (1980). Attention and the detection of signals. *J Exp Psychol, 109*(2), 160-174.

Ramos, B. P., & Arnsten, A. F. (2007). Adrenergic pharmacology and cognition: focus on the prefrontal cortex. *Pharmacol Ther, 113*(3), 523-536.

Sauer, J. M., Ring, B. J., & Witcher, J. W. (2005). Clinical pharmacokinetics of atomoxetine. *Clin Pharmacokinet, 44*(6), 571-590.

Schmajuk, M., Liotti, M., Busse, L., & Woldorff, M. G. (2006). Electrophysiological activity underlying inhibitory control processes in normal adults. *Neuropsychologia, 44*(3), 384-395.

Smith, A., Brice, C., Nash, J., Rich, N., & Nutt, D. J. (2003). Caffeine and central noradrenaline: effects on mood, cognitive performance, eye movements and cardiovascular function. *J Psychopharmacol, 17*(3), 283-292.

Svensson, T. H., Bunney, B. S., & Aghajanian, G. K. (1975). Inhibition of both noradrenergic and serotonergic neurons in brain by the alpha-adrenergic agonist clonidine. *Brain Res, 92*(2), 291-306.

Thiel, C. M., & Fink, G. R. (2008). Effects of the cholinergic agonist nicotine on reorienting of visual spatial attention and top-down attentional control. *Neuroscience, 152*(2), 381-390.

Thiel, C. M., Zilles, K., & Fink, G. R. (2005). Nicotine modulates reorienting of visuospatial attention and neural activity in human parietal cortex. *Neuropsychopharmacology, 30*(4), 810-820.

van der Lubbe, R. H. J., Neggers, S. F. W., Verleger, R., & Kenemans, J. L. (2006). Spatiotemporal overlap between brain activation related to saccade preparation and attentional orienting. *Brain Research, 1072*(1), 133-152.

Vossel, S., Thiel, C. M., & Fink, G. R. (2008). Behavioral and neural effects of nicotine on visuospatial attentional reorienting in non-smoking subjects. *Neuropsychopharmacology, 33*(4), 731-738.

Witte, E. A., Davidson, M. C., & Marrocco, R. T. (1997). Effects of altering brain cholinergic activity on covert orienting of attention: comparison of monkey and human performance. *Psychopharmacology (Berl), 132*(4), 324-334.

CHAPTER 3

THE EFFECT OF NORADRENERGIC ATTENUATION BY CLONIDINE ON INHIBITION IN THE STOP SIGNAL TASK.

Logemann, H.N.A., Böcker, K.B.E., Deschamps, P.K.H., Kemner, C., Kenemans, J.L.

Accepted (in revised form) by Pharmacology Biochemistry & Behavior

ABSTRACT

Understanding the neuropharmacology of inhibition is of importance to fuel optimal treatment for disorders such as Attention Deficit / Hyperactivity Disorder. The aim of the present study was to assess the effect of noradrenergic antagonism by clonidine on behavioral-performance and brain-activity indices of inhibition. A placebo-controlled, double blind, randomized, crossover design was implemented. Male (N=21) participants performed in a visual Stop Signal Task while EEG was recorded under clonidine in one session and under placebo in another. We expected that 100 μg clonidine would have a negative effect on EEG indices of inhibition, the stop N2 and stop P3. Furthermore, we expected that clonidine would negatively affect the behavioural measure of inhibition, the Stop Signal Reaction Time (SSRT). Behavioural analyses were performed on data of 17 participants, EEG analyses on a subset (N=13). Performance data suggested that clonidine negatively affected attention (response variability, omissions) without affecting inhibition as indexed by SSRT. Electrophysiological data show that clonidine reduced the stop P3, but not the stop N2, indicating a partial negative effect on inhibition. Results show that it is unlikely that the stop P3 reduction was related to the effect of clonidine on lapses of attention and on peripheral cardiovascular functioning. In conclusion, the current dose of clonidine had a negative effect on attention and a partial effect on inhibitory control. This inhibitory effect was restricted to the dorsal region of the prefrontal cortex (presumably the superior frontal gyrus) as opposed to the ventral region of the prefrontal cortex (right inferior frontal gyrus).

INTRODUCTION

Mechanisms of inhibition are of obvious importance to everyday functioning. Abnormal functioning of mechanisms of inhibition is associated with disorders such as Attention Deficit / Hyperactivity Disorder (ADHD) (Kenemans et al., 2005; Pliszka, Liotti, & Woldorff, 2000). Methylphenidate is a standard pharmacological treatment for ADHD, and has been shown to positively affect inhibition and attention (Kenemans et al., 2005). Methylphenidate facilitates dopaminergic, but also noradrenergic neurotransmission by blocking the reuptake of these neurotransmitters (Zetterstrom, Sharp, Collin, & Ungerstedt, 1988). However, a significant number of patients suffer from side effects, hence, it is important to disentangle the relative contributions to both the clinical effect and side effects (Barkley, 1998). A better understanding of the role of the dopaminergic and noradrenergic system in attention and inhibitory control may fuel the development of more optimal pharmacological treatment.

Atomoxetine has also been used as a pharmacological treatment for reducing ADHD symptoms and is thought to facilitate noradrenergic neurotransmission by blocking noradrenaline reuptake (Del Campo, Chamberlain, Sahakian, & Robbins, 2011). However, as a result of its effect on the noradrenaline transporter, atomoxetine does also increase prefrontal dopamine (Bymaster et al., 2002; Pliszka, 2005). Atomoxetine has been shown to positively affect inhibition, known to be a key component in ADHD (Chamberlain et al., 2007). More specifically, the effect of atomoxetine by enhancing noradrenergic neurotransmission on inhibition has been investigated using the Stop Signal Task (SST) (Chamberlain et al., 2007; Chamberlain et al., 2006). In the SST, participants are required to respond to go stimuli which are infrequently followed by a rare stop signal after which a response to the go stimulus has to be withheld. This task yields the Stop Signal Reaction Time (SSRT), which is believed to be an index of inhibitory motor control. Results showed that atomoxetine decreased the SSRT, indicating facilitation of inhibition. However, in a more recent study, Graf et al. (2011) found that atomoxetine actually increased commission errors in a flanker go/no-go task. The number of such errors constitutes an alternative indication of failing inhibitory control. It must be noted though that it has convincingly been argued by Eagle et al. (2008) that the specific inhibitory component being taxed differs between the go/no-go and SST paradigm, which might explain differences between results of Chamberlain et al. (2006) and Graf et al. (2011).

Although speculation, in this vein, a reducing effect on one component of inhibition may result in a compensatory effect on the other. Alternatively, these seemingly contrasting findings may be explained by the inverted U relationship between NE levels and inhibitory performance. A relatively low dose of 60 mg apparently results in even more optimal NE levels both in healthy adults (Chamberlain et al., 2006) and adult ADHD patients (Chamberlain et al., 2007), but a relatively high dose of 80 mg (as in Graf et al., 2011) in healthy adults may yield supra-optimal NE levels with consequential reduced performance. In any case, it may be expected that decreased noradrenergic neurotransmission results in impaired inhibition. That is, NE antagonism would move the level of transmission away from the natural optimal level and result in monotonously decreasing performance. In the present study clonidine was used to attain a reduction in specifically noradrenergic transmission, and was compared to a placebo condition in which NE transmission was assumed to be at optimal levels. Clonidine is an agonist for both pre- and postsynaptic α2 receptors (Pliszka, 2005), and antagonizes spontaneous Locus Coeruleus – Noradrenaline (LC-NE) activity at low doses (Svensson, Bunney, & Aghajanian, 1975).

The preferred measure for stopping performance, stop-signal reaction time or SSRT, cannot be measured directly and has to be inferred from stop rates and go-reaction time data. This estimation method is based on assumptions that are hard to verify (Band, van der Molen, & Logan, 2003; Overtoom et al., 2002). In addition to behavioural measures, it is therefore advisable to use brain activity measures of inhibition. This also makes it possible to explain behaviour in terms of brain activity. Indeed, in an attempt to localize the facilitation of stopping by noradrenergic enhancement by atomoxetine Chamberlain et al. (2009) showed, utilizing fMRI, that atomoxetine enhanced activity in the right Inferior Frontal Gyrus (IFG). Importantly, the right Inferior Frontal Gyrus (IFG) has been implicated as the neuroanatomical correlate of inhibition and related process of disengaging and reorienting of attention (M. Corbetta, Patel, & Shulman, 2008; Maurizio Corbetta & Shulman, 2002). Research with patients with frontal-damage has indicated both right IFG and the superior frontal gyrus (SFG) to be implicated in intact inhibition (Aron, Fletcher, Bullmore, Sahakian, & Robbins, 2003; Floden & Stuss, 2006).

Electrophysiological indices of inhibition are readily recorded in the EEG. Most notable are the stop N2 and stop P3 Event Related Potentials (ERPs). Specifically, it has been

shown that a stop signal synchronized brain potential at approximately 200 ms latency is significantly more negative on successful stop trials as opposed to failed stop trials (Schmajuk, Liotti, Busse, & Woldorff, 2006). This difference wave is termed the stop N2. Furthermore, the right IFG has been implicated as the neurobiological correlate of the stop N2 (Schmajuk et al., 2006). The stop P3 is also modulated by stopping success, being larger for successful inhibitions as opposed to unsuccessful ones (Schmajuk et al., 2006). The stop P3 has been interpreted as reflecting inhibition (Lansbergen, Bocker, Bekker, & Kenemans, 2007) and is thought to originate from the Superior Frontal Gyrus (SFG) (Kenemans & Kähkönen, 2011). In sum, using the SST in combination with EEG, a ventral inhibitory system as well as a more dorsal inhibitory system can be assessed as indexed by respectively the stop N2 (right IFG) and stop P3 (SFG).

In the current study we assessed whether noradrenergic attenuation by clonidine would negatively affect inhibition. A placebo controlled double-blind crossover design was used. Participants performed in the SST task while EEG was recorded. To our knowledge it is unknown whether clonidine is harmful for a fetus, which is why only men were included. With respect to brain activity measures of inhibition, it was expected that clonidine would attenuate the stop N2 and stop P3. With respect to behavioural measures, it was expected that clonidine would increase the SSRT. In addition, performance (omission errors, reaction-time fluctuation), as well as subjective measures of alertness were recorded to monitor the potentially sedative effects of clonidine.

METHODS

Participants and drug treatment

It was required that participants pass a medical screening consisting of an interview and a cardiovascular assessment. Specifically, participants with a blood pressure below 100 mmHg systolic and/or 70 mmHg diastolic and/or heart rate below 60 or above 100 bpm were excluded. Participants had normal or corrected to normal vision. A total of 21 male healthy subjects (age, M: 22 SD: 3) were included in the study. The study was approved by the local medical ethics committee of the University Medical Center Utrecht, and conducted in accordance with the declaration of Helsinki. Clonidine was used to attenuate noradrenergic

signaling. According to the Summary of Product Characteristics (Centrafarm Services B.V., Etten-Leur, The Netherlands) for clonidine, maximum plasma levels are reached after 1 to 3 hours. Clonidine is relatively long acting and, according to the SPC, has a half-life of approximately 9 hours. Initially a dosage of 200 μg of clonidine was used. However, the first two participants receiving clonidine experienced significant side effects and did not complete the experiment. More specifically, one participant became unwell, possibly due to the significant drop in systolic and diastolic blood pressure and the other participant fainted (possibly a vasovagal collapse). Because of medical ethical concerns, the experiment was continued with 100 μg clonidine. From this sample, two participants were excluded. One participant had to vomit after capsule intake, the other participant presented with cardiac arrhythmia and slow pulse after the pretest (but before treatment). In total, 17 subjects completed the experiment.

Stop Signal Task (SST)

The SST was modeled after the SST reported in Schmajuk et al. (2006). The primary task consisted of a dual choice task in which go-stimuli (the letters "X" and "O"; visual angles: (h) 1.4° x (w) 1.4° and 1.4° x 1.3° respectively) were presented randomly and sequentially. The letters were presented for 150 ms, centrally and slightly above a continuously present fixation cross. Participants had to discriminate between the go stimuli by pressing the left or right button on a response board with the left or right index finger. The trial-to-trial interval was varied between 1.5 to 1.8 seconds. The experiment consisted of a pretest (before capsule administration) and posttest (after capsule administration). The pretest consisted of 4 blocks, one practice block, consisting of 126 go trials, and three stop signal blocks consisting of 128 trials. In 25 percent of trials in the stop signal blocks, a go stimulus was followed by a stop stimulus consisting of a "$" sign (visual angle: 1.7° x 0.8°), presented at the same location as the go stimuli. The first block was used as a practice block and to establish a baseline average reaction time. If participants slowed more than 1.5 times this baseline reaction time in subsequent stop-signal blocks, participants were instructed to speed up, but only if more than 40% inhibitions were made. After the practice block, a base stop signal block was presented in which the go-stop Stimulus Onset Asynchrony (SOA) was fixed at 250 ms. Subsequently, two experimental stop signal blocks followed. After each stop-signal block, the go-stop SOA was

dynamically adjusted based on the stop rate in the previous block to ensure an approximate 50% stop rate (De Jong, Coles, & Logan, 1995). If the stop rate was below 40% (even after dynamic SOA adjustment), participants were instructed to respond slightly slower to the go-stimuli. The posttest was similar to the pretest but consisted of 9 blocks. The posttest started with a practice block followed by the base stop signal block and three experimental stop signal blocks. After the stop signal blocks, the response-stimulus assignment was switched and four equivalent stop signal blocks (base block plus three experimental blocks) were presented again. In both the pretest and posttest, the stop signal was jittered over 99ms below and above the set SOA in the experimental stop signal blocks to allow ADJAR filtering of overlapping EEG signals (see below). Furthermore, for each stop-signal block, trials were semi-randomized so that no more than three stop signals could occur on successive trials.

Procedure

Participants performed in two sessions separated in time by at least one week. The sessions differed with respect to the administered capsule(s) (clonidine/placebo). The experimental procedure was as follows: After signing the informed consent and upon passing the medical screening, participants were included in the study. Participants first filled out the Profile of Mood States Questionnaire (POMS). The POMS was used as a test of effectiveness of the drug manipulation at the subjective level. Afterwards participants performed in the SST pretest and a second task (visual-spatial cuing, described elsewhere). Approximately 10 minutes before capsule administration, blood pressure and heart rate were assessed. Subsequently, the capsule(s) were administered at t=0:00. At t=1:40 the POMS was filled out again and blood pressure and heart rate were assessed at t=1:50. Participants performed in the SST posttest at t=2:00. The order of tasks across sessions was the same and counterbalanced across participants. The SST posttest always started with four equivalent blocks with respect to response-stimulus assignment as used in the pretest. In between tasks there was a 20 minute break. Five minutes after completion of both tasks, at t=4:10, blood pressure and heart rate was assessed and participants were dismissed.

EEG acquisition

EEG was recorded from 64 electrodes in a cap (10/20 system) using the ActiveTwo system (Biosemi Inc., Amsterdam) (Metting van Rijn, Peper, & Grimbergen, 1990). HEOG was recorded from electrodes positioned at the outer canthi of the eyes. VEOG was recorded from electrodes positioned infra- and supraorbitally to the eye. The sampling rate was 2048 Hz, with DC-400 Hz filtering.

Behavioral analysis

The complete sample (N=17) was used for the behavioral analyses. The most relevant outcome was the SSRT. The SSRT was estimated in accordance with De Jong et al. (1990). The proportion of stop trials with no responses was calculated. This proportion of successful stops was corrected for omissions in accordance with Tannock et al. (1989). Reaction times of valid responses (defined as a response in the 150 ms – 1500 ms time interval) on go trials were rank ordered from shortest to longest reaction times. The specific point on this RT vector was then calculated by multiplying the length of the RT vector with 1 minus the corrected stop rate. The SSRT for the specific block was determined by subtracting the average go-stop interval from the RT at the determined vector point. Finally, the SSRT of all experimental blocks was averaged, yielding one averaged SSRT. Errors of omission and the standard deviation of the RT (SDRT; response variability) plausibly reflecting sustained attention were also analyzed (Berwid et al., 2005; Johnson et al., 2008). More specifically, the values of these variables for each experimental block were averaged across blocks yielding the average error of omission and average SDRT per session and per participant. Drug effects on errors of omission were in turn correlated with other drug effects to test whether the latter could be indirectly attributed to drug induced lapses of attention.

EEG analyses

EEG analyses were performed using Brain Vision Analyzer. EEG was re-referenced to the right mastoid and down sampled offline to 250 Hz. A 2 Hz (12 dB/oct), 50 Hz notch filter and 30 Hz low pass filter were used offline. EEG was segmented in 2600ms go-stimulus locked epochs with a 100 ms baseline. These epochs were corrected for ocular activity using the Gratton and Coles algorithm (Gratton, Coles, & Donchin, 1983). Four participants were

excluded due to artifacts mostly due to excessive frontal EMG. For the remaining sample, electrodes FP1, FP2, FPz, AF7, AF8, AFz, and F6 were excluded from the analyses due to artifacts on these electrodes (mostly muscle-activity related). Epochs containing remaining artifacts were removed using a difference criterion of 100 μv. Subsequently, only epochs containing stop signals were used for further processing. For each subject, epochs containing failed stops, and (separately) epochs containing successful stops were averaged time-locked to the go stimulus and to the stop stimulus. Subsequently, Adjacent Response (ADJAR) filtering level 2 was performed to remove go-stimulus related motor activity from the stop-stimulus locked ERPs (Woldorff, 1993). For the processed stop-ERPs the baseline was set at 0-50ms to remove any remaining go-stimulus related activity (Lansbergen et al., 2007). For statistical analyses, stop-stimulus locked ERPs were used.

Based on Schmajuk et al. (2006), and after visual inspection of the grand average waveforms and electrophysiological topography, time windows and specific electrodes for the stop (successful minus failed stops) N2 and stop P3 were chosen. The stop N2 was analyzed at electrode FC4 in the interval 172 – 192 ms. More specifically, the mean amplitude within this time interval was computed for each participant and per condition (clonidine, placebo) and these values were used for the statistical analyses. With respect to the stop P3, separate time windows were chosen with respect to the drug condition since, after visual inspection of grand average waveforms, the latency of the stop P3 peak seemed to differ between conditions. For placebo the time window 232 – 248 ms was used, whereas for clonidine the time window 244 – 260 ms was used. The stop P3 was analyzed at location Cz, irrespective of drug condition.

In order to account for variance attributable to experimental error and individual electrophysiological variability, peak analyses were performed. More specifically, for peak analyses, the maximum (not mean) amplitude of the stop N2 and stop P3 within slightly extended time windows (to account for variability of peaks) was determined for each participant and each condition and these values were used for the statistical analyses.

Profile Of Mood States (POMS) questionnaire

The POMS implemented in the current study was an abbreviated version translated to Dutch (Wald, 1984; Wald & Mellenbergh, 1990). The POMS consists of 6 dimensions: depression (8 items), anger (7 items), vigor (5 items), fatigue (6 items), tension (6 items), and total mood

disturbance. Each scale ranged from 0 (low) to 4 (high). The score on a dimension was the sum of the numbers on the scales for the items pertaining to that specific dimension. Total mood disturbance was calculated by subtracting vigor from the sum of tension, depression, anger, and fatigue.

Statistical analyses

Planned comparisons (2x2 repeated-measures ANOVAs) were performed with respect to performance, cardiovascular, and POMS variables with factor time (pre - post) and factor drug (placebo – clonidine). With respect to the electrophysiological variables (with the exception of the peak analyses), planned comparisons (also repeated-measures ANOVAs) were performed with factors time (pre – post), stop (successful – failed), and drug (placebo – clonidine). With respect to the stop P3 peaks, planned comparisons (repeated-measures ANOVAs) were performed with factors time (pre – post), and drug (placebo – clonidine). As individual variability with respect to responsiveness may be expected, cardiovascular data were used as a proxy for individual responsivity. Correlation analyses were performed with cardiovascular variables on the one hand and the dependent variables on the other. Post-hoc analyses included performance analyses for post-treatment measurement only, analysis of uncorrected SSRTs, correlation analysis between omissions and the stop P3 and lastly, analysis of performance data for the (limited) sample for which electrophysiological data was statistically analyzed.

RESULTS

Behavioral data

The stop rate (corrected for omissions) during the pretest for placebo, pretest for clonidine, posttest for placebo and posttest for clonidine was acceptable, respectively 50% (SD: 13), 37% (SD: 12), 47% (SD: 7), and 49% (SD: 8). Performance data are depicted in figure 1. The interaction between factor time (pre-post) and drug (clonidine-placebo) was not significant with respect to SSRT, mean RT, Standard Deviation of Reaction Time (SDRT) and error rates. However, the time x drug interaction was significant for omissions. The proportion of omissions increased more from pretest to posttest after clonidine as compared to placebo

(F(1,16) = 4.9, p = 0.042). Separate analyses for the pre- and post-data showed that SDRT, error rates, and percentage omissions were higher following clonidine as opposed to placebo (respectively: F(1,16) = 11.7, p = 0.003; F(1,16) = 5.7, p = 0.030; F(1,16) = 7.0, p = 0.017) for post-measurement only. Lastly, the time x drug interaction, and the effect of drug for post-treatment separately, was not significant with respect to the uncorrected SSRTs.

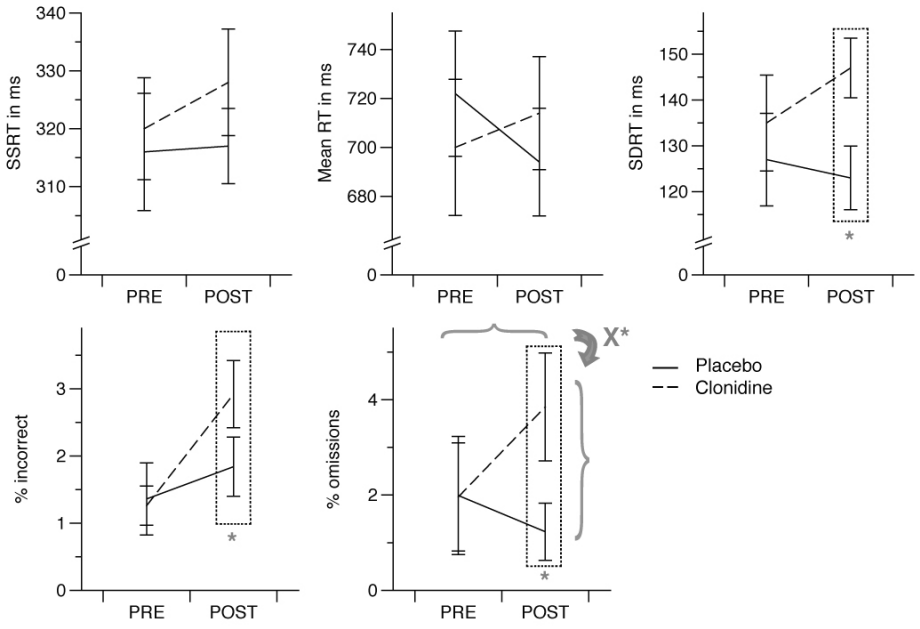

Figure 1. Performance data (SSRT, Mean RT, SDRT, %correct, %omissions) for placebo and clonidine, separately for pre- and post-measurement. A significant time x drug interaction is defined by an 'X*', a significant post-treatment effect is denoted by a dotted box.

Electrophysiological data

For both placebo and clonidine, the stop signal related averaged (over participants) difference wave (successful minus failed stops) at electrode FC4 and Cz, is presented in figure 2. The stop N2 and stop P3 are depicted in respectively the upper and lower panel of this figure. The topographies of the stop N2 and stop P3 are depicted in figure 3. The time window of the stop P3 was extended (only) for the topographical depiction in order to account for the slight difference in latency of the peak of this component between conditions.

Overall, the stop N2 was significant. In other words, the N2 was significantly larger for successful inhibitions as opposed to failed inhibitions (Mean amplitude analysis: $F(1,12) = 15.0$, $p = 0.002$; peak amplitude analysis: $F(1,12) = 37.9$, $p < 0.001$). The topographical map shows a right frontal maximum of this stop N2 under placebo. However, the stop N2 was not affected by clonidine. The P3, irrespective of drug, was significantly enhanced by stopping success (mean amplitude analysis: $F(1,12) = 30.6$, $p < 0.001$; peak amplitude analysis: $F(1,12) = 77.1$, $p < 0.001$). The topographies of the stop P3 for clonidine and placebo show a central distribution. Peak analyses showed that the stop P3 was significantly reduced after clonidine as compared to placebo (mean amplitude analysis: $F(1,12) = 0.0$, $p =$ ns. ; peak analyses: $F(1,12) = 6.6$, $p = 0.025$). Additional correlation analyses revealed no significant association between the clonidine induced increase in omission rate and the clonidine induced decrease of stop P3.

Figure 2. Stop signal locked difference waves (successful minus failed inhibitions) for placebo and clonidine (upper panel: stop N2, lower panel: stop P3).

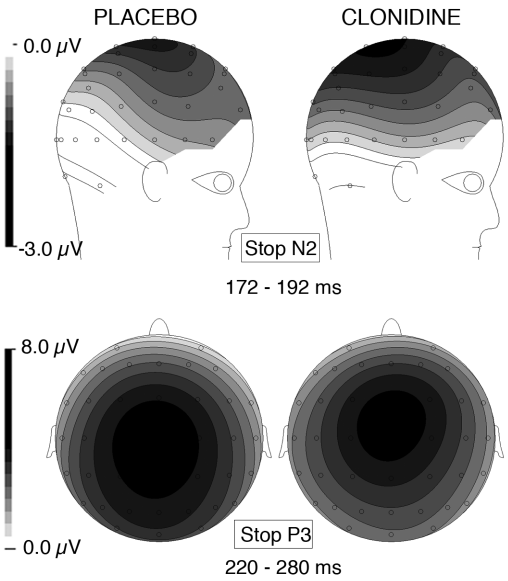

Figure 3. The distribution of the Stop N2 (upper panel) and stop P3 (lower panel), for placebo and clonidine.

Cardiovascular data and individual differences

As shown in figure 4, clonidine did not affect pulse, but significantly lowered both diastolic and systolic blood pressure ($F(1,16) = 58.3$, $p < 0.001$, $F(1,16) = 12.2$, $p = 0.003$, respectively). Taking the peripheral response to clonidine as a proxy for individual sensitivity to the drug, correlations between blood pressure responses and clonidine effects on the variables of interest (post-treatment: SSRT, stop N2, stop P3) were analyzed. These analyses indicated no significant correlations.

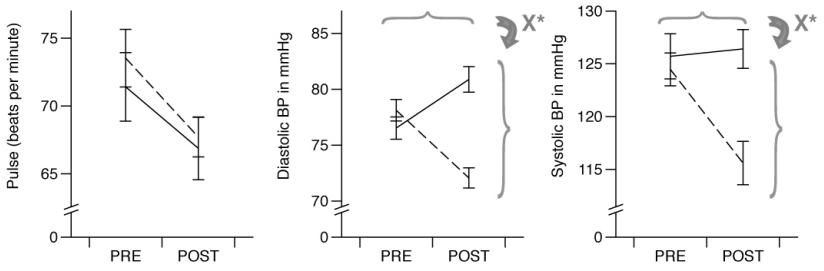

Figure 4. Cardiovascular effects pre- and post-measurement, for clonidine and placebo. An 'X*' denotes a significant time x drug interaction.

Subjective Measures

The effect of clonidine on the POMS scales is depicted in figure 5. Results show that clonidine significantly decreased vigor, and increased fatigue (respectively: $F(1,15) = 4.6$, $p = 0.048$; $F(1,15) = 16.9$, $p = 0.001$). Clonidine did neither affect depression, anger nor tension. Total Mood Disturbance in which these above factors are all included, increased significantly after clonidine ($F(1,15) = 10.8$, $p = 0.005$).

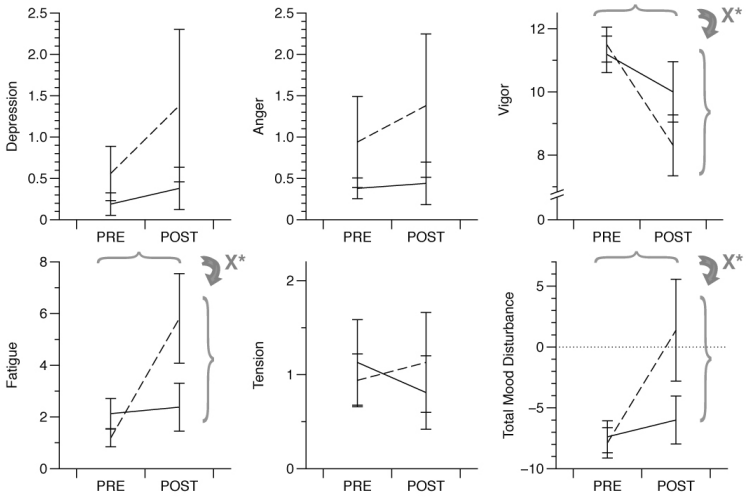

Figure 5. At pre- and post-measurement, values on the scales of the POMS separately for clonidine and placebo. An 'X*' denotes a significant time x drug interaction.

DISCUSSION

In the current study the effect of noradrenergic attenuation by clonidine on inhibitory processes was investigated using the SST in combination with EEG. It was expected that clonidine would impair inhibition as reflected in an attenuated stop N2, stop P3 and behaviourally, in an increased SSRT.

Electrophysiological data indicate that inhibition was partially negatively affected. More specifically, clonidine negatively affected the stop P3, without affecting the stop N2 or the SSRT. Results from performance data suggest clonidine negatively affected attention as evidenced by the clonidine induced suggested increase in response variability and increase in omissions. As there was no sign of a correlation between the drug induced effect on the stop P3 on the one hand and the drug induced effect on omission rate on the other, an explanation of the drug effect on stop P3 in terms of lapses of attention is not plausible. Furthermore, there was no correlation between individual differences in cardiovascular response to clonidine and the electrocortical or performance dependent variables. In other words, a more pronounced peripheral responsivity to clonidine is not associated with a stronger drug effect in the CNS as assessed with the behavioural and ERP measures.

One important question is why clonidine affected the stop P3 (albeit limited to peak analyses) but did not affect the SSRT. The validity of SSRT as an index for inhibitory motor control depends on assumptions, which prove hard to verify (Band et al., 2003; Overtoom et al., 2002). In the occasion that the validity of the SSRT is compromised, independent electrocortical indices of behavioral inhibition could reveal a drug effect that would not be apparent in SSRT. With respect to the SSRT, simulations have revealed that the currently used procedures for adjusting stop-signal delay and SSRT estimation result in a biased SSRT estimate to the extent that some stop signals might not trigger behavioral inhibition at all (instead of simply being too slow, Band et al. (2003)). Specifically, an increase in failures to process the stop signal results in over estimation of SSRT. It is conceivable that such failures to engage in stop-signal processing are more frequent under clonidine, which would mirror the increased omission rate (for go stimuli) under clonidine. However, this would actually result in an overestimation of the SSRT under clonidine and a higher likelihood of finding a difference in SSRT with placebo. Hence, it is unlikely that the lack of a drug effect on SSRT is due to

problems with SSRT estimation. Another possibility is that the stop P3 as an electrophysiological index of inhibition may be a relatively non-specific proxy for inhibitory control in contrast to the SSRT. That is, SSRT specifically relates to speed of inhibition, the stop P3 may convey a more general behavioural interrupt signal as suggested by Kenemans & Kähkönen (2011), which is not directly reflected in SSRT. It is conceivable that clonidine affects this more non-specific component rather than speed of inhibition.

Lastly, although speculative, it could be that the SSRT, like the stop N2, is mostly sensitive to manipulations of activity of the right IFG (which is in line with Aron et al. (2003)) and this region may be more sensitive to dopaminergic manipulations as opposed to noradrenergic manipulations which may primarily affect the SFG as indexed by the stop P3.

Importantly, one may argue that the clonidine effects in the current study are specific to attention. Performance data suggests clonidine affects attention, which in turn might result in a compensatory effect in mechanisms of inhibition, which might explain effects in our electrophysiological data. However, there are two issues here. Firstly, based on behavioural data alone it is difficult to specifically disentangle effects pertaining to attention from effects related to inhibition. Secondly, it has been shown that response variability correlates with inhibitory related activity which may likely indicate a compensatory effect (Bellgrove, Hester, & Garavan, 2004). However, in that case, increased response variability is likely to be associated with an increase in inhibitory activity, not a decrease as was found in our study. Taken together, it seems unlikely that the effects on inhibition are indirect and resulting from a clonidine induced detrimental effect on attention.

With respect to performance data, it should be noted that responses with a reaction time exceeding 1500 ms were deemed invalid responses (i.e. waiting for the stop-signal). We chose this cut-off value, since we anticipated a general slowing of reaction time as a result of possible fatigue related to the length of the experiment and sedation under clonidine. The cut-off threshold was based on previous literature, i.e. Lansbergen et al. (2007), but may be considered relatively liberal. More specifically, it may be argued that in some cases participants could have been waiting for the stop-signal, and this in turn may have affected performance and EEG data. However, we deem this unlikely for two reasons. Firstly, as described in the methods section, participants were specifically instructed to speed up responding to go-stimuli if performance with respect to speed-of-responding was somewhat diminished. Secondly, the

stop rate was below 50% for all conditions. Hence, if participants were consistently waiting for a stop-signal, the stop rate would be significantly increased, maybe even approaching 100%.

As the effects of clonidine on electrophysiology were investigated in a limited sample (N=13), it should be noted that the behavioural effects (prepost x drug interactions and drug interactions for post-test only, pertaining to SSRT, MeanRT, SDRT, error rates, and %omissions) in this sample mostly mirrored effects in the complete sample. Some minor differences were evident though. With respect to the limited sample, the prepost x drug interaction with respect to SDRT was significant. Furthermore, and as may be expected with a smaller sample size, the p value pertaining to the effect of clonidine on percentage omissions and error rates was somewhat reduced: $p = 0.06$ for the prepost x drug interaction pertaining to omissions, and $p = 0.06$ for the prepost x drug interaction pertaining to error rates (post treatment only).

Initially, for the first two participants, 200 μg clonidine was administered and although sedation and fatigue have been reported for 200 μg (Kennedy, Gnam, Ralevski, & Brown, 1995), adverse effects seemed more pronounced. Hence, as a result of medical ethical concerns, a lower dose of clonidine was implemented. This relative low dose may have resulted in stronger individual variability in responsiveness to the drug and may be reflected in the dissociation with the clonidine effect on the stop P3 on the one hand and stop N2 and SSRT on the other. However, it must be noted that even with 100 μg, clear and significant reductions in blood pressure were found. In addition, shifts of subjective state were also significantly more pronounced under clonidine relative to placebo, especially with respect to experienced enhanced fatigue and reduced vigor. Weight and body mass index may also influence the strength of a drug effect and therefore contribute to individual differences in drug response, but were not assessed in this study. Instead we used cardiovascular variables as a proxy for individual responsivity and assumed a relation between the peripheral drug effect and central effect.

The clonidine effect on the stop P3 was significant with peak analysis, but not with analysis of mean amplitudes. Peak analysis is principally less biased and therefore more sensitive because it takes into account individual differences in latency. On the other hand, compared to mean-amplitude analysis, peak analysis induces more variance in the parameter estimates because fewer samples are taken into account. This may contribute to not finding an

effect of clonidine for the stop N2 peaks. As evident from figures 3 and 5, signal-to-noise ratios for the stop P3 may be much higher than those for the stop N2. Therefore, peak estimation for the stop N2 may have been more sensitive to noise, which would reduce the power of detecting drug effects.

As discussed in the introduction, a more dorsally based and a more ventrally based prefrontal mechanism of inhibitory control have been postulated, thought to be reflected in the stop P3 and the stop N2, respectively. The selective norepinephrine reuptake inhibitor atomoxetine has been reported to result in shorter SSRTs while enhancing right IFG (ventral PFC) activity. However, high-dose atomoxetine, which may enhance NE activity beyond optimal conditions, has been reported to result in opposite effects (Graf et al., 2011). Another complication is that atomoxetine also blocks dopamine reuptake (Bymaster et al., 2002; Pliszka, 2005). In the current study we assumed that clonidine specifically attenuates noradrenergic activity to a suboptimal level. Clonidine 100 μg did not affect the stop N2, whereas it specifically attenuated the stop P3. Hence, dependence of inhibitory control on NE mechanisms that can be detected with the current low dose of clonidine concerns specifically the dorsal inhibition system as manifest in the stop P3.

It is possible that the stop N2 mechanism is part of a right-hemisphere vigilance or circuit-breaker mechanism that also contains a posterior component in the temporal-parietal junction (TPJ; (M. Corbetta et al., 2008; Maurizio Corbetta & Shulman, 2002; Posner & Petersen, 1990)). To the extent that the IFG component of this system, as indexed by the stop N2, does not depend on the NE system, the TPJ component may still do so. One possible ERP substrate for the TPJ component is the Late Positive Deflection (LPD), observed to be larger to events outside as opposed to those within the focus of attention (Mangun & Hillyard, 1991). Therefore, the LPD may be an interesting target for further research into the role of NE in inhibition and vigilance in general.

As to the P3 modulation by stopping success (stop P3), it has been suggested that such modulation is caused by enhanced negativity (or in a similar vein, reduced positivity) in case of a failed inhibition, i.e. an error (Error-Related Negativity or ERN (Falkenstein, Hohnsbein, Hoormann, & Blanke, 1991; Gehring, Goss, Coles, Meyer, & Donchin, 1993)). Lansbergen and colleagues (2007) showed that healthy volunteers characterized by long SSRTs had reduced stop P3s, relative to healthy fast stoppers. However, this reduced stop P3 was not

related to reduced ERNs, as these (when derived by the contrast between failed stops and correct-go ERPs) did not differ between slow and fast stoppers. Importantly, in ADHD patients, the stop P3 is reduced (Bekker, Kenemans, Hoeksma, Talsma, & Verbaten, 2005; Liotti et al., 2007). However, methylphenidate does not affect the stop P3, while it does normalize other inhibition-related ERPs, among which the stop N2 (Overtoom et al., 2009; Pliszka et al., 2007). To the extent that some ADHD patients present with anomalous functioning of the ventral inhibition mechanism (stop N2), and others with a compromised dorsal mechanism (stop P3), the latter may benefit more from medication balanced towards manipulation of noradrenalin neurotransmission. This conclusion is supported by the fact that NE projections from LC predominantly target dorsal cortical regions, rather than ventral ones (Gordon, Allen, & Trombley, 1988).

The Locus Coeruleus - Norepinephrine (LC-NE) system has been hypothesized to function in two modes (Aston-Jones and Cohen, 2005). The first one is the phasic mode, in which tonic noradrenergic activity is low but phasic responses to relevant stimuli are high; this mode is thought to support 'exploitation', the maintenance of a relatively rigid focus on a relatively specific attentional set. The second mode is the tonic mode, in which tonic noradrenergic activity is high but phasic responses to relevant stimuli are low. Conceptually, stop-signal stimuli should invoke an explorative response, in the sense of 'circuit breaking' or 'disengagement' (from the current attentional set). The present study suggests that noradrenergic antagonism by clonidine affected both the tonic and the phasic mode of the LC-NE system. Firstly, clonidine increased the number of omissions within the primary focus of attention, which could be related to deficient exploitation and therefore to a modulation of the phasic mode. Secondly, to the extent that inhibition and disengagement (in the context of either attentional or inhibitory processing) are linked, the drug effect on the stop P3 is indicative of a modulation of the tonic mode of the LC-NE system. From an alternative view, the conceptual link between disengagement and inhibition may be questioned. Inhibition may depend on a more or less continuous monitoring for stop signals, in parallel or even integrated with the attentional focus on go stimuli. Such 'dual-task integration' (Kramer, Wickens, & Donchin, 1985; Wester, Verster, Volkerts, Bocker, & Kenemans, 2010), rather than dis- and re-engagement, could then exclusively rely on the phasic NE mode'. It may however also be viewed as encompassing, in addition to an attentional focus on the primary go task, an ongoing

readiness for disengagement. Clonidine then may act to reduce both the phasic NE mode (evident in in increased variability of reaction times) and the tonic NE mode (as manifest in reduced stop P3).

To conclude, the present results suggest that clonidine negatively affects a dorsal inhibitory system (SFG) as evident in the stop P3 reduction. The presently used dose did not affect the ventral frontal inhibitory system (right IFG) as indexed by the stop N2. Future studies using other manipulations of NE transmission (e.g., moderate doses of atomoxetine) should reveal how strong this dissociation between norepinephrine effects on dorsal versus ventral inhibitory mechanisms really is.

ACKNOWLEDGMENTS

This study was financially supported by the open MAGW grant from NWO (Dutch organization for scientific research), NWO: 400-07-130

REFERENCES

Aron, A. R., Fletcher, P. C., Bullmore, E. T., Sahakian, B. J., & Robbins, T. W. (2003). Stop-signal inhibition disrupted by damage to right inferior frontal gyrus in humans. *Nat Neurosci, 6*(2), 115-116.

Band, G. P., van der Molen, M. W., & Logan, G. D. (2003). Horse-race model simulations of the stop-signal procedure. *Acta Psychol (Amst), 112*(2), 105-142.

Barkley, R. A. (1998). *Attention deficit hyperactivity disorder: A handbook for diagnosis and treatment* (2 ed.). New York: Guildford.

Bekker, E. M., Kenemans, J. L., Hoeksma, M. R., Talsma, D., & Verbaten, M. N. (2005). The pure electrophysiology of stopping. *Int J Psychophysiol, 55*(2), 191-198.

Bellgrove, M. A., Hester, R., & Garavan, H. (2004). The functional neuroanatomical correlates of response variability: evidence from a response inhibition task. *Neuropsychologia, 42*(14), 1910-1916.

Berwid, O. G., Curko Kera, E. A., Marks, D. J., Santra, A., Bender, H. A., & Halperin, J. M. (2005). Sustained attention and response inhibition in young children at risk for Attention Deficit/Hyperactivity Disorder. *J Child Psychol Psychiatry, 46*(11), 1219-1229.

Bymaster, F. P., Katner, J. S., Nelson, D. L., Hemrick-Luecke, S. K., Threlkeld, P. G., Heiligenstein, J. H., et al. (2002). Atomoxetine increases extracellular levels of norepinephrine and dopamine in prefrontal cortex of rat: a potential mechanism for efficacy in attention deficit/hyperactivity disorder. *Neuropsychopharmacology, 27*(5), 699-711.

Chamberlain, S. R., Del Campo, N., Dowson, J., Muller, U., Clark, L., Robbins, T. W., et al. (2007). Atomoxetine improved response inhibition in adults with attention deficit/hyperactivity disorder. *Biol Psychiatry, 62*(9), 977-984.

Chamberlain, S. R., Hampshire, A., Muller, U., Rubia, K., Del Campo, N., Craig, K., et al. (2009). Atomoxetine modulates right inferior frontal activation during inhibitory control: a pharmacological functional magnetic resonance imaging study. *Biol Psychiatry, 65*(7), 550-555.

Chamberlain, S. R., Muller, U., Blackwell, A. D., Clark, L., Robbins, T. W., & Sahakian, B. J. (2006). Neurochemical modulation of response inhibition and probabilistic learning in humans. *Science, 311*(5762), 861-863.

Corbetta, M., Patel, G., & Shulman, G. L. (2008). The reorienting system of the human brain: from environment to theory of mind. *Neuron, 58*(3), 306-324.

Corbetta, M., & Shulman, G. L. (2002). Control of goal-directed and stimulus-driven attention in the brain. *Nature Reviews, 3*, 201 - 215.

De Jong, R., Coles, M. G., & Logan, G. D. (1995). Strategies and mechanisms in nonselective and selective inhibitory motor control. *J Exp Psychol Hum Percept Perform, 21*(3), 498-511.

De Jong, R., Coles, M. G., Logan, G. D., & Gratton, G. (1990). In search of the point of no return: the control of response processes. *J Exp Psychol Hum Percept Perform, 16*(1), 164-182.

Del Campo, N., Chamberlain, S. R., Sahakian, B. J., & Robbins, T. W. (2011). The roles of dopamine and noradrenaline in the pathophysiology and treatment of attention-deficit/hyperactivity disorder. *Biol Psychiatry, 69*(12), e145-157.

Eagle, D. M., Baunez, C., Hutcheson, D. M., Lehmann, O., Shah, A. P., & Robbins, T. W. (2008). Stop-signal reaction-time task performance: role of prefrontal cortex and subthalamic nucleus. *Cereb Cortex, 18*(1), 178-188.

Falkenstein, M., Hohnsbein, J., Hoormann, J., & Blanke, L. (1991). Effects of crossmodal divided attention on late ERP components. II. Error processing in choice reaction tasks. *Electroencephalogr Clin Neurophysiol, 78*(6), 447-455.

Floden, D., & Stuss, D. T. (2006). Inhibitory control is slowed in patients with right superior medial frontal damage. *J Cogn Neurosci, 18*(11), 1843-1849.

Gehring, W. J., Goss, B., Coles, M. G. H., Meyer, D. E., & Donchin, E. (1993). A Neural System for Error Detection and Compensation. *Psychological Science, 4*(6), 385-390.

Gordon, B., Allen, E. E., & Trombley, P. Q. (1988). The role of norepinephrine in plasticity of visual cortex. *Prog Neurobiol, 30*(2-3), 171-191.

Graf, H., Abler, B., Freudenmann, R., Beschoner, P., Schaeffeler, E., Spitzer, M., et al. (2011). Neural correlates of error monitoring modulated by atomoxetine in healthy volunteers. *Biol Psychiatry, 69*(9), 890-897.

Gratton, G., Coles, M. G., & Donchin, E. (1983). A new method for off-line removal of ocular artifact. *Electroencephalogr Clin Neurophysiol, 55*(4), 468-484.

Johnson, K. A., Barry, E., Bellgrove, M. A., Cox, M., Kelly, S. P., Daibhis, A., et al. (2008). Dissociation in response to methylphenidate on response variability in a group of medication naive children with ADHD. *Neuropsychologia, 46*(5), 1532-1541.

Kenemans, J. L., Bekker, E. M., Lijffijt, M., Overtoom, C. C., Jonkman, L. M., & Verbaten, M. N. (2005). Attention deficit and impulsivity: selecting, shifting, and stopping. *Int J Psychophysiol, 58*(1), 59-70.

Kenemans, J. L., & Kähkönen, S. (2011). How human electrophysiology informs psychopharmacology: from bottom-up driven processing to top-down control. *Neuropsychopharmacology, 36*(1), 26-51.

Kennedy, S. H., Gnam, W., Ralevski, E., & Brown, G. M. (1995). Melatonin responses to clonidine and yohimbine challenges. *J Psychiatry Neurosci, 20*(4), 297-304.

Kramer, A. F., Wickens, C. D., & Donchin, E. (1985). Processing of stimulus properties: evidence for dual-task integrality. *J Exp Psychol Hum Percept Perform, 11*(4), 393-408.

Lansbergen, M. M., Bocker, K. B., Bekker, E. M., & Kenemans, J. L. (2007). Neural correlates of stopping and self-reported impulsivity. *Clin Neurophysiol, 118*(9), 2089-2103.

Liotti, M., Pliszka, S. R., Perez, R., 3rd, Luus, B., Glahn, D., & Semrud-Clikeman, M. (2007). Electrophysiological correlates of response inhibition in children and adolescents with ADHD: influence of gender, age, and previous treatment history. *Psychophysiology, 44*(6), 936-948.

Mangun, G. R., & Hillyard, S. A. (1991). Modulations of sensory-evoked brain potentials indicate changes in perceptual processing during visual-spatial priming. *J Exp Psychol Hum Percept Perform, 17*(4), 1057-1074.

Metting van Rijn, A. C., Peper, A., & Grimbergen, C. A. (1990). High-quality recording of bioelectric events. Part 1. Interference reduction, theory and practice. *Med Biol Eng Comput, 28*(5), 389-397.

Overtoom, C. C., Bekker, E. M., van der Molen, M. W., Verbaten, M. N., Kooij, J. J., Buitelaar, J. K., et al. (2009). Methylphenidate restores link between stop-signal sensory impact and successful stopping in adults with attention-deficit/hyperactivity disorder. *Biol Psychiatry, 65*(7), 614-619.

Overtoom, C. C., Kenemans, J. L., Verbaten, M. N., Kemner, C., van der Molen, M. W., van Engeland, H., et al. (2002). Inhibition in children with attention-deficit/hyperactivity disorder: a psychophysiological study of the stop task. *Biol Psychiatry, 51*(8), 668-676.

Pliszka, S. R. (2005). The neuropsychopharmacology of attention-deficit/hyperactivity disorder. *Biol Psychiatry, 57*(11), 1385-1390.

Pliszka, S. R., Liotti, M., Bailey, B. Y., Perez, R., 3rd, Glahn, D., & Semrud-Clikeman, M. (2007). Electrophysiological effects of stimulant treatment on inhibitory control in children with attention-deficit/hyperactivity disorder. *J Child Adolesc Psychopharmacol, 17*(3), 356-366.

Pliszka, S. R., Liotti, M., & Woldorff, M. G. (2000). Inhibitory control in children with attention-deficit/hyperactivity disorder: event-related potentials identify the processing component and timing of an impaired right-frontal response-inhibition mechanism. *Biol Psychiatry, 48*(3), 238-246.

Posner, M. I., & Petersen, S. E. (1990). The attention system of the human brain. *Annu Rev Neurosci, 13*, 25-42.

Schmajuk, M., Liotti, M., Busse, L., & Woldorff, M. G. (2006). Electrophysiological activity underlying inhibitory control processes in normal adults. *Neuropsychologia, 44*(3), 384-395.

Svensson, T. H., Bunney, B. S., & Aghajanian, G. K. (1975). Inhibition of both noradrenergic and serotonergic neurons in brain by the alpha-adrenergic agonist clonidine. *Brain Res, 92*(2), 291-306.

Tannock, R., Schachar, R. J., Carr, R. P., Chajczyk, D., & Logan, G. D. (1989). Effects of methylphenidate on inhibitory control in hyperactive children. *J Abnorm Child Psychol, 17*(5), 473-491.

Wald, F. D. M. (1984). *De verkorte POMS. Master's Thesis.*, University of Amsterdam., Amsterdam.

Wald, F. D. M., & Mellenbergh, G. J. (1990). Instrumenteel onderzoek. *Nederlands tijdschrift voor de psychologie, 45*, 86-90.

Wester, A. E., Verster, J. C., Volkerts, E. R., Bocker, K. B., & Kenemans, J. L. (2010). Effects of alcohol on attention orienting and dual-task performance during simulated driving: an event-related potential study. *J Psychopharmacol, 24*(9), 1333-1348.

Woldorff, M. G. (1993). Distortion of ERP averages due to overlap from temporally adjacent ERPs: analysis and correction. *Psychophysiology, 30*(1), 98-119.

Zetterstrom, T., Sharp, T., Collin, A. K., & Ungerstedt, U. (1988). In vivo measurement of extracellular dopamine and DOPAC in rat striatum after various dopamine-releasing drugs; implications for the origin of extracellular DOPAC. *Eur J Pharmacol, 148*(3), 327-334.

CHAPTER 4

THE EFFECT OF THE AUGMENTATION OF CHOLINERGIC NEUROTRANSMISSION BY NICOTINE ON EEG INDICES OF VISUOSPATIAL ATTENTION.

Logemann, H.N.A., Böcker, K.B.E., Deschamps, P.K.H., Kemner, C., Kenemans, J.L.

Submitted

ABSTRACT

The cholinergic system has been implicated in visuospatial attention but the exact role remains unclear. In visuospatial attention, bias refers to neuronal signals that modulate the sensitivity of sensory cortex, while disengagement refers to the decoupling of attention making reorienting possible. In the current study we investigated the effect of facilitating cholinergic neurotransmission by nicotine (Nicorette Freshmint 2mg, polacrilex chewing gum) on behavioural and electrophysiological indices of bias and disengagement. Sixteen non-smoking participants performed in a Visual Spatial Cueing (VSC) task while EEG was recorded. A randomized, single blind, crossover design was implemented. Based on the scarce literature, it was expected that nicotine would specifically augment disengagement related processing, especially manifest as an increase of the modulation of the Late Positive Deflection (LPD) by validity of cueing. No effect was expected on bias related components (cue-locked: EDAN, LDAP; target-locked: P1 and N1 modulations). Weak indications for a reduction of the reaction time validity effect by nicotine were observed only for a selected sample of subjects. Nicotine reduced the result of bias as indexed by a reduced P1 modulation by validity, especially in subjects with strong peripheral responses to nicotine. Nicotine did not affect ERP manifestations of the directing of bias (EDAN, LDAP) or disengagement (LPD).

INTRODUCTION

Bias and disengagement are central components of visuospatial attention (M. Corbetta, Patel, & Shulman, 2008; Maurizio Corbetta & Shulman, 2002). Bias refers to neuronal signals that increase the sensitivity of sensory cortex and results in facilitated processing of stimuli within the focus of attention. Disengagement refers to the decoupling of attention, which enhances processing of previously unattended stimuli. Anomalous functioning of mechanisms of bias and disengagement can have profound implications. More specifically, bias and disengagement related dysfunctions are thought to be key components in Attention Deficit / Hyperactivity Disorder (ADHD) (Kenemans et al., 2005). Smoking has been associated with ADHD pathology, and it has been argued that ADHD patients smoke to alleviate cognitive dysfunctions related to ADHD pathology (Kollins, McClernon, & Fuemmeler, 2005; Potter, Newhouse, & Bucci, 2006). Nicotine is effectively administered via smoking and augments cholinergic neurotransmission. Previous studies have documented positive effects of nicotine on attentional performance (Heishman, Kleykamp, & Singleton, 2010; Newhouse, Potter, & Singh, 2004; Rezvani & Levin, 2001), and importantly, the cholinergic system has been implicated in visuospatial attention (Maurizio Corbetta & Shulman, 2002), but the exact role of the cholinergic system in visuospatial attention is still unclear.

Visuospatial attention has been investigated using the Visual Spatial Cueing (VSC) task (Posner, Snyder, & Davidson, 1980). In this task, a cue signals the likely location of a subsequently presented target to which a response is usually required. The relevant behavioural outcome is termed the validity effect. This variable represents the benefit of valid cueing as opposed to invalid cueing in terms of the response time to the target. Several studies have investigated the effect of cholinergic challenge on performance in the VSC task and revealed that nicotine reduces the validity effect (Meinke, Thiel, & Fink, 2006; Thiel, Zilles, & Fink, 2005; Vossel, Thiel, & Fink, 2008; Witte, Davidson, & Marrocco, 1997). This reduction has been interpreted as reflecting enhanced disengagement (Witte et al., 1997), but also as reflecting reduced bias (Meinke et al., 2006). Indeed, a reduction of the validity effect may imply either enhanced disengagement, or reduced bias, or both. Without explicit reference to brain activity indices of either mechanism, it is impossible to disentangle the effect(s) of cholinergic manipulation by nicotine. A number of studies revealed attenuated activity in the

inferior parietal cortex in invalid trials after nicotine challenge (Thiel & Fink, 2008; Thiel et al., 2005; Vossel et al., 2008). This may be interpreted, in line with Yu et al. (2005), as reduced reliance on the cue in response to nicotine, which may be viewed as a reduction of bias. However, these studies failed to show that nicotine affects the bias-related occipital modulation (Thiel & Fink, 2008; Vossel et al., 2008); such reduced modulation would be expected from a bias account of nicotine's performance effects.

In sum, performance data suggest that nicotine either reduces bias and/or enhances disengagement. fMRI data, providing reference to brain activity, are equivocal as they do not provide a clear indication in favor of either hypothesis. Whereas the fMRI data provide some indication for nicotine affecting bias, an EEG study by Meinke et al. (2006) provided support for an effect on disengagement. These researchers combined EEG with the VSC task and investigated electrophysiological indices of visuospatial attention. More specifically, the effect of nicotine on the target-locked P1 and N1 modulations by validity was assessed. It has been suggested that the P1 reflects the suppression of unattended stimuli, whereas the N1 reflects the enhanced sensory processing of attended stimuli (Meinke et al., 2006). In other words, The P1 and N1 modulations reflect the result of bias. No effect of nicotine was evident with respect to these ERPs, which is in line with the fMRI results described above. However, nicotine positively affected a later positive deflection with a frontocentral distribution, and this nicotine induced enhancing effect was specific for invalid trials. Although reported in a post-hoc manner, and not described in detail, this component may be related to the Late Positive Deflection, which is larger for invalid trials as compared to valid trials. Since invalid trials necessitate disengaging from the current attentional locus (Maurizio Corbetta & Shulman, 2002), it follows that an enhanced brain response to invalidly cued targets may likely reflect a disengagement mechanism (Mangun & Hillyard, 1991; Meinke et al., 2006).

The aim of the current study was to further investigate whether nicotine would indeed facilitate disengagement, without affecting bias. Importantly, both the directing of attention (initiation of bias), as well as the result of bias, and disengagement can be assessed separately using EEG in combination with the VSC task. Relevant EEG indices of visuospatial attention are, firstly, the cue-locked ERPs. These are the Early Directing Attention Negativity (EDAN) and Late Directing Attention Positivity (LDAP), which reflect the parietal response to the cue assumed to be instrumental in directing of attention (or, in other words, the

initiation of bias) (van der Lubbe, Neggers, Verleger, & Kenemans, 2006). Secondly, the relevant target-locked ERP parameters are the P1, N1 and LPD modulation by validity (as described above). With respect to nicotine, a randomized single-blind placebo controlled design was used. Firstly, it was hypothesized that nicotine would reduce the behavioural validity effect. Secondly, it was hypothesized that this effect would be paralleled by a specific increase in disengagement related activity. That is, it was expected that nicotine would enhance the modulation of the LPD by validity, without affecting the bias related ERPs (cue-locked EDAN, LDAP; and target locked, P1 and N1 modulations by validity).

METHODS

Participants

Sixteen healthy non-smoking male participants participated. Participants were recruited from Utrecht University. Inclusion criteria were age between 18 – 40 years old. All participants had normal, or corrected to normal vision. Exclusion criteria were previous use of nicotine chewing gum, current medication use and contraindications mentioned in the Summary of Product Characteristics (SPC) of Nicorette Freshmint 2mg. Prior to participation all participants provided informed consent. The study was approved by the medical ethics committee of the University Medical Center Utrecht, The Netherlands, and conducted in accordance with the Declaration of Helsinki.

Nicotine administration

Nicotine was administered via polacrilex gum (Nicorette Freshmint 2mg). Based on Thiel et al. (2007), participants were requested to chew once every three seconds for thirty minutes. With this chewing rate plasma levels can be expected to be 3.57 ng/ml after 25 minutes of chewing (Vossel et al., 2008). The placebo consisted of conventional chewing gum (Sportlife smashmint)

Visual Spatial Cueing (VSC) task

VSC task parameters were based on Van der Lubbe et al.(2006) and Mangun et al. (1991). On each trial, a fixation dot was presented for 600 ms. This fixation dot was then replaced by a

diamond (width 1.3°, height 0.7°) in which one half, pointing to one visual hemifield, was colored green, and the other half, pointing to the other hemifield, was colored red. This cue was presented for 400 ms, and was then replaced by the fixation dot, again 600 ms in duration. The fixation dot was subsequently followed by one of two possible targets (white bars) differing in height (width 0.8°, height 2° or 2.4°), presented in either the left or right visual hemifield (6.4° relative to the center of the screen). Each trial was 3100 ms in duration.

The 'pretest' version (presented before nicotine or placebo administration) of this task consisted of two sequences of trials. Blocks always started with an instruction. The first block was a practice block consisting of 32 trials of which 8 were trials in which the target was invalidly cued (invalid trials). The subsequent block was an experimental block (used for data analysis), and consisted of 256 trials of which 64 were invalid trials. The 'posttest; version always started with a block in which the response-target assignment and definition of the cue (red half or green half of the diamond), stated in the instruction, was equal to the blocks in the pretest. The in total four experimental blocks in the posttest differed from each other with respect to specific response-target assignment (left or right button response to short or long bar) and cue definition (red half of the diamond, or green half of the diamond). Specific block order was counterbalanced over participants. For each run of the task, trials were semi-randomized within the experimental blocks.

Procedure

Participants performed in two sessions that differed with respect to the administered drug (nicotine or placebo). Sessions were separated by at least one week. After signing the informed consent and after passing the medical interview, CO levels were assessed to verify smoking abstinence. Subsequently, participants filled out the Profile of Mood States (POMS) questionnaire, used to assess the subjective effect of nicotine, and performed in the pretest version of the VSC task and Stop Signal Task (SST, data presented elsewhere). Following the pretests, cardiovascular variables (heart rate and blood pressure) were assessed, and the EEG cap was mounted. Subsequently, the chewing gum was administered. At 20 minutes following drug administration (t=0.20h), participants were requested to fill out the POMS again. Cardiovascular assessment took place again at t=0.25h, and at t=0.30h participants performed in the posttest version of the VSC and SST (task order was counterbalanced over participants).

Within tasks, participants were allowed short breaks between blocks. Between the tasks, there was a ten minute break. Finally, at t=2.35h, cardiovascular assessment took place and participants were dismissed.

EEG acquisition.

EEG was acquired with the ActiveTwo system (Biosemi Inc., Amsterdam) (Metting van Rijn, Peper, & Grimbergen, 1990), using 64 electrodes mounted in a 10/20 cap. Electrodes for monitoring eye movements were placed at the outer canthi of the eyes (horizontal eye movements, HEOG), and supra- and infraorbitally to the eye (vertical, VEOG). Sampling was performed at 2048 Hz, and a default DC – 400 Hz online filter was used.

EEG analyses

EEG data was processed using Brain Vision Analyzer. Signals were referenced offline to the right mastoid. Subsequently, EEG data were down sampled to 250 Hz and filtered (offline) using a high pass filter of 0.1592 Hz (24 dB/oct), low pass filter of 30 Hz (12 dB/oct) and notch filter of 50 Hz. Data were then segmented into epochs ranging from -100 ms to 2300 ms cue-locked. Using the Gratton et al. (1983) algorithm, epochs were corrected for blinks (VEOG). Segments containing evidence of horizontal eye movement (HEOG) exceeding 60 μV were rejected (van der Lubbe et al., 2006), and segments containing remaining artifacts were removed using a maximal difference criterion of 100 μV. Epochs were further segmented into cue-locked epochs and target-locked epochs. These epochs were baseline-corrected with the baseline set at -100 to 0 ms.

The parietal response to the cue was calculated in accordance with Van der Lubbe et al. (2006). For each participant, activity at ipsilateral electrodes was subtracted from activity at contralateral electrodes relative to cue direction. The difference waves for cues pointing to the left visual hemifield were then averaged with difference waves for cues pointing to the right visual hemifield. The EDAN and LDAP were quantified in the 240 – 280 ms and 560 – 640 ms time window respectively, and analyzed at electrode pair PO7/PO8 (van der Lubbe et al., 2006).

With respect to the target-locked ERPs, epochs were averaged separately for the categories validly cued left, invalidly cued left, validly cued right, and invalidly cued right

targets. In line with Mangun et al. (1991), the P1 and N1 were initially analyzed at electrodes PO7 and PO8 and quantified as the mean amplitude in windows 92 – 132 ms and 140 – 188 ms respectively. However, after visual inspection of grand average waveforms, the latency of the 92 – 132 ms window for the P1 was adjusted to 100 – 136 ms to provide a better fit around the P1 differences waves (valid minus invalid, irrespective of visual field) for both conditions (placebo and nicotine). Furthermore, the electrophysiological distribution of the drug effect on the P1 amplitude modulation by validity indicated a stronger drug effect on the nearby electrodes O1 and O2, thus these electrodes were selected for analyses.

Lastly, in accordance with Mangun et al. (1991), the LPD was analyzed at electrode Cz, and quantified as the mean amplitude in time window 228 – 300 ms.

Sample selection

Additional analyses were performed on two split-half samples. This was done in order to account for individual variability in responsivity to nicotine. Firstly, diastolic blood pressure was used as a proxy for the strength of an nicotine induced central effect under the assumption of a positive correlation between peripheral responsivity and central responsivity to nicotine. Analyses were performed on half of the sample in which the enhancing effect of nicotine on diastolic blood pressure was most pronounced. It must be noted that the baseline cardiovascular data were missing for the first session for one participant. To account for these missing data, data from the baseline measurement from session 2 was used as an estimation of baseline data for session1. Secondly, analyses were performed on half of the sample showing the strongest effect (Cohen's d) of cueing on speed of responding (strongest validity effect) on the pretest (averaged across condition). This was done to ensure enough room for improvement with respect to the validity effect on reaction time, as not all participants show a clear attentional modulation in terms of behavioural performance. A similar strategy was applied by Thiel & Fink (2008).

RESULTS

Peripheral effects

Nicotine increased diastolic blood pressure and heart rate ($F(1,14) = 11.69$, $p < 0.01$ and $F(1,14) = 4.68$, $p < 0.05$, respectively). Systolic blood pressure was unaffected by nicotine.

Performance data

With respect to the entire sample (top panel, figure 1), the validity effect on reaction time was significant: Responses on valid trials were faster as opposed to invalid trials irrespective of drug, as indicated by a main effect of validity ($F(1,15) = 19.99$, $p < 0.001$. There was no effect of validity on the proportion of errors. Nicotine did not affect the validity effect on reaction time neither in the complete sample, nor in the sample of diastolic responders. However, nicotine reduced the validity effect on reaction time for half of the sample with the strongest validity effect on the pretest, but this effect was restricted to post-treatment data ($F(1,7) = 8{,}99$, $p < 0.05$). Nicotine did not affect the validity effect pertaining to the proportion of errors, neither for the complete sample, nor for the split-half samples.

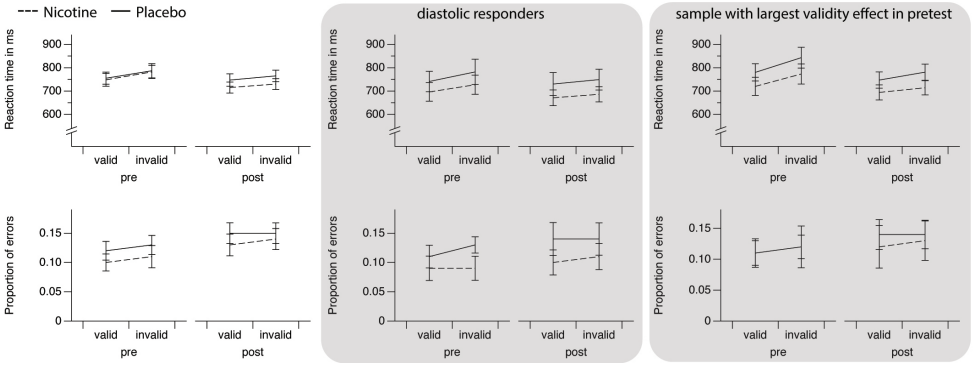

Figure 1. Reaction time and proportion of errors with respect to validly and invalidly cued targets, separately for placebo and nicotine and for the complete sample, for diastolic responders and for half of the sample in which the validity effect on RT was largest during the pretest.

Electrophysiological data

Cue-locked event related potentials

Cue-locked ERPs for the entire sample are depicted in figure 2. Neither the EDAN nor the LDAP was affected by nicotine. Furthermore, nicotine did not affect the cue-locked ERPs in any of the split-half post-hoc analyses.

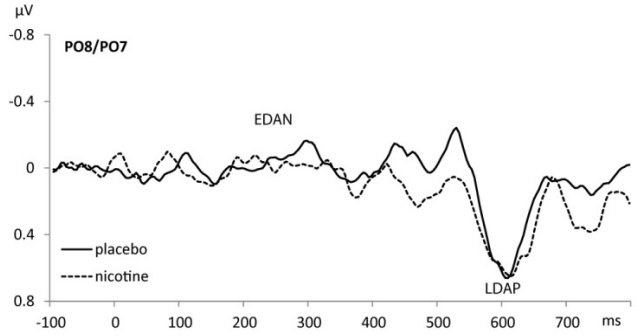

Figure 2. The EDAN and LDAP after placebo and nicotine for the complete sample (N=16).

Target-Locked event related potentials

The N1 and LPD modulations by validity were not affected by nicotine (figure 3). There was a trend towards significance with respect to the reducing effect of nicotine on the P1 modulation by validity (middle panel, figure 3; $F(1,15) = 3.66$, $p = 0.075$). As illustrated in figure 3 (lower panel), the nicotine induced reduction of validity effect with respect to the P1 was significant in strong diastolic responders ($F(1,7) = 5.99$, $p < 0.05$).

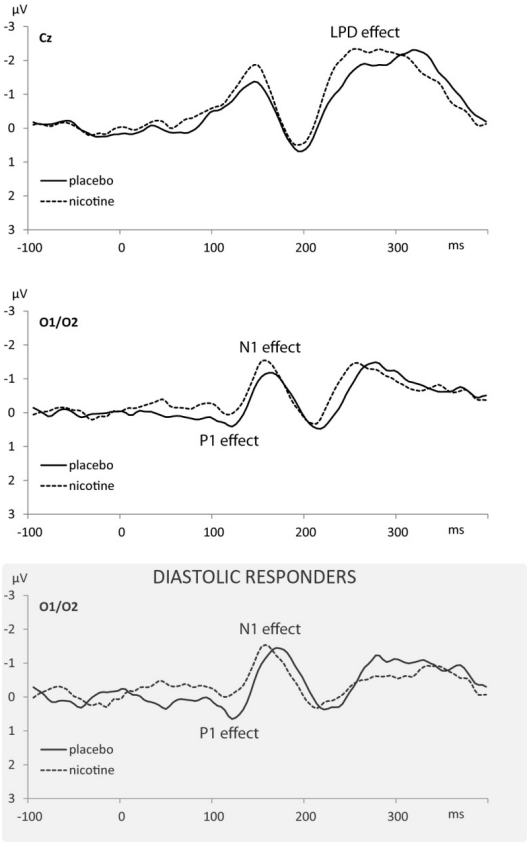

Figure 3. For the complete sample and separately for nicotine and placebo, the LPD effect (difference wave valid minus invalidly cued targets), P1 effect and N1 effect; lower panel depicts the N1 and P1 effect after nicotine and placebo for diastolic responders (N=8).

Subjective effects

Nicotine did not affect any of the POMS variables (depression $\eta^2_p = 0.001$, anger $\eta^2_p = 0.000$, vigor $\eta^2_p = 0.03$, fatigue $\eta^2_p = 0.004$, tension $\eta^2_p = 0.008$, TMD $\eta^2_p = 0.003$)

DISCUSSION

The exact role of the cholinergic system in visuospatial attention remains to be elucidated. Results from previous studies suggested that cholinergic agonism by nicotine results in augmented disengagement related processing. In the current study, facilitation of cholinergic neurotransmission by nicotine was further investigated with respect to behavioural and brain activity (electrophysiological) indices of bias and disengagement in a visuospatial cueing task. It was expected that nicotine would facilitate disengagement without affecting bias. Hence, firstly, a reduction of the behavioural validity effect was expected. Secondly, it was expected that this nicotine induced reduction, as a result of facilitated disengagement, would be reflected in an increase of the modulation of the LPD by cueing (validity). No nicotine-induced effect was expected to be evident at electrophysiological markers of bias, namely the EDAN, LDAP, N1 effect and P1 effect.

Although our nicotine manipulation had clear enhancing effects on heart rate and diastolic blood pressure, it hardly affected the neurocognitive parameters of interest. To scrutinize any possibility of a nicotine effect on attention-related variables, we took into account a number of individual differences with respect to baseline performance and cardiovascular sensitivity to nicotine. This analysis revealed that in certain subgroups nicotine reduced the validity effect on reaction time (RT, dependent on a strong baseline validity effect), and the validity effect on P1 (in strong diastolic responders). Nicotine did not affect the cue-related ERPs related to bias, nor did it affect disengagement related indices.

Referring to the model as laid out in the introduction; the reduced validity effect on RT can be interpreted in terms of either reduced bias or enhanced disengagement. The overall rationale of the current study was to use ERPs to be able to better decide between these two possibilities. No ERP evidence was found for nicotine effects on disengagement, and neither for such effects on cue-related biasing signals. The only effect of nicotine, albeit in a specific subgroup, concerned a reduction of the P1 effect, which can be taken as a reduction of the effect of the biasing signals on subsequent target processing in visual cortex. To the extent that the behavioral validity effect was reduced by nicotine, this reduction was paralleled by a reduction of target-processing modulation, or reduced differential sensitivity in visual cortex.

The lack of effect of nicotine on disengagement as reflected in the absence of a significant drug effect on the LPD modulation by validity seems to be inconsistent with a result from Meinke et al. (2006), in which a post-hoc effect was described on a later frontocentral component. However, this was a post-hoc finding and important specifics (such as latency and electrode(s) of interest) related to this component were not described.

With respect to the behavioural data, we only found a nicotine-induced reduction of the validity effect on reaction time in a specific subgroup. This contrasts with other studies that did report overall reductions of RT-validity effects under nicotine. There are two possible explanations for this contradiction. Firstly, the contradiction may be explained in terms of task difficulty. It has been argued that nicotine asserts a stronger effect on attentional variables when performance is more or less effortless (Meinke et al., 2006). In Meinke et al. (2006) two similar cueing experiments were used which differed in difficulty of the task. In the first, easier, experiment nicotine reduced the validity effect on reaction time. However, in the more taxing task, nicotine did not affect the validity effect on reaction time. In this last task, reaction time on the target seemed to be longer as opposed to the first task. In our experiment, general reaction time on targets was even more pronounced (>700ms). Assuming general reaction time here is, at least in part, indicative of task difficulty, our task may have been more taxing, resulting in a reduced effect of nicotine on the validity effect on reaction time. Secondly, in a related vein, another possibility for the lack of a drug effect on the validity effect on reaction time may be related to the size of the validity effect. In the current study the effect size of the validity effect for both the pretest and the posttest (placebo) was less pronounced (Cohen's d pretest: 0.29, posttest: 0.17) as compared to Meinke et al. (2006) (Cohen's d: 0.5 for experiment 1). Comparable to the procedure as maintained by Thiel et al. (2008), one solution to the relative small validity effect was to perform analyses on half of the sample which showed the strongest validity effect on RT on the pretests (Cohen's d effect size). In this sub-sample, nicotine reduced the validity effect. It must be noted though that this effect was restricted to the posttest only. The lack of the interaction with factor time (pretest – posttest) was not entirely unexpected since variance was higher in the pretest. Lower variance in the posttest is most likely related to the larger amount of trials (four blocks instead of one).

Extending to the electrophysiological data, it may be possible that the specific dosage of nicotine was too low to assert an effect. However, Nyberg et al. (1982) asserts that

increasing the dosage (4mg and higher) is not an option because of the increased chance of adverse effects in healthy non-smokers. Adding to this potential issue is the specific combination of the rather lengthy experiment and the relatively short half-life of nicotine. More specifically, in half of the sample a significant amount of nicotine has already been metabolized prior to the start of the VSC task. It may be argued that with relatively low blood plasma levels of nicotine, individual responsivity to the drug is relatively subtle and variance may be sizeable. To account for this, we performed split-half analyses on the sample in which nicotine asserted the strongest effect as evidenced by the nicotine induced effect on diastolic blood pressure. Interestingly, in the sample of diastolic responders, the hypothesized modulation by attention as hypothesized by previous authors (Thiel & Fink, 2008; Vossel et al., 2008) was clearly evident. More specifically, nicotine significantly reduced the P1 modulation by validity in these diastolic responders. Importantly, the same reduction of the P1 modulation by nicotine was evident as a trend towards significance in the complete sample. The N1 modulation by validity, however, was not significantly affected by nicotine in any of the samples. It has been argued that the P1 and N1 effects are part of a gating mechanism important in bias, in which the components serve differential roles (Meinke et al., 2006). The present results suggest that the P1 effect is more sensitive to cholinergic manipulation as opposed to the N1 effect.

Nicotine did not significantly affect the EDAN or the LDAP, important in the onset of bias, or directing of attention. This may seem to contrast the findings related to the P1 modulation by validity. However, as pointed out by Hopf et al. (2000), the EDAN and LDAP do not fully predict the P1 modulation. Other mechanisms (but not probed in our paradigm) may well be important in directing attention, and may still be affected by cholinergic manipulation; this may be subject for future investigations.

To conclude, our results suggest that facilitation of cholinergic neurotransmission by nicotine may reduce the bias related modulation of activity in visual cortex, rather than disengagement related processing. If anything, such cholinergic facilitation reduces attentional focus as reflected in reduced differential sensitivity in visual cortex, and reduced relative performance benefits for attended target stimuli.

ACKNOWLEDGMENTS

This study was financially supported by the open MAGW grant from NWO (Dutch organization for scientific research), NWO: 400-07-130. We thank R.H.J. van der Lubbe, PhD, for providing us with his original visuospatial cueing task. Furthermore, we would like to thank Z. Molnar-Logemann, MSc. for help with the POMS data analysis.

REFERENCES

Corbetta, M., Patel, G., & Shulman, G. L. (2008). The reorienting system of the human brain: from environment to theory of mind. *Neuron, 58*(3), 306-324.

Corbetta, M., & Shulman, G. L. (2002). Control of goal-directed and stimulus-driven attention in the brain. *Nature Reviews, 3*, 201 - 215.

Gratton, G., Coles, M. G., & Donchin, E. (1983). A new method for off-line removal of ocular artifact. *Electroencephalogr Clin Neurophysiol, 55*(4), 468-484.

Heishman, S. J., Kleykamp, B. A., & Singleton, E. G. (2010). Meta-analysis of the acute effects of nicotine and smoking on human performance. *Psychopharmacology (Berl), 210*(4), 453-469.

Hopf, J. M., & Mangun, G. R. (2000). Shifting visual attention in space: an electrophysiological analysis using high spatial resolution mapping. *Clin Neurophysiol, 111*(7), 1241-1257.

Kenemans, J. L., Bekker, E. M., Lijffijt, M., Overtoom, C. C., Jonkman, L. M., & Verbaten, M. N. (2005). Attention deficit and impulsivity: selecting, shifting, and stopping. *Int J Psychophysiol, 58*(1), 59-70.

Kollins, S. H., McClernon, F. J., & Fuemmeler, B. F. (2005). Association between smoking and attention-deficit/hyperactivity disorder symptoms in a population-based sample of young adults. *Arch Gen Psychiatry, 62*(10), 1142-1147.

Mangun, G. R., & Hillyard, S. A. (1991). Modulations of sensory-evoked brain potentials indicate changes in perceptual processing during visual-spatial priming. *J Exp Psychol Hum Percept Perform, 17*(4), 1057-1074.

Meinke, A., Thiel, C. M., & Fink, G. R. (2006). Effects of nicotine on visuo-spatial selective attention as indexed by event-related potentials. *Neuroscience, 141*(1), 201-212.

Metting van Rijn, A. C., Peper, A., & Grimbergen, C. A. (1990). High-quality recording of bioelectric events. Part 1. Interference reduction, theory and practice. *Med Biol Eng Comput, 28*(5), 389-397.

Newhouse, P. A., Potter, A., & Singh, A. (2004). Effects of nicotinic stimulation on cognitive performance. *Curr Opin Pharmacol, 4*(1), 36-46.

Nyberg, G., Panfilov, V., Sivertsson, R., & Wilhelmsen, L. (1982). Cardiovascular effect of nicotine chewing gum in healthy non-smokers. *Eur J Clin Pharmacol, 23*(4), 303-307.

Posner, M. I., Snyder, C. R., & Davidson, B. J. (1980). Attention and the detection of signals. *J Exp Psychol, 109*(2), 160-174.

Potter, A. S., Newhouse, P. A., & Bucci, D. J. (2006). Central nicotinic cholinergic systems: a role in the cognitive dysfunction in attention-deficit/hyperactivity disorder? *Behav Brain Res, 175*(2), 201-211.

Rezvani, A. H., & Levin, E. D. (2001). Cognitive effects of nicotine. *Biol Psychiatry, 49*(3), 258-267.

Thiel, C. M., & Fink, G. R. (2007). Visual and auditory alertness: modality-specific and supramodal neural mechanisms and their modulation by nicotine. *J Neurophysiol, 97*(4), 2758-2768.

Thiel, C. M., & Fink, G. R. (2008). Effects of the cholinergic agonist nicotine on reorienting of visual spatial attention and top-down attentional control. *Neuroscience, 152*(2), 381-390.

Thiel, C. M., Zilles, K., & Fink, G. R. (2005). Nicotine modulates reorienting of visuospatial attention and neural activity in human parietal cortex. *Neuropsychopharmacology, 30*(4), 810-820.

van der Lubbe, R. H. J., Neggers, S. F. W., Verleger, R., & Kenemans, J. L. (2006). Spatiotemporal overlap between brain activation related to saccade preparation and attentional orienting. *Brain Research, 1072*(1), 133-152.

Vossel, S., Thiel, C. M., & Fink, G. R. (2008). Behavioral and neural effects of nicotine on visuospatial attentional reorienting in non-smoking subjects. *Neuropsychopharmacology, 33*(4), 731-738.

Witte, E. A., Davidson, M. C., & Marrocco, R. T. (1997). Effects of altering brain cholinergic activity on covert orienting of attention: comparison of monkey and human performance. *Psychopharmacology (Berl), 132*(4), 324-334.

Yu, A. J., & Dayan, P. (2005). Uncertainty, neuromodulation, and attention. *Neuron, 46*(4), 681-692.

CHAPTER 5

THE EFFECT OF ENHANCING CHOLINERGIC NEUROTRANSMISSION BY NICOTINE ON EEG INDICES OF INHIBITION IN THE HUMAN BRAIN.

Logemann, H.N.A., Böcker, K.B.E., Deschamps, P.K.H., Kemner, C., Kenemans, J.L.

Submitted

ABSTRACT

The role of the cholinergic system in inhibition remains to be elucidated. Nicotine is a potent tool to augment this system, but most studies investigated its effects solely on behaviour. Reference to brain activity is important to specifically identify inhibition-related mechanisms. In the current study we investigated the effect of enhancing cholinergic neurotransmission by nicotine on inhibition. Participants (16 healthy non-smokers) performed in a stop task while EEG was recorded. A pre- versus post-treatment, within subjects, placebo controlled, single blind design was used. It was hypothesized that nicotine would decrease stop-signal reaction time (SSRT) and increase the amplitude of inhibition-related event related potentials, the stop N2 and stop P3. Nicotine shortened SSRT, but only when pretreatment values were not taken into account. After including a measure for sensitivity to nicotine based on diastolic blood pressure, highly responsive individuals turned out to have an enhanced stop P3 under nicotine indicative of enhanced inhibitory activity, possibly reflecting enhanced activation in the superior frontal gyrus.

INTRODUCTION

Elucidating the pharmacology of inhibition is of crucial importance. Anomalous inhibitory functioning is key to disorders such as ADHD (Kenemans et al., 2005). Studies have suggested a link between the cholinergic neurotransmitter system and problems of inhibition (Potter, Bucci, & Newhouse, 2012) . However, the exact role of acetylcholine in inhibition remains to be firmly established.

One very suited paradigm used to investigate inhibitory functioning is the Stop Signal Task (SST) (Logan, Cowan, & Davis, 1984; Schmajuk, Liotti, Busse, & Woldorff, 2006). In this task go stimuli are presented to which a response has to be made. In a minority of trials, the go stimulus is followed by a stimulus signaling to withhold a response to the go stimulus. The relevant behavioural output is the Stop Signal Reaction Time (SSRT). The SSRT is thought to indicate the amount of time needed to abort a response, and is thought to reflect inhibitory motor control (De Jong, Coles, & Logan, 1995; De Jong, Coles, Logan, & Gratton, 1990). Several studies have investigated the effect of the facilitation of cholinergic neurotransmission by nicotine on performance in the SST. More specifically, studies have shown improvements of inhibition in ADHD patients following nicotine, as reflected in reductions in SSRT (Potter et al., 2012; Potter & Newhouse, 2004, 2008). Interestingly, studies have shown that smoking is associated with ADHD (Kollins, McClernon, & Fuemmeler, 2005). This has been interpreted to be possibly related to self-medication (Potter, Newhouse, & Bucci, 2006). Indeed, this self-medication may be associated with alleviating inhibitory anomalies.

Cholinergic manipulation by nicotine has also been investigated in the normal healthy population. In general, nicotine does not seem to affect SSRT in healthy controls, neither in smokers nor in non-smokers (Bekker, Bocker, Van Hunsel, van den Berg, & Kenemans, 2005; Potter et al., 2012). It may be that in (some) normal healthy subjects there is not enough room for improvement with respect to SSRT (ceiling effect). Or, in other words, the effect of nicotine may depend on baseline SSRT. There is some support for this notion. In a sample of ADHD patients in which SSRTs are relatively long, nicotine has been shown to significantly reduce SSRT (Potter et al., 2012). Interestingly, opposite effects are evident with mecamylamine, which in effect attenuates cholinergic signaling (Potter et al., 2012). Mecamylamine has been shown to increase SSRT in healthy controls. However,

mecamylamine does not seem to affect SSRT in the ADHD sample, possibly resulting from a floor effect.

Although suggestive of an effect of nicotine on inhibition, one issue with the above studies is that no reference is made to brain activity indices of inhibition. More specifically, estimation of SSRT depends on assumptions about the independence between stop and go processes, which are often hard to verify (Band, van der Molen, & Logan, 2003; Overtoom et al., 2002). Also, effects on reaction time to go stimuli may bias the SSRT (Verbruggen, Chambers, & Logan). Using brain-activity measures, such as event-related brain potentials (ERPs), offers the possibility to assess brain responses specifically tied to the presentation of the stop stimulus and to the extent of successful stopping (De Jong et al., 1990; Bekker et al., 2005; Lansbergen et al., 2007). In addition, ERPs have revealed different mechanisms associated with inhibitory control (detailed below). To our knowledge no studies have been performed yet, specifically investigating the effect of nicotine on brain activity indices of inhibition. One isolated EEG study did suggest an effect following nicotine administration via polacrilex gum on brain activity reflections of inhibition in non-smoking healthy participants (Meinke, Thiel, & Fink, 2006). In this study, a visual spatial cueing (VSC) paradigm was combined with EEG. In the traditional VSC paradigm a cue signals the likely location of a target to which a response has to be made (Posner, Snyder, & Davidson, 1980). In general responses are faster to targets presented at the indicated locations as opposed to incongruently cued targets. This benefit of valid cueing in terms of RT is called the validity effect. Indeed several studies report a reduction of the validity effect following nicotine administration (Meinke et al., 2006; Thiel & Fink, 2008; Vossel, Thiel, & Fink, 2008). Interestingly although post-hoc, Meinke et al. (2006) reported that nicotine affected a late positive deflection (LPD) which was specific to invalid trials. Indeed, this component may be the LPD reported in Mangun et al. (1991), which may in turn be implicated in the disengaging of attention, which again in turn can be conceptually linked with inhibition (M. Corbetta, Patel, & Shulman, 2008; Maurizio Corbetta & Shulman, 2002). Although speculative, part of the modulation of the late positive deflection by validity as outlined by Mangun et al. (1991), may be related to the stop P3 which has been implicated in inhibition in the SST (Lansbergen, Bocker, Bekker, & Kenemans, 2007).

In the current study our goal was to specifically investigate the effect of enhancing cholinergic neurotransmission by nicotine on EEG indices of inhibition in the visual SST. Indeed, brain activity indices of inhibition have been reported with respect to the SST. Firstly, in response to the stop signal, a negative wave of about 200ms latency termed the N2 is evident. Stopping success significantly modulates this N2 and this effect is defined as the 'stop N2'. The generator of the stop N2 is thought to be the right Inferior Frontal Gyrus (rIFG) (Schmajuk et al., 2006). Optimal performance in the SST heavily relies on intact rIFG functioning, an area strongly implicated in inhibitory control (Aron, Fletcher, Bullmore, Sahakian, & Robbins, 2003). Secondly, a positive wave of about 300ms latency is present after the stop signal that is also modulated by stopping success (Lansbergen et al., 2007; Schmajuk et al., 2006). This effect is called the 'stop P3' and is thought to be generated by the Superior Frontal Gyrus (SFG) (Kenemans & Kähkönen, 2011). Stop N2 and stop P3 then, may reflect more ventrally and more dorsally based mechanisms of inhibition, respectively.

16 non-smoking healthy participants performed in the visual SST while EEG was recorded on two sessions separated by at least a week. A prepost single blind within subjects design was used. The hypothesis was that nicotine would improve inhibition. It was expected that nicotine would decrease SSRT, but that this decrease was dependent on baseline SSRT. Furthermore, we explored the possible effects of nicotine on the stop N2 and the stop P3.

METHODS

Participants

16 healthy adult non-smoking male subjects participated in the current study. All participants had normal or corrected to normal vision. Exclusion criteria were current medication use, previous use of nicotine chewing gum and conditions mentioned in the summary of product characteristics of Nicorette Freshmint 2mg. All participants signed the informed consent prior to participation. The medical ethics committee of the University Medical Center Utrecht approved the experiment. The study was conducted in accordance to the Declaration of Helsinki.

Drug administration

Nicotine polacrilex gum (Nicorette Freshmint 2mg) was used to manipulate the cholinergic system. A dosage of 2mg was chosen since 4 mg result in adverse effects in non-smokers (Nyberg, Panfilov, Sivertsson, & Wilhelmsen, 1982) and does not seem to result in a stronger central effect (Thiel & Fink, 2007). Nicotine has a half-life of approximately two hours (Benowitz, Porchet, Sheiner, & Jacob, 1988). Chewing instruction was based on Vossel et al. (2008). Chewing for 25 minutes at a rate of one chew per three seconds results in plasma values of 3.57 ng/ml. The placebo consisted of conventional chewing gum (Sportlife smashmint)

Stop Signal Task

The SST was similar to the stop task as described in Schmajuk et al. (2006). Go stimuli were presented randomly and sequentially and consisted of two letters, an X and an O (visual angles: (h) 1.4° x (w) 1.4° and 1.4° x 1.3° respectively), presented slightly above a fixation cross. In response to these letters a differential response was required (i.e. left button press after X, right button press after O, and vice versa). Letters were presented for 150ms and trials varied between 1.5 to 1.8 seconds duration. In 25% of trials in the so-called stop signal blocks, the go stimulus was followed by the letter "$" (visual angle: 1.7° x 0.8°), signaling to withhold a response. The experiment consisted of a pretest (before gum administration), and a posttest (after gum administration). The pretest consisted of, in chronological order, a no-stop-signal block consisting of 126 go trials (to estimate a baseline reaction time), a base stop-signal block (to estimate a SOA yielding 50% inhibitions for the subsequent block) and two experimental stop signal blocks, each consisting of 128 trials. If, in stop signal blocks, participants slowed for more than approximately 1.5 times the reaction time on the go stimuli in the no-stop-signal blocks, participants were instructed to speed up in subsequent stop signal blocks. However, since it is important that stop rates are sufficiently high, feedback to speed up was only given if more than 40% successful inhibitions (corrected for omissions) were made. In the base stop signal block, the stimulus onset asynchrony (SOA) between go and stop stimulus was fixed at 250ms. In the following experimental blocks the SOA was set, based on performance in the previous block, to yield a 50% inhibition rate. For the experimental blocks, the stop signal onset was jittered around 99ms around the SOA. If, even after the dynamic SOA correction,

the stop rate (corrected for omissions) in experimental blocks was under 40%, participants were instructed to respond slightly slower to the go stimuli. For all stop signal blocks, the trials were randomized with the exception that no more than three stop signal trials would occur in succession. The posttest differed with respect to the pretest in that three experimental blocks followed the base stop signal block. After these blocks the response-go-stimulus assignment switched and the base block plus three experimental blocks were presented again.

Procedure

The experiment consisted of two sessions separated by at least one week, and differed with respect to the type of chewing gum administered (nicotine / placebo). After participants signed the informed consent and passed the medical interview, CO levels were assessed to verify smoking abstinence. Subsequently, participants filled out the Profile Of Mood States (POMS) questionnaire, to assess subjective effects, and performed in two pretests, the SST and a Visual Spatial Cueing task (results described elsewhere). After the pretests, heart rate and blood pressure were assessed and the EEG cap was placed. Participants were then requested to start chewing the chewing gum at a rate of approximately one chew per three seconds. 20 minutes after administration of the gum (t=0.20h), the POMS was administered again and at t=0.25h, heart rate and blood pressure were assessed again. Participants performed in the posttests at t=0.30h for in total two hours. The duration of the SST was in total 50 minutes including 1.5 minute breaks between blocks. Between the two tasks participants were provided with a 10 minute break. Task order and block order within tasks (with respect to response-stimulus assignment) was counterbalanced across participants. Cardiovascular assessment took place again at t=2.35h, and afterwards participants were dismissed from the experiment.

EEG acquisition

EEG was recorded using the ActiveTwo system (Biosemi Inc., Amsterdam) (Metting van Rijn, Peper, & Grimbergen, 1990). 64 Electrodes were placed in a 10/20 cap, and HEOG and VEOG were recorded from the electrodes placed at the outer canthi of the eye and supra and infraorbitally to the eye. Sampling rate was set at 2048 Hz, and filtering was at DC to 400 Hz.

Behavioural Analysis

The SSRT was calculated following the procedure outlined by de Jong et al. (1990). The proportion of successful stop trials was calculated and corrected for omissions (Tannock, Schachar, Carr, Chajczyk, & Logan, 1989). Reaction times, within the 150 – 1500 ms range, on go trials were rank ordered from shortest to longest reaction times. The specific reaction time on this vector was determined by multiplying the number of reaction times by one minus the corrected stop rate. Finally, the SSRT was calculated by subtracting the average go – stop SOA from the determined reaction time on the RT vector. Per participant and separately for pre- and posttest and session, the SSRT values were averaged over experimental blocks.

EEG analysis

Brain Vision Analyzer was used for all EEG analyses. EEG was offline re-referenced to the right-mastoid and down sampled to 250 Hz. An offline high pass filter of 0.1592 Hz, low pass filter of 30 Hz and notch filter of 50 Hz were used. EEG was segmented in 2600ms go signal locked epochs and corrected for eye movements and blinks using the algorithm proposed by (Gratton, Coles, & Donchin, 1983). Epochs with remaining artifacts were rejected using a 100 microvolt difference criterion. Epochs were then separated into epochs containing only failed stops and epochs containing only successful stops. These epochs were baseline corrected and further segmented separately into 1652 ms go stimulus locked epochs and stop stimulus locked epochs. Subsequently, for each category these epochs were averaged. Adjacent Response Filtering (ADJAR) level II was used to remove response related electrophysiological activity from stop-signal locked averaged epochs (Woldorff, 1993). Corrected stop signal locked epochs were baseline corrected with baseline set at 0 – 50 ms to correct for possible remaining response related activity (Lansbergen et al., 2007). Analyses were performed only on the stop-signal locked epochs for failed and successful stops.

After inspection of grand average waveforms and based on Schmajuk et al. (2006), the stop N2 (stop = contrast successful minus failed stops) was quantified as the mean amplitude within 172 – 192 ms at electrode FC4. The stop P3 was initially analyzed at cz within window 220 – 280 ms. However, for the selected sample, this window was adjusted to 212 – 272 ms, so that the window of analysis fits exactly around the stop P3 peaks (peak nicotine: 240 ms, placebo: 244 ms).

Sample selection

Analyses were also performed on half of the sample in which the effect of nicotine was thought to be most pronounced. As nicotine has well known effects on cardiovascular variables (Fisher, Daniels, Jaworska, Knobelsdorf, & Knott, 2012; Wignall & de Wit, 2011), diastolic blood pressure was used as a proxy for individual drug-responsivity, under the assumption that the peripheral effect is positively correlated with the central effect of nicotine. Specifically, analyses were performed on the sample (n=8) in which diastolic blood pressure was most enhanced in response to nicotine. Blood pressure data for T1 at one session (before drug administration) was missing for one participant. For the split-half analysis, data from T1 from the other session was used for this participant. For the analyses of the effect of nicotine on cardiovascular measures in the entire sample, this participant was excluded.

Split-half analyses were performed comparing half of the sample with longest SSRTs on the pretest (slow stoppers) to half of the sample with the shortest SSRTs (fast stoppers) with respect to the effect of nicotine (versus placebo) on SSRT on the posttest, and on the stop N2 and stop P3. Furthermore, correlation analyses were performed with on the one hand SSRT on the pretest and on the other hand the nicotine induced effect (compared to placebo) on SSRT, stop N2 and stop P3.

RESULTS

Cardiovascular

Cardiovascular data are depicted in figure 1. Nicotine significantly increased diastolic blood pressure and heart rate as compared to placebo as indicated by a significant drug (placebo - nicotine) x time (pretest - posttest) interaction, respectively $F(1,14) = 11.69$, $p < 0.01$, $F(1,14) = 4.68$, $p < 0.05$. Systolic blood pressure was not affected by nicotine.

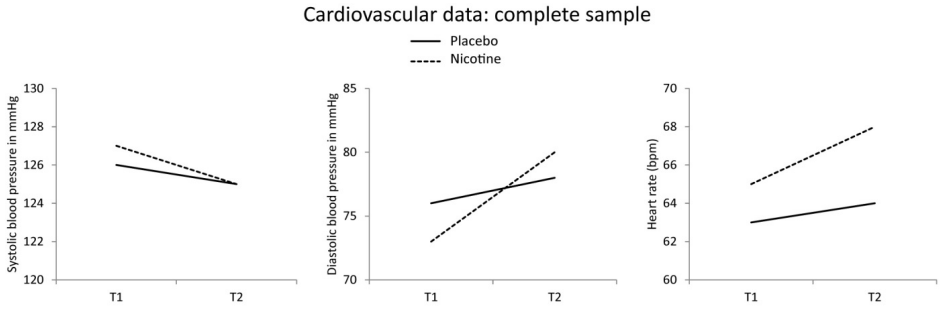

Figure 1. Blood pressure and heart rate at T1 and T2 after placebo and after nicotine.

Behaviour

Performance data for pre and post-measurements, separately for placebo and nicotine

In the total sample (figure 1) and in strong diastolic responders, nicotine did not affect any of the behavioural variables, or more specifically, none of the time (pre - post) x drug (placebo nicotine) interactions proved significant. It may be that the inclusion of pretest data induced increased variance, and this may obscure a possible drug induced effect. As can be seen in figure 1, an effect of drug on SSRT seems apparent. Hence, we investigated whether the inclusion of pretest data induced enhanced noise in the analysis pertaining to the drug effect on SSRT. Coefficients of variation (CVs; CV = σ/μ) were computed pertaining to this parameter for both post minus pretreatment values and for posttreatment values only. CVs were computed specifically for the placebo condition. For the contrast between pre- and posttreatment SSRT values, the CV was 3.54, for posttreatment data alone the CV was 0.14. Given that inclusion of pretreatment values in the design increases rather than decreases variance, a separate analysis of post-treatment data seemed justified. This resulted in a significant nicotine induced reduction of SSRT ($F(1,15) = 4.52$, $p = 0.05$).

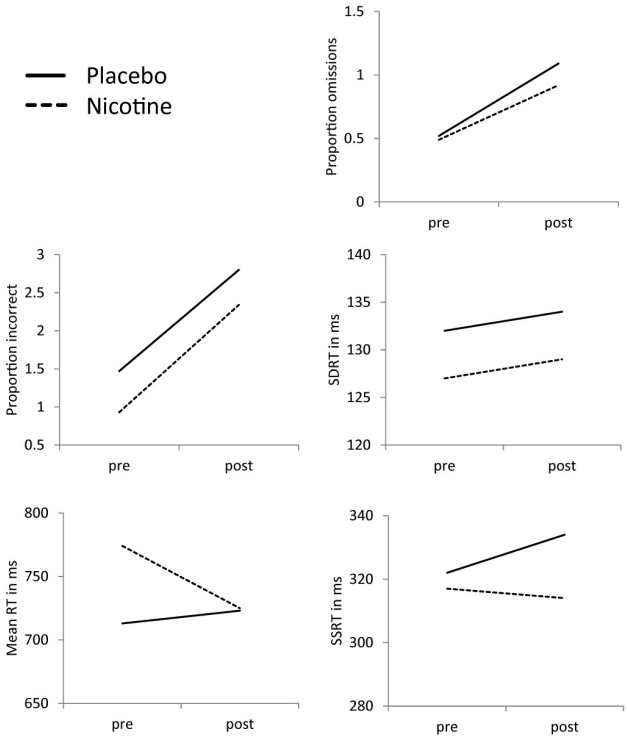

Figure 2. Performance data (n=16) pre and post administration of nicotine, for placebo and nicotine respectively.

EEG

Stop N2

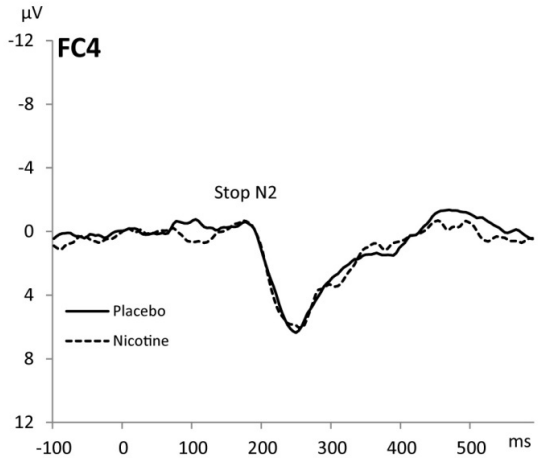

Figure 3. The Stop N2 (172 – 192 ms) for placebo and nicotine at electrode FC4.

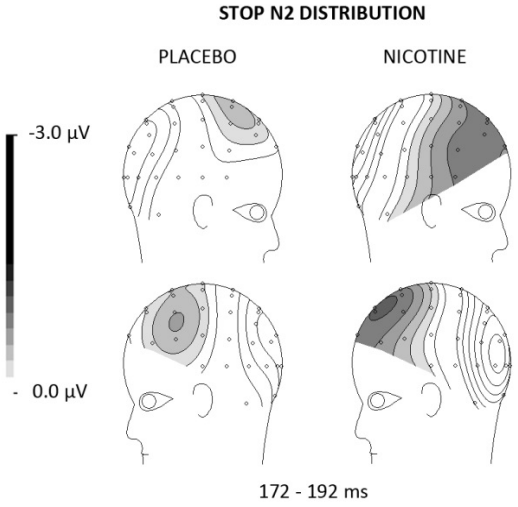

Figure 4. The distribution of the stop N2, for placebo and nicotine respectively.

The stop N2 (complete sample), and its distribution for nicotine and placebo are depicted in figures 3 and 4. The N2 was not significantly modulated by stopping success (no main effect of stopping success); hence the stop N2 was not significant. The drug x stopping success interaction was not significant, indicating that nicotine did not affect the stop N2. Nicotine did also not affect the stop N2 in strong diastolic responders. The lack of a stopping-success main effect was addressed further by a post-hoc analysis of half of the sample with the most pronounced stop N2 under placebo, which revealed a significant nicotine induced reduction of the stop N2 ($F(1,7) = 9.31$, $p < 0.05$). While this could indicate a floor effect with respect to the nicotine manipulation in the whole sample, another interpretation would be in terms of regression to the mean. One way to address this possibility is defining the subsample based on the largest stop N2s in the average across placebo and nicotine, for which the nicotine effect on the stop N2 was no longer significant. Admittedly, using this average to select the sample results in an a priori weakening of the nicotine effect on stop N2. It can be concluded however that, if anything, nicotine reduces rather than enhances stop N2.

Stop P3

As depicted in figure 5, stopping success significantly affected the P3 in the complete sample as quantified within the 220 – 280 ms interval at Cz ($F(1,15) = 57.61$, $p < 0.001$). For both nicotine and placebo, the stop P3 had a central distribution. In the complete sample, nicotine did not affect the stop P3. However, as depicted in figure 7, for half of the sample with the most pronounced effect of nicotine on diastolic blood pressure, nicotine did enhance the stop P3 (quantified in window 212 – 272 ms; ($F(1,7) = 6.99$, $p < 0.05$). Again, in this sample, the stop P3 had a central distribution (figure 8). Figure 7 suggests a short-latency effect of nicotine in the 88-148 ms interval, but post-hoc analysis of this difference did not reveal significance

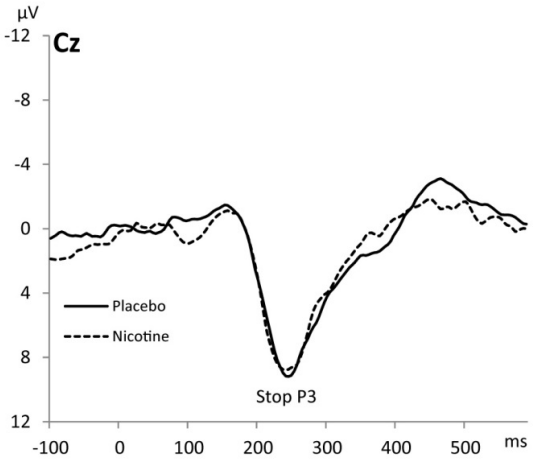

Figure 5. The stop P3 (220 – 280 ms) for placebo and nicotine at electrode Cz.

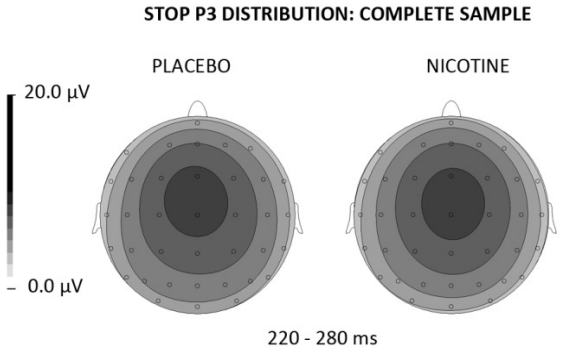

Figure 6. The distribution of the stop P3, separately for placebo and nicotine.

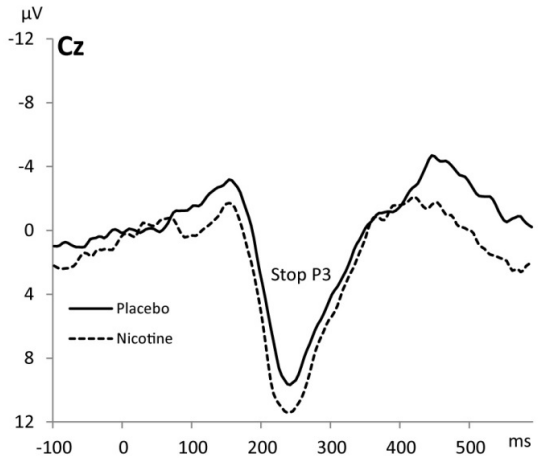

Figure 7. For half of the sample with the strongest effect of nicotine on diastolic blood pressure: The stop P3 (212 – 272 ms) for placebo and nicotine at electrode Cz.

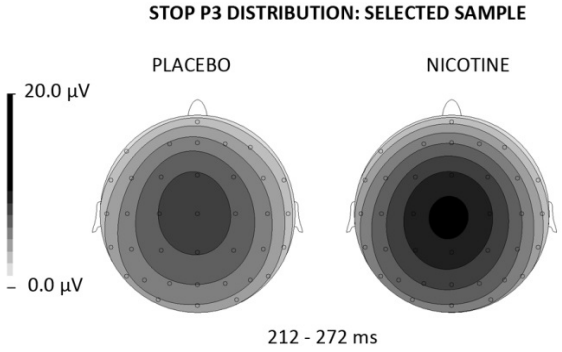

Figure 8. For half of the sample with the strongest effect of nicotine on diastolic blood pressure: The distribution of the stop P3, separately for placebo and nicotine.

Individual differences with respect to baseline SSRT and drug response

With respect to the complete sample, drug response did not depend on baseline impulsivity. Firstly, the group (slow versus fast stoppers) x prepost x drug interaction with respect to SSRT (posttest) and the stop N2 and stop P3 was not significant. Secondly, no correlation was evident between on the one hand, SSRT on the pretest and, on the other hand, drug response on SSRT (with and without inclusion of pretest data), stop N2 and stop P3.

DISCUSSION

Several studies suggested that cholinergic facilitation by nicotine positively affects inhibitory functioning (Potter et al., 2012; Wignall & de Wit, 2011). However, there is a significant lack of studies assessing the effect of cholinergic manipulation specifically on brain activity indices of inhibition. As outlined in the introduction, reference to brain activity is important since the relevant behavioural output in the SST, the SSRT, depends on assumptions which prove hard to verify. In the current study we combined the SST with EEG, and investigated the effect of nicotine on brain activity indices of inhibition in a sample of normal healthy participants. Results show that nicotine significantly affected cardiovascular measures, namely diastolic blood pressure and heart rate. Importantly, nicotine significantly augmented inhibition related electrophysiological activity as indicated by a nicotine induced enhancement of the stop P3, but the effect was restricted to strong diastolic responders. Nicotine significantly reduced SSRT, but the effect was restricted to post treatment data. Nicotine did not affect other performance measures, neither in the entire sample, nor in diastolic responders.

With respect to the lack of effect in the pre-post design (SSRT) or in the complete sample (stop P3), one possibility is that the effect of 2 mg nicotine in normal healthy participants is overall quite low in the SST, as is suggested by recent studies (Bekker et al., 2005; Potter et al., 2012; Wignall & de Wit, 2011). However, at least for healthy non-smokers, a higher dosage of 4mg does not seem to result in stronger central effects (Thiel & Fink, 2007) and, more importantly, is unadvisable due to increased adverse effects (Nyberg et al., 1982). Thus, it may be that in healthy non-smoking participants, there is not enough room for improvement. Indeed, Potter et al. (2012) reported a significant nicotine induced reduction in SSRT in impulsive participants (non-smokers), whereas in normal healthy controls (also non-

smokers) no effect of nicotine on SSRT was evident. In a related vein, we also investigated whether there was a difference in nicotine response on the SSRT, stop N2 and stop P3 comparing half of the sample with long SSRTs to half of the sample with short SSRTs. However, no group interaction was found, and also no correlation between the pretest mean SSRT and the drug induced response on SSRT, stop N2 or P3 was evident. Hence, the lack of effect of nicotine on the dependent variables may not be a result of a lack of room for improvement. It must be noted though, that in Potter et al. (2012) the high impulsive group (long SSRTs) were ADHD patients with a SSRT score > 1.5 SD above the mean SSRT norm, hence, in this group there may be much more room for improvement.

With respect to performance data, it must be noted that separate analysis of post-treatment data (excluding pre-treatment data) yielded a significant reducing effect of nicotine on SSRT. One argument for also analyzing post-treatment data (in the absence of evidence of a time x drug interaction) was that the pretest consisted of substantially less blocks (hence, trials) as compared to the posttest. Therefore, inclusion of pre-treatment data could have resulted in a reduced signal-to-noise ratio and thus, reduced statistical power. To address this issue, we calculated the coefficient of variation for the placebo condition pertaining to the SSRT, with and without inclusion of pretest data. As the signal-to-noise ratio was significantly reduced after inclusion of the pretest data, it seemed justified to also analyze the SSRT without inclusion of pretest data.

The significant reduction of SSRT under nicotine, albeit restricted to post-treatment data, seems to contrast previous studies with healthy non-smokers and smokers (Bekker et al., 2005; Potter et al., 2012; Wignall & de Wit, 2011), in which no effect on SSRT was evident. However, these studies administered nicotine via transdermal patches, not via chewing gum. Importantly, the time until maximum plasma levels of nicotine are reached (t max), as well as the duration of the effect, may differ widely between the two routes of administration (Gorsline, Okerholm, Rolf, Moos, & Hwang, 1992; Vossel et al., 2008). Hence, it may be speculated that a relatively more acute nicotinic challenge, such as with nicotine prolacrilex gum, may assert a stronger effect on SSRT.

One contributing factor to a possible small drug effect is the combination of the rather long experiment and the relatively short half-life (2 hours) of nicotine. Half of the sample started with the SST as the second task, and that implies that in these participants

almost half of the nicotine is already metabolized. It is plausible that with subtler drug effects in case of relatively low blood plasma levels of nicotine, interindividual variance is relatively high. As nicotine clearly affects cardiovascular variables (i.e. (Fisher et al., 2012; Wignall & de Wit, 2011)), our strategy was to use diastolic blood pressure as a proxy for individual responsivity to nicotine to account for this variability in responsivity. In the sample in which the diastolic enhancing effect of nicotine was most pronounced, a nicotine induced stop P3 enhancement was evident, but without an effect on the stop N2. It is tempting to conclude that in this sample, nicotine had a differential effect on the ventral versus dorsal mechanism of inhibition (M. Corbetta et al., 2008; Maurizio Corbetta & Shulman, 2002; Kenemans & Kähkönen, 2011). In other words, it might be suggested that nicotine affected the SFG, as indicated by its effect on the stop P3, while unaffecting the rIFG as indicated by the lack of effect on the stop N2. However, it should be stressed that the modulation by success of stopping with respect to the N2 was not significant for either the complete sample, or the split-half sample. Without a significant modulation of the N2 by stopping success, the effect of drug on the modulation, or stop N2 is difficult to assess. The P3 on the other hand, was significantly modulated by success in both the total sample, as well as in the split-half sample. The larger effect size makes a possible drug effect easier to detect. To provide a solution for the small stop N2, we performed the analysis pertaining to the effect of nicotine on the stop N2 on the sample in which the stop N2 was most pronounced at placebo. This yielded a significant reduction of the stop N2 under nicotine as compared to placebo. However, this may be a result of a regression to the mean effect. Performing the same analysis on the sample in which the stop N2 was most pronounced across drug conditions, did not yield a significant drug effect with respect to the stop N2. While this sample selection may have weakened a possible nicotine effect, it may be concluded that if anything the effect of nicotine is a reduction and not an enhancement of the stop N2.

With respect to the stop P3, the modulation of the P3 by stopping success could reflect error related negativity (ERN) in case of an error related to failed inhibition (Falkenstein, Hohnsbein, Hoormann, & Blanke, 1991; Gehring, Goss, Coles, Meyer, & Donchin, 1993). However, results by Lansbergen et al. (2007) suggested otherwise. In their experiment, slow and fast stoppers were contrasted in terms of SSRT and stop P3. Results showed that long SSRTs were associated with reduced stop P3s. However, the reduction in

stop P3s was not associated with ERN effects, as ERNs did not differ between slow and fast stoppers.

To conclude, in non-smokers nicotine significantly improved inhibition both at the behavioral level (SSRT) as well as at the level of a dorsally based electrocortical process related to inhibition (stop P3), although the latter effect was found only for stronger cardiovascular responders to nicotine. As to a more right-hemisphere ventrally based inhibitory mechanism (stop N2), no indication for enhancement by nicotine was observed.

ACKNOWLEDGMENTS

This study was financially supported by the open MAGW grant from NWO (Dutch organization for scientific research), NWO: 400-07-130.

REFERENCES

Aron, A. R., Fletcher, P. C., Bullmore, E. T., Sahakian, B. J., & Robbins, T. W. (2003). Stop-signal inhibition disrupted by damage to right inferior frontal gyrus in humans. *Nat Neurosci, 6*(2), 115-116.

Band, G. P., van der Molen, M. W., & Logan, G. D. (2003). Horse-race model simulations of the stop-signal procedure. *Acta Psychol (Amst), 112*(2), 105-142.

Bekker, E. M., Bocker, K. B., Van Hunsel, F., van den Berg, M. C., & Kenemans, J. L. (2005). Acute effects of nicotine on attention and response inhibition. *Pharmacol Biochem Behav, 82*(3), 539-548.

Benowitz, N. L., Porchet, H., Sheiner, L., & Jacob, P., 3rd. (1988). Nicotine absorption and cardiovascular effects with smokeless tobacco use: comparison with cigarettes and nicotine gum. *Clin Pharmacol Ther, 44*(1), 23-28.

Corbetta, M., Patel, G., & Shulman, G. L. (2008). The reorienting system of the human brain: from environment to theory of mind. *Neuron, 58*(3), 306-324.

Corbetta, M., & Shulman, G. L. (2002). Control of goal-directed and stimulus-driven attention in the brain. *Nature Reviews, 3*, 201 - 215.

De Jong, R., Coles, M. G., & Logan, G. D. (1995). Strategies and mechanisms in nonselective and selective inhibitory motor control. *J Exp Psychol Hum Percept Perform, 21*(3), 498-511.

De Jong, R., Coles, M. G., Logan, G. D., & Gratton, G. (1990). In search of the point of no return: the control of response processes. *J Exp Psychol Hum Percept Perform, 16*(1), 164-182.

Falkenstein, M., Hohnsbein, J., Hoormann, J., & Blanke, L. (1991). Effects of crossmodal divided attention on late ERP components. II. Error processing in choice reaction tasks. *Electroencephalogr Clin Neurophysiol, 78*(6), 447-455.

Fisher, D. J., Daniels, R., Jaworska, N., Knobelsdorf, A., & Knott, V. J. (2012). Effects of acute nicotine administration on behavioral and neural (EEG) correlates of working memory in non-smokers. *Brain Res, 1429*, 72-81.

Gehring, W. J., Goss, B., Coles, M. G. H., Meyer, D. E., & Donchin, E. (1993). A Neural System for Error Detection and Compensation. *Psychological Science, 4*(6), 385-390.

Gorsline, J., Okerholm, R. A., Rolf, C. N., Moos, C. D., & Hwang, S. S. (1992). Comparison of plasma nicotine concentrations after application of nicoderm (nicotine transdermal system) to different skin sites. *J Clin Pharmacol, 32*(6), 576-581.

Gratton, G., Coles, M. G., & Donchin, E. (1983). A new method for off-line removal of ocular artifact. *Electroencephalogr Clin Neurophysiol, 55*(4), 468-484.

Kenemans, J. L., Bekker, E. M., Lijffijt, M., Overtoom, C. C., Jonkman, L. M., & Verbaten, M. N. (2005). Attention deficit and impulsivity: selecting, shifting, and stopping. *Int J Psychophysiol, 58*(1), 59-70.

Kenemans, J. L., & Kähkönen, S. (2011). How human electrophysiology informs psychopharmacology: from bottom-up driven processing to top-down control. *Neuropsychopharmacology, 36*(1), 26-51.

Kollins, S. H., McClernon, F. J., & Fuemmeler, B. F. (2005). Association between smoking and attention-deficit/hyperactivity disorder symptoms in a population-based sample of young adults. *Arch Gen Psychiatry, 62*(10), 1142-1147.

Lansbergen, M. M., Bocker, K. B., Bekker, E. M., & Kenemans, J. L. (2007). Neural correlates of stopping and self-reported impulsivity. *Clin Neurophysiol, 118*(9), 2089-2103.

Logan, G. D., Cowan, W. B., & Davis, K. A. (1984). On the ability to inhibit simple and choice reaction time responses: a model and a method. *J Exp Psychol Hum Percept Perform, 10*(2), 276-291.

Mangun, G. R., & Hillyard, S. A. (1991). Modulations of sensory-evoked brain potentials indicate changes in perceptual processing during visual-spatial priming. *J Exp Psychol Hum Percept Perform, 17*(4), 1057-1074.

Meinke, A., Thiel, C. M., & Fink, G. R. (2006). Effects of nicotine on visuo-spatial selective attention as indexed by event-related potentials. *Neuroscience, 141*(1), 201-212.

Metting van Rijn, A. C., Peper, A., & Grimbergen, C. A. (1990). High-quality recording of bioelectric events. Part 1. Interference reduction, theory and practice. *Med Biol Eng Comput, 28*(5), 389-397.

Nyberg, G., Panfilov, V., Sivertsson, R., & Wilhelmsen, L. (1982). Cardiovascular effect of nicotine chewing gum in healthy non-smokers. *Eur J Clin Pharmacol, 23*(4), 303-307.

Overtoom, C. C., Kenemans, J. L., Verbaten, M. N., Kemner, C., van der Molen, M. W., van Engeland, H., et al. (2002). Inhibition in children with attention-deficit/hyperactivity disorder: a psychophysiological study of the stop task. *Biol Psychiatry, 51*(8), 668-676.

Posner, M. I., Snyder, C. R., & Davidson, B. J. (1980). Attention and the detection of signals. *J Exp Psychol, 109*(2), 160-174.

Potter, A. S., Bucci, D. J., & Newhouse, P. A. (2012). Manipulation of nicotinic acetylcholine receptors differentially affects behavioral inhibition in human subjects with and without disordered baseline impulsivity. *Psychopharmacology (Berl), 220*(2), 331-340.

Potter, A. S., & Newhouse, P. A. (2004). Effects of acute nicotine administration on behavioral inhibition in adolescents with attention-deficit/hyperactivity disorder. *Psychopharmacology (Berl), 176*(2), 182-194.

Potter, A. S., & Newhouse, P. A. (2008). Acute nicotine improves cognitive deficits in young adults with attention-deficit/hyperactivity disorder. *Pharmacol Biochem Behav, 88*(4), 407-417.

Potter, A. S., Newhouse, P. A., & Bucci, D. J. (2006). Central nicotinic cholinergic systems: a role in the cognitive dysfunction in attention-deficit/hyperactivity disorder? *Behav Brain Res, 175*(2), 201-211.

Schmajuk, M., Liotti, M., Busse, L., & Woldorff, M. G. (2006). Electrophysiological activity underlying inhibitory control processes in normal adults. *Neuropsychologia, 44*(3), 384-395.

Tannock, R., Schachar, R. J., Carr, R. P., Chajczyk, D., & Logan, G. D. (1989). Effects of methylphenidate on inhibitory control in hyperactive children. *J Abnorm Child Psychol, 17*(5), 473-491.

Thiel, C. M., & Fink, G. R. (2007). Visual and auditory alertness: modality-specific and supramodal neural mechanisms and their modulation by nicotine. *J Neurophysiol, 97*(4), 2758-2768.

Thiel, C. M., & Fink, G. R. (2008). Effects of the cholinergic agonist nicotine on reorienting of visual spatial attention and top-down attentional control. *Neuroscience, 152*(2), 381-390.

Verbruggen, F., Chambers, C. D., & Logan, G. D. Fictitious inhibitory differences: how skewness and slowing distort the estimation of stopping latencies. *Psychol Sci, 24*(3), 352-362.

Vossel, S., Thiel, C. M., & Fink, G. R. (2008). Behavioral and neural effects of nicotine on visuospatial attentional reorienting in non-smoking subjects. *Neuropsychopharmacology, 33*(4), 731-738.

Wignall, N. D., & de Wit, H. (2011). Effects of nicotine on attention and inhibitory control in healthy nonsmokers. *Exp Clin Psychopharmacol, 19*(3), 183-191.

Woldorff, M. G. (1993). Distortion of ERP averages due to overlap from temporally adjacent ERPs: analysis and correction. *Psychophysiology, 30*(1), 98-119.

CHAPTER 6

DIFFERENCES BETWEEN NICOTINE-ABSTINENT SMOKERS AND NON-SMOKERS IN TERMS OF VISUOSPATIAL ATTENTION AND INHIBITION BEFORE AND AFTER NICOTINE ADMINISTRATION

Logemann, H.N.A., Böcker, K.B.E., Deschamps, P.K.H., Kemner, C., Kenemans, J.L.

ABSTRACT

Health hazards of smoking are well known, but many people still smoke. Nicotine facilitates cholinergic neurotransmission. This system is implicated in visuospatial attention and inhibition, however the exact role is still unclear. Two key mechanisms in visuospatial attention are bias and disengagement. Bias refers to neuronal signals that enhance the sensitivity of sensory cortex; disengagement is the decoupling of attention. Previous studies suggest that nicotine affects disengagement and (related) inhibition. Excessive smoking is associated with numerous psychopathologies among which those involving attention deficit, and the condition of abstinence in 'healthy' smokers may resemble that of deficits in bias and/ or disengagement. Accordingly, nicotine abstinent smokers as opposed to non-smokers may present more room for improvement by the acute effect of nicotine on these mechanisms. In the current study, 16 smokers and 16 non-smokers (male students) performed in a visuospatial cueing (VSC) task, to assess bias and disengagement, and stop signal task (SST) to assess inhibition. A single blind, prepost crossover design was used. It was hypothesized that, in a baseline comparison, for nicotine abstinent smokers the stop signal reaction time (SSRT) in the SST would be enhanced (reflecting poor inhibitory motor control), as would be the validity effect on reaction time in the VSC (indicating poor disengagement). Furthermore, it was hypothesized that a nicotine induced effect on SSRT and on the validity effect would be enhanced in smokers. Results indicated no baseline differences between groups. Post-hoc analyses suggested that nicotine reduces SSRT more in non-smokers, relative to smokers.

INTRODUCTION

Today, in spite of the well known related health hazards many people still smoke. Nicotine facilitates cholinergic neurotransmission (Wonnacott, Irons, Rapier, Thorne, & Lunt, 1989), and nicotine induced cognitive benefits have been reported (Heishman, Kleykamp, & Singleton, 2010; Newhouse, Potter, & Singh, 2004; Rezvani & Levin, 2001). Acetylcholine has been implicated in (visuospatial) attention and inhibition, however the exact role is still unknown. Interestingly, smoking is correlated with Attention Deficit / Hyperactivity Disorder, which is marked by anomalous functioning of mechanisms of attention and inhibition and it has been suggested that ADHD patients smoke to self-medicate and thus alleviate some of the pathology (Kollins, McClernon, & Fuemmeler, 2005; Potter, Newhouse, & Bucci, 2006). It is tempting to suggest that nicotine abstinent smokers may, in some way resemble (sub-clinical) ADHD patients.

Two mechanisms are of crucial importance in visuospatial attention, bias and disengagement (M. Corbetta, Patel, & Shulman, 2008; Maurizio Corbetta & Shulman, 2002). Bias refers to neuronal signals that modulate the sensitivity of sensory cortex and the ensuing enhanced neural processing of the stimulus to which attention is directed to, whereas disengagement refers to the decoupling of attention. A classic paradigm to investigate visuospatial attention is the visuospatial cueing task, also called Posner Paradigm (Posner, Snyder, & Davidson, 1980). In this task a cue points to the right or left visual hemifield. In the majority of trials, a target, to which a response is required, is presented at the cued location. The benefit in terms of reaction time (RT) of valid cueing is termed the validity effect. It has been shown that nicotine effectively reduces the validity effect (Meinke, Thiel, & Fink, 2006; Thiel & Fink, 2008; Vossel, Thiel, & Fink, 2008). More specifically, nicotine seems to reduce RTs on invalid trials, suggesting a facilitation of disengagement (Meinke et al., 2006; Thiel & Fink, 2008; Witte, Davidson, & Marrocco, 1997). It should be noted that a reduction in validity effect may theoretically also imply reduced bias. However, recent fMRI studies did not find an effect of nicotine on attentional modulation in the occipital cortex, which would reflect bias (Thiel & Fink, 2008; Vossel et al., 2008). One isolated EEG study however, suggested a possible effect on disengagement related electrophysiological activity, but this result was not described in detail and seemed post-hoc (Meinke *et al.*, 2006).

Inhibition has been investigated with the Stop Signal Task (SST) (De Jong, Coles, Logan, & Gratton, 1990; Logan, Cowan, & Davis, 1984). In the SST, go stimuli are presented to which a response is required. In a minority of trials, the go stimulus is followed by a stimulus signaling to withhold a prepotent response. The relevant behavioural outcome is the Stop Signal Reaction Time (SSRT), thought to reflect inhibitory motor control. There is an obvious conceptual link between inhibition and disengagement. Importantly, inhibition (as indexed by the SSRT) is negatively affected by disruptions of the right Inferior Frontal Gyrus (Aron, Fletcher, Bullmore, Sahakian, & Robbins, 2003), the same neurobiological substrate as associated with disengagement and reorienting of attention (M. Corbetta et al., 2008; Maurizio Corbetta & Shulman, 2002). Indeed, nicotine effects on inhibition mirror those on disengagement. More specifically, in the SST, nicotine has been shown to reduce the SSRT in ADHD patients indicating improved inhibition (Potter, Bucci, & Newhouse, 2012; Potter & Newhouse, 2004, 2008) and as mentioned, in healthy participants nicotine decreases reaction times on invalidly cued targets in the VSC, indicating facilitated disengagement. It should be noted that Bekker et al. (2005) did not find an effect of nicotine on SSRT in healthy nicotine abstinent smokers. This may be related to individual differences in baseline stopping performance and associated room for improvement. Results of Potter et al. (2012) indicate that the reduction of SSRTs in response to nicotine may be restricted to groups in which there is sufficient room for improvement. Specifically, Potter et al. (2012) compared nonsmoking ADHD patients with relatively long SSRTs to healthy nonsmoking controls with respect to effect of nicotine on SSRT. Nicotine reduced SSRTs only in the ADHD group, indicating facilitated inhibition only for slow stoppers. An obvious hypothesis follows that, in relation to room for improvement, the acute effect of nicotine would be enhanced in nicotine abstinent smokers as opposed to non-smokers. Alternatively, it has been argued that smokers show drug-tolerance (Srivastava, Russell, Feyerabend, Masterson, & Rhodes, 1991). This would lead to the hypothesis that nicotine asserts a stronger effect in non-smokers than in (nicotine abstinent) smokers (hypothesis 2).

In sum, smoking has been associated with ADHD that is marked by deficits in bias, disengagement and inhibition. Nicotine seems to positively affect both disengagement and inhibition. Hence, it may be that smokers smoke (at least in part) to alleviate some (subclinical) deficits of disengagement and inhibition. In a similar vein, the acute effect of nicotine on these

parameters is likely to be larger for smokers as opposed to nonsmokers since (if the first hypothesis is true) smokers have more room for improvement, which would be in line with Potter et al. (2012). In the current study, 16 smokers and 16 nonsmokers performed in a VSC task and SST task in a prepost, single blind, placebo controlled, crossover experimental setup. It was hypothesized that on baseline, smokers would have a longer SSRT and a larger validity effect as opposed to nonsmokers. Furthermore, it was hypothesized that the acute effect of nicotine on these parameters would be larger for smokers as opposed to nonsmokers.

METHODS

Participants

Healthy male students were recruited from Utrecht University. In total 16 smokers and 16 non-smokers participated. The age range was 18 – 29 years old (M: 22 ± 3). Non-smokers were on average 21 years old (± 3), and smokers were on average 24 years old (± 3). Participants had normal or corrected to normal vision. Informed consent was given prior to participation. The study was approved by the medical ethics committee of the University Medical Center Utrecht, and conducted in accordance with the declaration of Helsinki.

Drug treatment

Acetylcholine neurotransmission was facilitated using nicotine polacrilex gum (Nicorette Freshmint 2mg). A dosage of 2mg was implemented, since higher doses of 4mg and higher do not seem to result in a stronger central effect, and may result in adverse effects in healthy non-smoking subjects (Nyberg, Panfilov, Sivertsson, & Wilhelmsen, 1982; Thiel & Fink, 2007). Participants were instructed to chew once every three seconds. This instruction was based on Vossel et al. (2008) and results in plasma values of 3.57 ng/ml (Vossel *et al.*, 2008). Blood plasma peak concentrations of nicotine will be reached after 30 minutes of chewing, and nicotine has a half-life of approximately 2 hours according to the Summary of Product Characteristics (SPC) of Nicorette Freshmint 2mg. Sportlife smashmint was used as the placebo gum.

Tasks

Visuospatial Cueing (VSC) Task

The VSC task was a combination of the paradigm of Van den Lubbe et al. (2006), and Mangun et al. (1991). Each trial started with a fixation dot which was presented for 600 ms. Subsequently, the dot was replaced by the cue presented for 400 ms. The cue was a diamond (width 1.3°, height 0.7°) in which the left and right half of differed in color (green/red). After the cue, the fixation dot was again presented for 600 ms and followed by one of two potential targets. The targets were white bars differing in height (width 0.8°, height 2.4°) and were presented in the left or right visual hemifield (6.4° from the center of the screen). The total duration of one single trial was 3100 ms.

The pretest version of the task consisted of two blocks, each block started with an instruction. The first block served as a practice block and consisted of 32 trials, of which 8 were invalid trials. Invalid trials were trials in which the target was invalidly cued, valid trials were trials in which the target was validly cued. The second block was the experimental block that was used for data analysis, and consisted of 256 trials of which 64 were invalid trials. The posttest version of this task started with the block in which cue-color specification and response-target assignment was equal to the blocks in the pretest. The four experimental blocks in the posttest differed from each other in terms of cue-color specification and response-target assignment. Block order was counterbalanced over participants and for each task-run; trials were semi-randomized within experimental blocks.

Stop Signal Task

The SST was modeled after Schmajuk et al. (2006). Stimuli to which a response was required (go stimuli) were presented at random and sequentially. Go stimuli consisted of an X (height and width 1.4°) or an O (height 1.4° and width 1.3°) and were presented slightly above the fixation cross which was presented at the center of the screen. In 25% of trials in the stop signal blocks, a stop signal, the letter "$" (height 1.7°, width 0.8°) was presented after the go stimulus. Participants were required to withhold a response after a stop signal. Go stimuli and stop stimuli were presented for 150 ms and trial duration varied between 1500 ms to 1800 ms. The pretest consisted of a block without stop signals consisting of 126 go trials (for estimating the baseline reaction time), a base stop signal block (for estimating the optimal SOA for

approximately 50% inhibitions in a subsequent block) and two experimental stop signal blocks (for data analysis), each consisting of 128 trials. Participants were instructed to speed up in stop signal blocks if reaction times in these blocks were more than 1.5 times the reaction time in the go stimulus only block (baseline reaction time). However, if less than 40% successful stops were made in the stop signal block, this feedback was not given. In the base stop signal block, the go-stop interval was set at 250 ms. In subsequent experimental blocks, the SOA was determined based on performance in the previous block to yield approximately 50% successful stops. In experimental blocks, the SOAs varied within a window of 99ms around the predetermined average SOA (rectangular distribution). If the stop rate (corrected for omissions) was below 40 percent, even after dynamic SOA correction, participants were instructed to make slightly slower responses to the go stimuli. With the exception of the go stimulus only block, trials were semi-randomized with the prerequisite that no more than three stop signals occur in succession. The posttest differed from the pretest in that three experimental stop signal blocks followed the base stop signal block. Subsequently, the stimulus-response assignment switched and the base block and three experimental stop signal blocks were presented again.

Procedure

Participants performed in two sessions that were separated by at least one week. Sessions differed with respect to the administered drug. Upon arrival at the lab, participants were informed again and after signing the informed consent and passing the medical interview, CO levels were assessed. Subsequently the Profile of Mood States (POMS) questionnaire was filled out and the pretest versions of the VSC and Stop Signal Task were performed. Next, blood pressure and heart rate were assessed, and an EEG cap was placed (EEG results described elsewhere) after which the chewing gum was administered (placebo or nicotine). Participants were requested to fill out the POMS again 20 minutes after drug administration (t=0.20h). At t=0.25h cardiovascular variables were assessed again, and participants performed the posttest version of the VSC and SST at t = 0.30h. The task order (VSC/SST) was counterbalanced over participants. In each task, participants were allowed to take short breaks between the blocks and between the tasks there was room for a ten minute break. Lastly, cardiovascular variables were assessed again at t=2.35h, and participants were dismissed from the experiment.

Data analyses

Stop Signal Task

The SSRT was estimated in accordance with De Jong et al. (1990). The proportion of successful stops was calculated and corrected for omissions (Tannock, Schachar, Carr, Chajczyk, & Logan, 1989). A reaction time vector was created with reaction times within a 150 ms – 1500 ms time interval on trials without stop signals (go trials) and these RTs were rank ordered ranging from shortest to longest. The x^{th} RT on this vector was calculated by multiplying the length of the RT vector by 1 minus the corrected (for omissions) percentage of inhibitions. The SSRT was then determined by subtracting the average SOA (go-stop interval) from the x^{th} RT. Lastly, the SSRTs in the experimental blocks were averaged resulting in the final averaged SSRT. For baseline analysis (of group differences without nicotine application) data from the pretest blocks (both sessions) and posttest blocks from the placebo session were averaged, separately for smokers and non-smokers.

Visuospatial Cueing Task

Reaction times on valid trials and invalid trials separately were averaged per participant and session across blocks. Reaction times outside the 100 ms – 1500 ms range were considered invalid and were not included in the analyses. The validity effect was defined as the difference in reaction time on invalidly cued targets minus validly cued targets. For baseline analysis, the same strategy was used as for analyses of the SSRT.

Post-Hoc analyses

In order to ensure sufficient room for improvement for a possible drug effect, analyses were also performed on split-half samples. More specifically, with respect to the VSC task, half of the sample (irrespective of group) with the largest validity effect size (Cohen's d) on the pretest (averaged across sessions) was selected for analyses pertaining to the validity effect. This selection resulted in a equal sample size across groups (8 smokers, 8 nonsmokers). The same strategy was used for the SST; half of the sample with the longest SSRTs was selected. This also resulted in an equal sample size across group. Analyses pertaining to the SSRT were performed on this selection.

RESULTS

Cardiovascular effects

For one participant, pretest cardiovascular values were missing for one session, so this participant was excluded. Baseline values (mean value of pretest placebo and pretest nicotine) differed between smokers and non-smokers with respect to diastolic blood pressure. More specifically, diastolic blood pressure was significantly lower for smokers as opposed to non-smokers ($F(1,29) = 7.66$, $p = 0.01$). Neither systolic, nor heart rate differed significantly between smokers and non-smokers at baseline. With respect to the acute effect of nicotine, as depicted in figure 1, diastolic blood pressure and heart rate increased in response to nicotine as evidenced by a significant time x drug interaction ($F(1,29) = 20.13$, $p < 0.001$, $F(1,29) = 10.69$, $p < 0.01$, respectively).

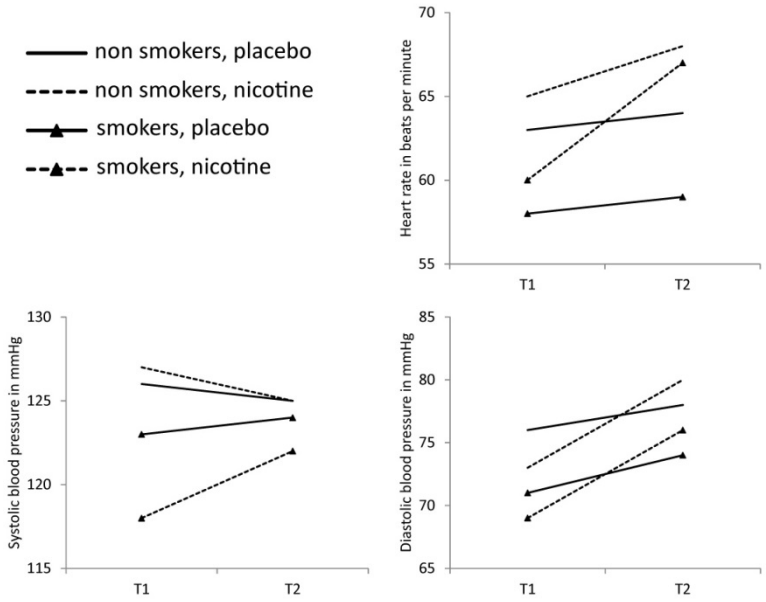

Figure 1. Systolic, diastolic blood pressure and heart rate at pre-treatment (T1) and post-treatment (T2) separately for smokers and non-smokers and separately for placebo and nicotine session.

Baseline differences smokers versus nonsmokers

With respect to the VSC task, reaction times were significantly modulated by cueing (main effect of validity, $F(1,30) = 33.69$, $p < 0.001$). However, no difference with respect to the validity effect was evident comparing smokers to non-smokers (smoker x validity interaction, $\eta_p^2 = 0.008$). Furthermore, with respect to the SST, smokers did not differ from nonsmokers in terms of SSRT (main effect of smoker, $\eta_p^2 = 0.027$).

Acute effect of nicotine on smokers and non smokers, performance data in the VSC task

As shown in figure 2, with respect to the VSC task, reaction times were again significantly modulated by cueing as indicated by a main effect of validity ($F(1,30) = 38.48$, $p < 0.001$). However, nicotine did not affect this validity effect irrespective of factor smoker. More specifically, the interaction time (pre – post) x drug x validity with respect to RT was not significant. Furthermore, the time x drug x validity x smoker interaction with respect to RT was not significant, indicating no differences between smokers and non-smokers with respect to the nicotine induced effect on the validity effect. Neither split-half analysis based on the validity effect for the pretest, nor post-hoc analysis of post-treatment only, indicated otherwise.

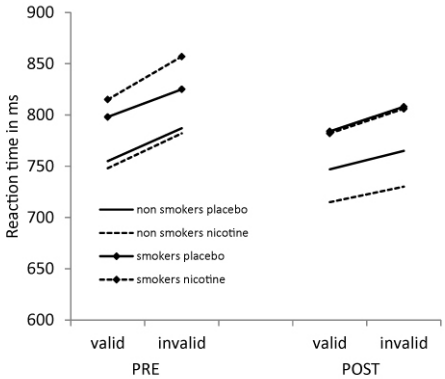

Figure 2. Reaction times on validly and invalidly cued targets prior to (PRE), and after (POST) drug administration, separately for smokers and non smokers.

Acute effect of nicotine on smokers and non-smokers, performance data in the SST

With respect to the SST task, overall, nicotine did not affect SSRT, or more specifically, the time x drug interaction was not significant. Furthermore, the time x drug x smoker interaction with respect to SSRT was not significant. However, separate post-hoc analysis of post treatment SSRTs indicated a trend towards a drug x smoker interaction ($F(1,30) = 3.66$, $p = 0.065$), showing a relative reduction in nonsmokers as opposed to smokers (figure 3). This drug x smoker (post-treatment only) interaction was significant for the split-half sample with relatively long SSRTs in the pretest ($F(1,14) = 5.48$, $p < 0.05$).

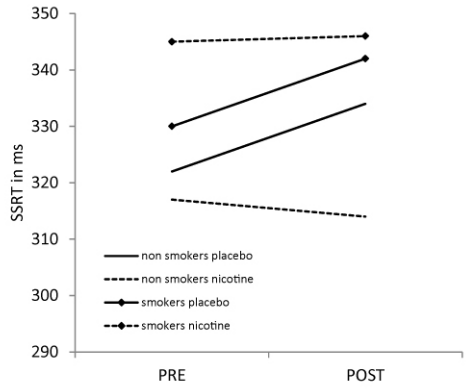

Figure 3. The SSRT in ms before (PRE) and after (POST) drug administration, separately for smokers and non-smokers.

Subjective effects

No time x drug x smoker interaction was evident with respect to any of the POMS variables (depression, $\eta_p^2 = 0.003$; anger, $\eta_p^2 = 0.013$; vigor, $\eta_p^2 < 0.001$; fatigue, $\eta_p^2 = 0.007$; tension, $\eta_p^2 = 0.033$, total mood disturbance, $\eta_p^2 = 0.007$).

DISCUSSION

Earlier studies suggest that nicotine positively affects disengagement and inhibition, but not bias. Furthermore, it has been implied that many ADHD patients smoke in order to alleviate related anomalies. It may be that smokers smoke (at least in part) to alleviate deficient functioning of mechanisms of disengagement and inhibition. If so, it is likely that nicotine abstinent smokers as opposed to nonsmokers present with more room for improvement with respect to disengagement and inhibition, and an acute effect of nicotine on these variables may be enhanced in smokers. In the current study these hypotheses were tested in a placebo controlled within-subjects crossover design. No baseline differences between smokers and nonsmokers with respect the validity effect in the VSC and SSRT in the SST were evident. With respect to the acute effect of nicotine, although restricted to post-treatment data only, results indicated that nicotine reduced SSRTs in the SST more in nonsmokers as opposed to smokers. Nicotine did not affect the validity effect on reaction time in the VSC task.

In contrast to our hypothesis, results did not indicate that nicotine abstinent smokers present with anomalies of disengagement and inhibition as compared to nonsmokers. Hence, we cannot conclude that nicotine abstinent smokers smoke to alleviate problems pertaining to these parameters; our results do not support the notion that nicotine abstinent smokers have more room for improvement (and a drug effect) with respect to disengagement and inhibition. With respect to differential effects of nicotine on bias, disengagement and inhibition, results did suggest a difference between smokers and nonsmokers. More specifically, with respect to the SST, the nicotine induced reduction of SSRT seemed more pronounced for nonsmokers as opposed to smokers, which is in contrast to our hypothesis. It should be noted though that the time x drug x smoker interaction with respect to SSRT was not significant. However, we did analyze post-treatment data separately since SSRTs were averaged over more blocks in post-treatment data, most likely resulting in a more reliable estimate of SSRT. For the post-treatment data, the drug x smoker interaction was trending towards significance. Indeed, results by Potter (2012) indicated that nicotine affects SSRT, but only in slow stoppers. Hence, we performed a split-half analysis in which we performed the analyses pertaining to SSRT only for the sample with relatively long SSRTs. For this sample (coincidentally equally distributed across groups, 8 smokers versus 8 nonsmokers), the drug x smoker interaction was significant.

The question remains why SSRT seems more strongly reduced in nonsmokers as opposed to smokers. Clearly, as evident from baseline analysis, results cannot be explained in terms of room for improvement. However, results may be explained by drug-tolerance. It may be that the effect of nicotine is less pronounced in smokers as opposed to nonsmokers as a result of repeated administration of the drug in smokers. It must be noted here that we did not find evidence of differences in peripheral responsiveness to the drug between smokers and nonsmokers. However, such effects of chronic nicotine use may be different with respect to central mechanisms. It would be interesting to investigate the effect of (a) higher dosage(s) in nicotine abstinent smokers on SSRT, which may not be possible in nonsmokers since it has been reported that side-effects may be sizeable in the absence of stronger central effects (Nyberg et al., 1982; Thiel & Fink, 2007).

With respect to the results of the VSC task, these seem to contradict results from previous studies. More specifically, we did not find evidence for an overall reduction in validity effect as a result of cholinergic challenge by nicotine. Indeed, results of Thiel et al. (2008) indicated that not all participants show clear evidence of attentional modulation as reflected in a benefit of cueing on reaction times. Thiel et al. (2008) showed that the effect of nicotine on the validity effect was restricted to the sample from which participants were excluded who did not show evidence of a clear attentional modulation. This seems straightforward since no drug effect can be assessed on the validity effect if no clear validity effect is present. In a similar vein, we performed the analyses in the split-half sample with the largest validity effect size (Cohen's d) in the pretest (averaged across session). However, even though this yields room for a possible drug effect, overall nicotine induced effects or differential effects comparing smokers to nonsmokers were not evident. One plausible explanation follows from Meinke et al. (2006) in which the authors assert that nicotine induced effects on performance are restricted to when performance is effortless. Assuming that general reaction time is (at least in part) indicative of task difficulty, results of Meinke et al. (2006) support this notion. In Meinke et al. two experiments were performed which differed with respect to task difficulty. In the first experiment, reaction times were much shorter as compared to the second experiment. Only in the first experiment a nicotine-induced effect on the validity effect was evident. Reaction times in the present experiment were even longer than in Meinke et al. (2006), indicating that the present task may be have been even more taxing. It would be interesting to

tease apart the exact link between processing effort, related task difficulty and nicotine effects. Lastly, in view of the lack of an overall nicotine induced effect on the validity effect, it may not be surprising that we did not find differences between smokers and nonsmokers with respect to the effect of nicotine on the validity effect.

To conclude, no direct evidence was found to suggest that nicotine abstinent smokers differ from nonsmokers on performance measures of selective attention and inhibition. With respect to the acute effect of nicotine, results suggest that nicotine may facilitate inhibition more in nonsmokers than in smokers. This difference might be explained in terms of drug-tolerance in smokers. Nicotine did not seem to affect attentional mechanisms, and no differences between smokers and nonsmokers were evident with respect to the effect of nicotine on these mechanisms. However, nicotine might have a larger effect on processing when it is effortless, and our specific VSC task variant may have been too taxing for a nicotine induced effect to be revealed.

ACKNOWLEDGMENTS

This study was financially supported by the open MAGW grant from NWO (Dutch organization for scientific research), NWO: 400-07-130

REFERENCES

Aron, A. R., Fletcher, P. C., Bullmore, E. T., Sahakian, B. J., & Robbins, T. W. (2003). Stop-signal inhibition disrupted by damage to right inferior frontal gyrus in humans. *Nat Neurosci, 6*(2), 115-116.

Bekker, E. M., Bocker, K. B., Van Hunsel, F., van den Berg, M. C., & Kenemans, J. L. (2005). Acute effects of nicotine on attention and response inhibition. *Pharmacol Biochem Behav, 82*(3), 539-548.

Corbetta, M., Patel, G., & Shulman, G. L. (2008). The reorienting system of the human brain: from environment to theory of mind. *Neuron, 58*(3), 306-324.

Corbetta, M., & Shulman, G. L. (2002). Control of goal-directed and stimulus-driven attention in the brain. *Nature Reviews, 3*, 201 - 215.

De Jong, R., Coles, M. G., Logan, G. D., & Gratton, G. (1990). In search of the point of no return: the control of response processes. *J Exp Psychol Hum Percept Perform, 16*(1), 164-182.

Heishman, S. J., Kleykamp, B. A., & Singleton, E. G. (2010). Meta-analysis of the acute effects of nicotine and smoking on human performance. *Psychopharmacology (Berl), 210*(4), 453-469.

Kollins, S. H., McClernon, F. J., & Fuemmeler, B. F. (2005). Association between smoking and attention-deficit/hyperactivity disorder symptoms in a population-based sample of young adults. *Arch Gen Psychiatry, 62*(10), 1142-1147.

Logan, G. D., Cowan, W. B., & Davis, K. A. (1984). On the ability to inhibit simple and choice reaction time responses: a model and a method. *J Exp Psychol Hum Percept Perform, 10*(2), 276-291.

Mangun, G. R., & Hillyard, S. A. (1991). Modulations of sensory-evoked brain potentials indicate changes in perceptual processing during visual-spatial priming. *J Exp Psychol Hum Percept Perform, 17*(4), 1057-1074.

Meinke, A., Thiel, C. M., & Fink, G. R. (2006). Effects of nicotine on visuo-spatial selective attention as indexed by event-related potentials. *Neuroscience, 141*(1), 201-212.

Newhouse, P. A., Potter, A., & Singh, A. (2004). Effects of nicotinic stimulation on cognitive performance. *Curr Opin Pharmacol, 4*(1), 36-46.

Nyberg, G., Panfilov, V., Sivertsson, R., & Wilhelmsen, L. (1982). Cardiovascular effect of nicotine chewing gum in healthy non-smokers. *Eur J Clin Pharmacol, 23*(4), 303-307.

Posner, M. I., Snyder, C. R., & Davidson, B. J. (1980). Attention and the detection of signals. *J Exp Psychol, 109*(2), 160-174.

Potter, A. S., Bucci, D. J., & Newhouse, P. A. (2012). Manipulation of nicotinic acetylcholine receptors differentially affects behavioral inhibition in human subjects with and without disordered baseline impulsivity. *Psychopharmacology (Berl), 220*(2), 331-340.

Potter, A. S., & Newhouse, P. A. (2004). Effects of acute nicotine administration on behavioral inhibition in adolescents with attention-deficit/hyperactivity disorder. *Psychopharmacology (Berl), 176*(2), 182-194.

Potter, A. S., & Newhouse, P. A. (2008). Acute nicotine improves cognitive deficits in young adults with attention-deficit/hyperactivity disorder. *Pharmacol Biochem Behav, 88*(4), 407-417.

Potter, A. S., Newhouse, P. A., & Bucci, D. J. (2006). Central nicotinic cholinergic systems: a role in the cognitive dysfunction in attention-deficit/hyperactivity disorder? *Behav Brain Res, 175*(2), 201-211.

Rezvani, A. H., & Levin, E. D. (2001). Cognitive effects of nicotine. *Biol Psychiatry, 49*(3), 258-267.

Schmajuk, M., Liotti, M., Busse, L., & Woldorff, M. G. (2006). Electrophysiological activity underlying inhibitory control processes in normal adults. *Neuropsychologia, 44*(3), 384-395.

Srivastava, E. D., Russell, M. A., Feyerabend, C., Masterson, J. G., & Rhodes, J. (1991). Sensitivity and tolerance to nicotine in smokers and nonsmokers. *Psychopharmacology (Berl), 105*(1), 63-68.

Tannock, R., Schachar, R. J., Carr, R. P., Chajczyk, D., & Logan, G. D. (1989). Effects of methylphenidate on inhibitory control in hyperactive children. *J Abnorm Child Psychol, 17*(5), 473-491.

Thiel, C. M., & Fink, G. R. (2007). Visual and auditory alertness: modality-specific and supramodal neural mechanisms and their modulation by nicotine. *J Neurophysiol, 97*(4), 2758-2768.

Thiel, C. M., & Fink, G. R. (2008). Effects of the cholinergic agonist nicotine on reorienting of visual spatial attention and top-down attentional control. *Neuroscience, 152*(2), 381-390.

van der Lubbe, R. H. J., Neggers, S. F. W., Verleger, R., & Kenemans, J. L. (2006). Spatiotemporal overlap between brain activation related to saccade preparation and attentional orienting. *Brain Research, 1072*(1), 133-152.

Vossel, S., Thiel, C. M., & Fink, G. R. (2008). Behavioral and neural effects of nicotine on visuospatial attentional reorienting in non-smoking subjects. *Neuropsychopharmacology, 33*(4), 731-738.

Witte, E. A., Davidson, M. C., & Marrocco, R. T. (1997). Effects of altering brain cholinergic activity on covert orienting of attention: comparison of monkey and human performance. *Psychopharmacology (Berl), 132*(4), 324-334.

Wonnacott, S., Irons, J., Rapier, C., Thorne, B., & Lunt, G. G. (1989). Presynaptic modulation of transmitter release by nicotinic receptors. *Prog Brain Res, 79*, 157-163.

CHAPTER 7

THE EFFECTS OF HALOPERIDOL 2MG ON COGNITION, MOTOR, CARDIOVASCULAR, AND SUBJECTIVE VARIABLES: A SMALL-SAMPLE PILOT STUDY

Logemann, H.N.A., Böcker, K.B.E., Deschamps, P.K.H., van Harten, P.N.,
Koning, J., Kemner, C., Kenemans, J.L.

ABSTRACT

The exact role of dopamine in attention and inhibition remains to be elucidated. Studies suggest that dopaminergic antagonism by 2mg haloperidol may have a detrimental effect on the control and disengagement of attention, and also on inhibition. This hypothesis was subjected to a preliminary dose-finding pilot in a pre- versus post-treatment, placebo controlled, double blind, cross-over design. Specifically, we focused on the efficacy of 2mg haloperidol in influencing these cognitive parameters as pitted against the occurrence of (undesired) cardiovascular and subjective effects. Furthermore, we assessed the sensitivity of two motor parameters ('velocity scaling' and spontaneous motor activity) as potential proxies for central responsivity. Ten subjects performed in the Visual Spatial Cueing (VSC) task, the Stop Signal Task (SST), and motor, cardiovascular, and subjective variables were assessed. Two participants withdrew from the experiment. It was hypothesized that the benefit of cueing on RT in the VSC would be reduced under haloperidol (reflecting decreased bias and/or enhanced disengagement), while the Stop Signal Reaction Time in the SST (reflecting inhibition capacity, and disengagement) would be increased. Results indicate clear effects on motor parameters and support the possibility of a reduction of the validity effect under haloperidol. Reaction time variability in the SST increased under haloperidol. Results suggest that velocity scaling (reflecting bradykinesia) may be a useful proxy for drug responsivity and suggest that haloperidol may negatively affect bias, but not disengagement/inhibition. Although sedation was evident, side effects were considered minor under 2mg haloperidol.

INTRODUCTION

Attention and inhibition are important for everyday functioning. Deficits of attention and inhibition can be profound and have been associated with disorders of attention such as Attention Deficit / Hyperactivity Disorder (ADHD) (Kenemans *et al.*, 2005). The dopaminergic neurotransmitter system has been implicated in attention and inhibition; however the exact role remains to be elucidated. This is important since methylphenidate, which affects both dopamine and noradrenaline, is used to treat the symptoms of ADHD, but in at least 20 percent of patients methylphenidate yields an unfavorable balance between desired effects and undesired side effects (Barkley, 1998; Swanson et al., 1998).Hence, it is important to investigate the role(s) of these neurotransmitter systems in order to fuel optimal (pharmacological) treatment of disorders of attention such as ADHD. The present study addressed the effect of dopaminergic manipulation in healthy subjects in two tasks aimed at assessing attention and inhibition, the Visual Spatial Cueing (VSC task) and the Stop Signal Task (SST). Here we report on the effects of haloperidol 2mg in a small sample (N=8). This preliminary analysis served to assess the power to detect relevant effects of a low dose of haloperidol w, weighted against the possible undesired side effects that may occur with either the current or higher dosages. These side effects (cardiovascular, sedation, motor impairment) were addressed explicitly as well.

A classic way to study attention is to use the Visual Spatial Cueing paradigm (Posner, Snyder, & Davidson, 1980). In this task, a cue signals the likely location of an upcoming target presented in either the left or right visual field, to which a response is required. In a minority of trials, the target is invalidly cued, that is, presented at the location opposite to the cued location. The relevant outcome parameter is termed the "validity effect" which is the benefit in terms of reaction time with respect to responses to validly cued targets as compared to invalidly cued targets. The validity effect is thought to reflect two underlying mechanisms: Bias, referring to the neuronal signals that modulate the sensitivity of sensory cortex, and disengagement, referring to the decoupling of attention from an invalidly cued location (M. Corbetta, Patel, & Shulman, 2008; Maurizio Corbetta & Shulman, 2002). A state manipulation that reduces bias results in a reduced validity effect, but so does a state variable that enhances disengagement.

Conceptually, disengagement (from the current task set) also plays a role when an ongoing response suddenly has to be inhibited because of a sudden change in task demands. This situation is usually captured in the Stop Signal Task (SST) (De Jong, Coles, Logan, & Gratton, 1990; Logan, Cowan, & Davis, 1984). In this task, go stimuli are presented and in a minority of trials the go stimulus is followed by a stop stimulus. The stop stimulus signals to withhold a planned response. The relevant outcome measure is the Stop Signal Reaction Time (SSRT), which is thought to be an index of inhibition (De Jong, Coles, & Logan, 1995; De Jong et al., 1990).

Both disengagement and reorienting of attention have been associated with active neurons in the right Inferior Frontal Gyrus (IFG) (M. Corbetta et al., 2008; Maurizio Corbetta & Shulman, 2002). For example, it has been shown that lesions of the right IFG have a detrimental effect on inhibition as indexed by SSRT in the SST (Aron, Fletcher, Bullmore, Sahakian, & Robbins, 2003). Furthermore, pharmacological effects (at least with respect to the cholinergic system) on disengagement as assessed by performance measures mirror those on inhibition. More specifically, several studies show that nicotine reduces the validity effect in the VSC task (Meinke, Thiel, & Fink, 2006; Thiel, Zilles, & Fink, 2005; Vossel, Thiel, & Fink, 2008; Witte, Davidson, & Marrocco, 1997) and suggest that this effect is a result of faster responses in invalid trials (Meinke et al., 2006; Thiel & Fink, 2008; Witte et al., 1997). Hence, this reduction may be due to enhanced disengagement. It has also been shown that, at least for groups with enough room for improvement, nicotine decreases the SSRT in the SST, indicating improvement of inhibition (Potter, Bucci, & Newhouse, 2012).

As mentioned, methylphenidate augments both dopaminergic as well as noradrenergic neurotransmission. Coull et al. (2001) investigated the effect of clonidine induced attenuation of noradrenergic neurotransmission on visuospatial attention. A variant of the VSC task was combined with fMRI to provide specific reference to brain activity indices of bias and disengagement. It was shown that noradrenergic antagonism results in a reduction of bias. While noradrenergic neurotransmission may be primarily related to bias, dopaminergic neurotransmission may be associated more with disengagement and inhibition. Lijfijt et al. (2006) suggest this is indeed the case. In a study by Lijfijt et al. (2006), dopamine-metabolite levels correlated significantly with SSRT in the SST, but not with reaction time (RT) to go stimuli. For norepinephrine-metabolite levels, the reverse pattern was found. This dissociation

suggests a link between dopamine and inhibition on the one hand, and norepinephrine and focus on, or bias for the ongoing task set on the other.

From the inferred link between dopaminergic neurotransmission on the one hand, and disengagement and inhibition on the other, it may be expected that attenuation of dopaminergic neurotransmission yields an increase in the VSC validity effect. However, this is opposite to what has been reported by Clark et al. (1989). These authors reported that dopaminergic antagonism by droperidol resulted in a reduction of the validity effect, not an increase. Even more unexpected, droperidol specifically reduced the difference between invalid-cue and 'neutral-cue' (a cue without direction information) conditions, while the difference between valid and neutral was unaffected. While at first sight this points to a specific effect of enhanced disengagement, this can also be explained as an effect on bias. This is because this pattern of effects will also emerge when a state manipulation reduces the bias against an uncued location, without affecting the bias towards the cued location.

In sum, dopaminergic attenuation may compromise bias as well as disengagement and inhibition. The present study addressed this issue by comparatively assessing the effects of 2mg haloperidol on VSC validity effects (reflecting bias and disengagement), SST SSRT (reflecting disengagement and inhibition), as well as SST go-reaction-time parameters (reflecting bias to an ongoing task set). The current report concerns a cross-sectional analysis of a sample of 8 healthy volunteers. Special heed was paid to cardiovascular, motor and subjective side effects of this relatively low dosage of haloperidol.

METHODS

Sample

Healthy male students were recruited from Utrecht University. Ten subjects participated in the study. All participants gave informed consent prior to participation. It was required to pass a short medical interview prior to the experiment. The study was approved by the Medical Ethics Committee from the University Medical Center Utrecht and conducted in accordance with the declaration of Helsinki. Two participants did not complete the experiment. One participant withdrew because of reasons not related to the experiment; the other participant withdrew because of experienced side effects.

Pharmacological manipulation

Haloperidol is a potent dopamine D2 receptor antagonist (Kapur, Zipursky, Jones, Remington, & Houle, 2000). Both 2mg haloperidol and placebo were encapsulated (DB capsule, AA Swedish orange). The placebo contained no active substance, and consisted of a mixture of lactose monohydrate DC and magnesium stearate (0.5%).

Tasks

Visual Spatial Cueing Task

Our specific implementation of the VSC task was a combination of the VSC task as described in Van den Lubbe et al. (2006) and the task as described in Mangun et al. (1991). Trials always started with a fixation dot, presented for 600 ms, after which a cue was presented for 400 ms. The cue consisted of a diamond (width 1.3°, height 0.7°) for which the left and right half differed in terms of color (red vs green). The cue was followed by the fixation dot (presented again for 600ms) and subsequently by one of two possible targets, either a short white bar (width 0.8°, height 2.0°) or a long white bar (width 0.8°, height 2.4°), presented in either the left or right visual hemifield (6.4° from the center of the screen) for 100 ms. Subsequently the fixation dot was presented for 1400 ms. The total duration of one trial was 3100 ms. The pretest version consisted of two blocks in total and an instruction was presented prior to each block. The first block was used as a practice block and consisted of 32 trials of which 8 were invalid trials. Valid trials were trials in which the location of the target was congruently cued (target presented at location indicated by the cue), invalid trials were trials in which the location of the target was opposite to the location indicated by the cue. The second block was used for data-analysis and consisted of 256 trials of which 75% were valid trials. The posttest version consisted of in total four blocks (no practice block) that differed with respect to the specific stimulus-response assignment and color-cue assignment. The posttest started with the block equal to the block of the pretest in terms of the stimulus-response and color-cue assignment. Within blocks trials were semi-randomized for each task-run and specific block order was counterbalanced across participants.

Stop Signal Task

An SST similar to Schmajuk et al. (2006) was implemented. In this task go stimuli were presented centrally, randomly, sequentially and slightly above a fixation cross. These stimuli consisted of the letters X (height and width 1.4°) and O (height 1.4° and width 1.3°), and a differential response was required after presentation of these stimuli (left or right button press). In 25% of trials in a stop signal block, the go stimulus was followed by a stop stimulus (letter '$', height 1.7°, width 0.8°) that signaled that the go response should be withheld. Both stimuli were presented for 150 ms and trials varied between 1.5 – 1.8 seconds. The pretest consisted of 126 go trials (no stop trials), a base stop signal block (used to estimate the optimal Stimulus Onset Asynchrony (SOA) to yield 50 percent inhibitions in a subsequent block) and lastly, two experimental stop signal blocks (for data-analysis, 128 trials in each block). Since participants often delay responding to go stimuli in stop signal blocks, participants were instructed to speed up responding to go stimuli in stop signal blocks if the average reaction time to go stimuli in these blocks was more than 1.5 times the reaction time in the first block (block without the stop trials). In the base stop signal block, the go-stop stimulus interval was set at 250 ms and in subsequent blocks this SOA was based on the performance in the previous block so that approximately 50 percent inhibitions were made. Furthermore, in experimental blocks, the exact onset of the stop signal was varied 99 ms above and 99ms below the average SOA. If participants made less than 40 percent inhibitions in stop signal blocks, they were instructed to react slightly slower to the go stimuli. Trials in stop signal blocks were randomized pseudo-randomly so that no more than three stop signals would be presented in succession. The difference between the posttest and pretest was that for the posttest, the base stop block was followed by three experimental blocks (equal to the pretest in terms of stimulus-response assignment). Afterwards, the stimulus-response assignment switched and another four stop signal blocks (base plus experimental blocks) were presented. Block order in terms of stimulus-response assignment was counterbalanced across participants.

Motoric parameters

Motor disturbances under haloperidol are related to dopamine receptor occupancy; specifically, extrapyramidal side effects occur with 78 - 80% receptor occupancy (Kapur et al., 2000; Nyberg, Nordstrom, Halldin, & Farde, 1995). Hence, the effect of haloperidol on motor activity may be used as an index for individual responsivity under the assumption that a stronger drug induced motor effect is positively correlated with a stronger central effect. Firstly, haloperidol induced akathisia/dyskinesia, resulting in possible increases in spontaneous motor activity, was assessed using an Actigraph (Actigraph GT3X+,Actigraph, LLC; Pensacola, FL, USA) . The Actigraph stores acceleration data (in gravitational unit G) at a sample rate of 100 Hz.

Secondly, haloperidol induced bradykinesia has been proposed to be reflected in a decrease in velocity scaling (Caligiuri, Lohr, & Ruck, 1998). In the present study, and in accordance with Caligiuri et al. (1998), velocity scaling was assessed in the following manner: Participants were instructed to move a cursor, by means of flexing a handle (like turning a key), as quickly and accurately as possible to a target on a computer screen. In a typical trial, a target was presented at the center of the screen for 2 seconds, after which it was presented to a position either 25 or 45 degrees (to the right for left hand movements, and to the left for right hand movements) from the midline wrist flexion, where it remained for 2 seconds after which it was presented again at the center of the screen. The interstimulus interval was 2 seconds, and for each hand 32 movements were performed.

Cardiovascular and subjective parameters

Cardiovascular variables (diastolic- and systolic blood pressure, and heart rate) were assessed using an automatic blood pressure monitor (Microlife, BP3AC1-1PC). Subjective effects were assessed using the Profile of Mood States (POMS) questionnaire (Wald, 1984; Wald & Mellenbergh, 1990).

Procedure

After participants signed the informed consent and passed the medical interview, the Actigraph was placed on the right ankle. The Actigraph collected acceleration data along three axes during the entire experiment. Participants filled out the POMS questionnaire and

performed for about 10 minutes the Velocity Scaling task. Subsequently, participants performed the pretest version of the SST and VSC, which took approximately 30 minutes. Approximately 10 minutes prior to drug administration, blood pressure and heart rate were assessed. About 1 hour and 50 minutes after drug administration (t=1h50m), an EEG cap was placed (EEG data presented elsewhere). At t=2h45m, participants filled out the POMS again, 5 minutes later cardiovascular variables were assessed, and at t=3h00m participants performed the posttest version of the VSC task and SST. With respect to the VSC task, HEOG was carefully monitored for evidence of horizontal saccades. In accordance to van der Lubbe et al. (2006), we defined a saccade as a peak (positive or negative polarity) exceeding 60 microvolts relative to baseline. Between blocks, participants had short breaks and between tasks there was room for a longer break (20 minutes). Upon completion of both tasks, Velocity Scaling was assessed again (at t=5h20m), and at t=5h30m blood pressure and heart rate were measured. The total duration of the experiment was approximately 6.5 hours.

Data analyses

Spontaneous motor activity

The Actigraph stores acceleration data on 3 axes (in units of gravity, G). Data from 1 minute to 11 minutes after the start of the first pretest (VSC or SST task; approximately 35 minutes prior to capsule administration), and after the start of the first posttest (approximately at t = 3:00 relative to capsule ingestion) were used for analyses. The sample rate was set at 100 Hz and raw data were integrated for pretest and posttest in epochs of 10 seconds. For these epochs, the output across the three axes was averaged. Lastly, these 10 second epochs within a 10 minute time-frame (separately for pre and posttest) were averaged to yield one measure of spontaneous motor activity.

Velocity scaling

The ability to adjust the speed of movement in relation to target distance (velocity scaling) was used to quantify bradykinesia (Caligiuri *et al.*, 1998). Velocity scaling values are expressed in degrees/second/degree (Caligiuri *et al.*, 1998). Scaling values for movements towards targets were averaged with values for return movements. Importantly velocity scaling is thought to be a measure of bradykinesia for which a lower velocity scaling value represents more bradykinesia (Caligiuri *et al.*, 1998).

POMS

The Profile of Mood States (POMS) questionnaire consists of six scales, depression (8 items), anger (7 items), vigor (5 items), fatigue (6 items), tension (6 items), and total mood disturbance. Each scale ranges from from 0 to 4. The total score on a scale was determined by the sum of the item values for that scale. The Effect size (partial eta squared) was calculated for the drug effect on each scale.

Stop Signal Task

In line with de Jong et al. (1990), the SSRT was calculated as follows. The corrected (for omissions) proportion of successful stops was calculated using Tannock et al. (1989). RTs in go-trials in the 150 to 1500 ms window were ordered from short to long RTs. The n^{th} point on this vector was determined by multiplying the total number of RTs by one minus the stop rate (corrected for omissions). The SSRT was then calculated by subtracting the average go-stop interval from the reaction time at the n^{th} point on the RT vector.

Visual Spatial Cueing Task

Reaction times within the 100 ms – 1500 ms range were considered valid, and were included in the analyses. For each participant, session and time (pre/post), reaction times on (separately) valid trials and invalid trials were averaged across blocks. The validity effect was defined as the difference between the averaged reaction time on invalidly cued targets minus validly cued targets.. HEOG data were inspected to assess the number of saccades per participant and session. Results from the entire sample (n=8) were reported, as well as results from the sample

(n=5) from which participants were excluded who made saccades in more than 50% of trials in either the first or second session.

Statistical Analyses

Repeated measures ANOVAs were used to test each drug x time interaction for the relevant variables. With respect to data from the computer tasks, analyses were restricted to post-treatment data if no baseline difference (haloperidol-placebo) was evident. Unnecessary inclusion of pretest data would result in a decreased signal-to-noise ratio as a result of increased variance since the pretests consisted of fewer trials as compared to the posttests.

For each statistical outcome, we reported the (SPSS) partial squared eta, which is calculated from the F value and degrees of freedom between (dfb) and degrees of freedom within (dfw): $\eta p2 = (dfb* F)/(dfw + dfb * F)$. To provide a frame of reference, a partial eta squared (SPSS) exceeding 0.1, 0.2, 0.3, or 0.4 implies that a sample of respectively 74, 35, 22, or 15 participants is sufficient to detect such effect with 80 percent statistical power and alpha set at 0.05, using the current implemented design.

RESULTS

Cardiovascular data

No drug (placebo – haloperidol) x time (pre – post) interaction was evident with respect to any of the cardiovascular variables (figure 1). The partial eta squared (η_p^2) effect size for the drug x time interaction pertaining to systolic and diastolic blood pressure and heart rate was, 0.088, 0.058, and 0.002, respectively. Heart rate decreased significantly over time (main effect of time: $F(1,7) = 30.65$, $p < 0.01$).

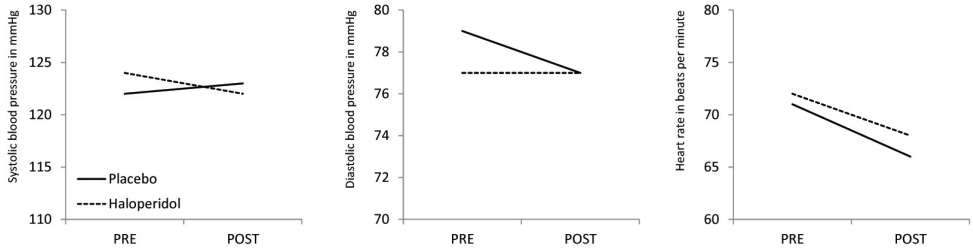

Figure 1. Cardiovascular data, pre- and post-administration of the capsules.

Motor effects

Effects on spontaneous motor activity and velocity scaling are depicted in figure 2, in upper and lower panels, respectively. Spontaneous motor activity increased over time irrespective of drug ($F(1, 7) = 12.83$, $p < 0.01$. Haloperidol relative to placebo, did not affect spontaneous motor activity ($F(1,7) < 1$; drug x time η_p^2: 0.095). Velocity scaling was negatively affected under haloperidol as indicated by a significant drug x time interaction ($F(1,7) = 7.63$, $p = 0.028$). As depicted in table 1, the haloperidol (relative to placebo) induced effect on velocity scaling was inversely related to the change with respect to spontaneous motor activity under haloperidol (relative to placebo); the Pearson correlation was -0.774, $p = 0.024$.

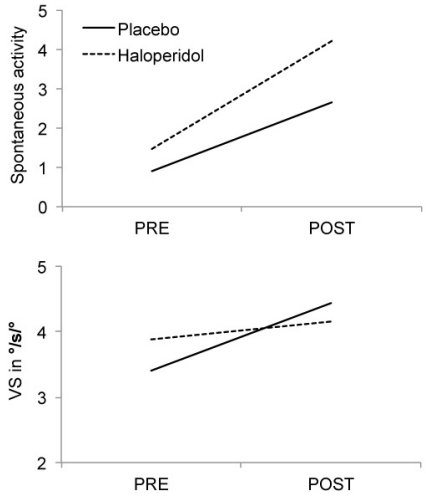

Figure 2. Spontaneous motor activity (10s accumulated G, upper panel) and Velocity Scaling (VS, lower panel), at pre- and post-administration, separately for placebo and haloperidol.

Table 1. For spontaneous motor activity (upper panel), and velocity scaling (lower panel): change from pre-test to post-test for placebo and haloperidol. Haloperidol induced change is change from pre to post under haloperidol minus pre to post change after placebo.

SPONTANEOUS MOTOR ACTIVITY						
Placebo			Haloperidol			
PRE	POST	POST-PRE	PRE	POST	POST-PRE	**Haloperidol induced change**
0,14	2,63	2,49	0,23	4,35	4,12	**1,63**
0	0,01	0,01	0	8,38	8,38	**8,37**
0	0	0	0,48	0,33	-0,15	**-0,15**
0	0	0	0	0,12	0,12	**0,12**
0	1,72	1,72	0,14	1,4	1,26	**-0,46**
5,69	8,38	2,69	10,13	14,46	4,33	**1,64**
1,29	3,33	2,04	0,41	0,97	0,56	**-1,48**
0	5,18	5,18	0,36	3,73	3,37	**-1,81**
VELOCITY SCALING						
Placebo			Haloperidol			
PRE	POST	POST-PRE	PRE	POST	POST-PRE	**Haloperidol induced change**
3,55	4,03	0,48	4,03	4,11	0,08	**-0,4**
6,03	10,59	4,56	6,12	8,25	2,13	**-2,43**
4,9	5,35	0,45	5,29	5,36	0,07	**-0,38**
2,62	2,82	0,2	3,59	2,8	-0,79	**-0,99**
2,78	2,81	0,03	2,22	2,4	0,18	**0,15**
2,04	2,86	0,82	2	2,36	0,36	**-0,46**
2,41	2,74	0,33	3,73	3,54	-0,19	**-0,52**
2,92	4,28	1,36	4,09	4,49	0,4	**-0,96**

Subjective effects

POMS data are depicted in figure 3. A time x drug trend towards significance pertaining to a reduction of vigor after haloperidol was evident ($F(1,7) = 3.72$, $p = 0.095$; $\eta_p^2 = 0.347$). No effect was evident on any of the other POMS variables (drug x time effect sizes: depression, $\eta_p^2 = 0.125$; anger, $\eta_p^2 < 0.001$; fatigue, $\eta_p^2 = 0.042$; tension, $\eta_p^2 = 0.042$; and total mood disturbance, $\eta_p^2 = 0.231$) The drug x time effect size with respect to total mood disturbance implies that a haloperidol induced increase in total mood disturbance may be detected in a sample size above 30.

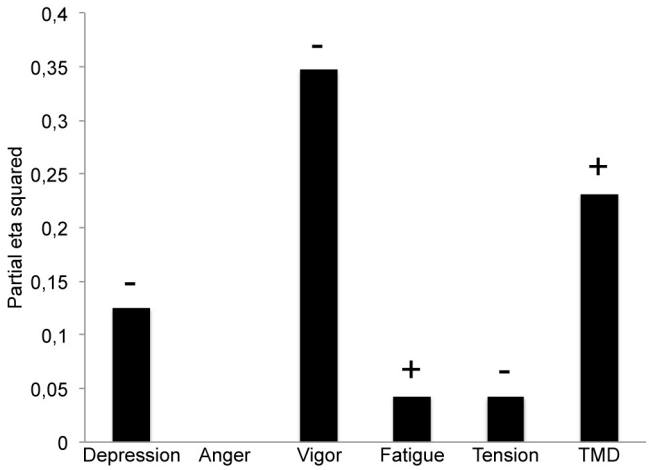

Figure 3. The drug x time effect size for each POMS variable; an increase or decrease is depicted as a plus or minus sign respectively. TMD = Total Mood Disturbance (composite score).

Adverse reactions

No serious adverse events occurred following the administration of 2 mg haloperidol. One participant withdrew from the experiment because of modest extrapyramidal side effects (tremor).

Performance data

Visual Spatial Cueing Task

Performance data pertaining to the VSC task are depicted in figure 4 for both the complete and the restricted sample (from which participants who made excessive saccades were excluded). Overall the validity effect on reaction time was trending towards significance as indicated by a main effect of validity on reaction time ($F(1,7) = 4.11$, $p = 0.082$; $\eta_p^2 = 0.370$), the validity effect on error rate was not significant ($\eta_p^2 = 0.024$). For the restricted sample these effect sizes were larger (respectively $\eta_p^2 = 0.531$; 0.286) and the effect of validity on reaction time was again trending to significance ($F(1,4) = 4.54$, $p = 0.10$). There was no baseline (= pretest data) difference between conditions (placebo - haloperidol) with respect to the validity effect on reaction time and errors (drug x validity interaction, respectively $\eta_p^2 = 0.151$, 0.040 for the complete sample, and $\eta_p^2 = 0.056$, $\eta_p^2 < 0.001$ for the restricted sample). Post-treatment data indicated a trend towards a reduction of the validity effect on reaction time under haloperidol (drug x validity interaction: $F(1,7) = 4.04$, $p = 0.084$; $\eta_p^2 = 0.366$), no effect of haloperidol was evident with respect to the validity effect on the error rate ($\eta_p^2 = 0.145$). For the restricted sample, the effect size (η_p^2) of the drug x validity interaction pertaining to reaction time was 0.222 and that for the error rate 0.309.

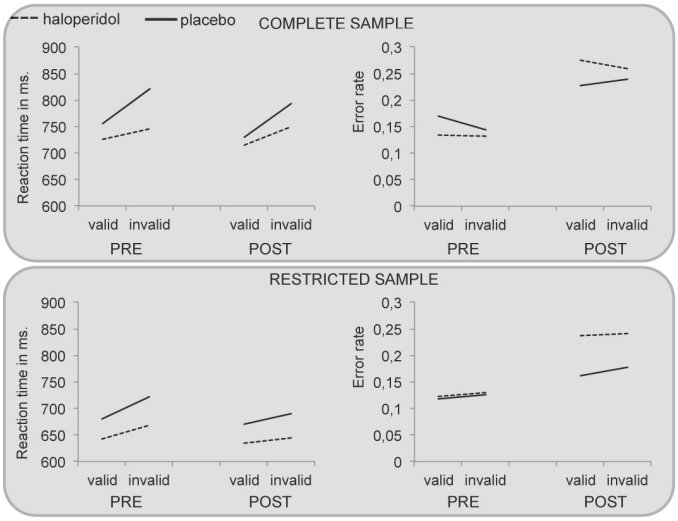

Figure 4. The validity effect on reaction time (left panel) and error rate (right panel) for the pretest and posttest version of the VSC task, separately for haloperidol and placebo.

Stop Signal Task

There was no baseline difference between conditions (drug - placebo) with respect to any of the SST variables (SSRT, mean RT, SDRT, error rate, omissions). With respect to the post-treatment data, haloperidol negatively affected both the standard deviation of reaction time (SDRT), as well as the error rate (respectively, $F(1,7) = 6.27$, $p = 0.041$; $F(1,7) = 8.19$, $p = 0.024$), while the other variables were not affected by haloperidol (main effect of drug: SSRT, $\eta_p^2 = 0.076$; mean RT, $\eta_p^2 = 0.028$; omission rate, $\eta_p^2 = 0.022$).

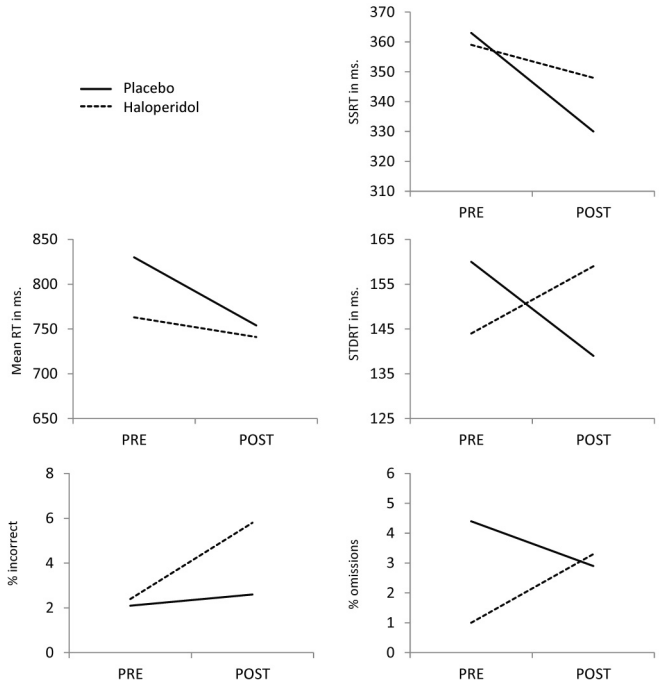

Figure 5. Performance data (SSRT, meanRT, SDRT, error rate and omission rate) for placebo and haloperidol for the pretest and posttest version of the SST.

DISCUSSION

The present work presents a preliminary analysis of the effects of 2mg haloperidol in two tasks aimed at assessing attention and inhibition, as well as cardiovascular, motoric, and subjective parameters in a cross-sectional design. The results indicate that D2 receptor blockade by 2mg haloperidol may affect the 'bias' (or 'top-down' control) aspect of attention but not the disengagement aspect or inhibition. In addition, haloperidol 2mg did not exert cardiovascular effects. Results suggest that 2 mg haloperidol may produce subjective sedation (vigor scale in the POMS). Furthermore, clear signs of basal-ganglia dysfunction were evident, manifested in reduced velocity scaling (Caligiuri *et al.*, 1998). We conclude that specific effects within the attention-inhibition spectrum can be detected with a 2 mg dosage in a larger sample. Given the current effect size for the drug x validity effect on reaction time with respect to the post-treatment data, a sample size of 31 would be sufficient. Applying higher dosages also seems to be unnecessary or even undesirable, given the effects on basal-ganglia function that were clearly detected with 2 mg, as well as the detectable subjective sedation and adverse reactions in one out of ten participants.

The validity effect on reaction time in the visual spatial cueing (VSC) task reflects the combined contribution of both bias and disengagement, which refer to the neuronal signals that modulate the sensitivity of sensory cortex, and to the decoupling of attention from an invalidly cued location, respectively. A clear trend ($\eta_p^2 = 0.366$ in the complete sample and $\eta_p^2 = 0.222$ for the restricted sample) towards a reduced validity effect on reaction time under haloperidol was observed, reflecting either reduced bias or enhanced disengagement. The reduced validity effect was accompanied by a significant increase in reaction time variability in the stop signal task (SST). Furthermore, the primary inhibition measure, stop-signal reaction time (SSRT) was not at all affected by haloperidol. This pattern of results is consistent with an involvement of D2-receptor mechanisms in the bias aspect of attention, without any involvement in inhibition. To the extent that inhibition also includes a disengagement component, it may be deduced that disengagement was also unaffected by 2 mg haloperidol.

We did not find longer SSRTs in the SST under haloperidol, and given the small effect size (partial eta squared for the main effect of drug in post-treatment data = 0.076) it is unlikely that such an effect will be found with larger sample sizes. The absence of drug effects

on SSRT may be related to the particular SST that is used. In typical visual-go/ auditory-stop variants, a high amount of proactive top-down control is possible which results in fast inhibitory signals in response to any auditory stimulus (Kenemans & Kähkönen, 2011; Overtoom et al., 2009). This kind of top-down inhibitory control depends on intactness of the right inferior frontal gyrus (Aron *et al.*, 2003). In contrast, the visual-go/ visual-stop variant of the SST, like the one that was presently used, prompts a much more reactive inhibition strategy (and the conceptually associated disengagement) that is mainly initiated after a relatively difficult detection of the visual stop stimulus in the context of the visual go stimulus. This reactive mechanism was also reflected in the brain potentials as reported in the original Schmajuk et al. (2006) article, which featured stop-related activity over right frontal cortex only after the stop signal had been presented. Both inferior PFC (Aron *et al.*, 2003) and superior PFC have been implicated (Floden & Stuss, 2006). Methylphenidate (Overtoom et al. (2009) and Pliszka et al. (2007), in patients) and atomoxetine (Chamberlain et al. (2009); Chamberlain et al. (2006) , healthy volunteers) improve stopping performance in both proactive and reactive inhibition eliciting contexts, which could be related to blockades of more subcortically based dopamine transporters or more cortically located norepinephrine transporters (Schulz *et al.*, 2012). In contrast, D2 receptor mechanisms (mostly, but not exclusively, subcortical) could be involved mainly or solely in proactive inhibition. This would fit the present absence of haloperidol effects on stopping, and also its alleged (based on the present results) role in top-down control of attention ('bias').

In previous studies we used cardiovascular measures as proxies for individual responsivity to noradrenergic and cholinergic manipulations (respectively 100 μg clonidine, Logemann et al., in revision; 2 mg nicotine in nicotine prolacrilex chewing gum, Logemann et al., submitted). In the present study attenuation of dopaminergic signaling by 2 mg haloperidol did not elicit pronounced effects on cardiovascular variables. Sedating effects, therefore, cannot be ascribed to peripheral feedback mechanisms, and probably have a more central origin. The sedative effects of haloperidol were mirrored by a significant haloperidol induced attenuation of velocity scaling, or in other words, increased bradykinesia. This suggests that velocity scaling as a measure of drug induced bradykinesia may be used (instead of cardiovascular variables) as a proxy for individual responsivity to the drug under the assumption that a stronger drug induced effect on motor variables is positively correlated with

a stronger central effect. Although the straightforward analysis of spontaneous motor activity did not seem to suggest a possible effect of 2mg haloperidol, the pre to post change under haloperidol relative to placebo was inversely correlated with the haloperidol-induced change pertaining to velocity scaling. However, even though spontaneous motor activity, as assessed by the Actigraph, may be affected by the current dose of haloperidol, our results suggest that velocity scaling may be a more sensitive proxy for central responsiveness to haloperidol.

In sum, this preliminary report suggests sufficient power for larger sample sizes to detect effects of 2mg haloperidol on relevant parameters of attention. While the current dosage seems adequate to detect cognitive effects, it also induced a number of side effects (sedation, motor impairment) that may reach unacceptable levels with dosages exceeding 2 mg.

ACKNOWLEDGMENTS

This study was financially supported by the open MAGW grant from NWO (Dutch organization for scientific research), NWO: 400-07-130

REFERENCES

Aron, A. R., Fletcher, P. C., Bullmore, E. T., Sahakian, B. J., & Robbins, T. W. (2003). Stop-signal inhibition disrupted by damage to right inferior frontal gyrus in humans. *Nat Neurosci, 6*(2), 115-116.

Barkley, R. A. (1998). *Attention deficit hyperactivity disorder: A handbook for diagnosis and treatment* (2 ed.). New York: Guildford.

Caligiuri, M. P., Lohr, J. B., & Ruck, R. K. (1998). Scaling of movement velocity: a measure of neuromotor retardation in individuals with psychopathology. *Psychophysiology, 35*(4), 431-437.

Chamberlain, S. R., Hampshire, A., Muller, U., Rubia, K., Del Campo, N., Craig, K., et al. (2009). Atomoxetine modulates right inferior frontal activation during inhibitory control: a pharmacological functional magnetic resonance imaging study. *Biol Psychiatry, 65*(7), 550-555.

Chamberlain, S. R., Muller, U., Blackwell, A. D., Clark, L., Robbins, T. W., & Sahakian, B. J. (2006). Neurochemical modulation of response inhibition and probabilistic learning in humans. *Science, 311*(5762), 861-863.

Clark, C. R., Geffen, G. M., & Geffen, L. B. (1989). Catecholamines and the covert orientation of attention in humans. *Neuropsychologia, 27*(2), 131-139.

Corbetta, M., Patel, G., & Shulman, G. L. (2008). The reorienting system of the human brain: from environment to theory of mind. *Neuron, 58*(3), 306-324.

Corbetta, M., & Shulman, G. L. (2002). Control of goal-directed and stimulus-driven attention in the brain. *Nature Reviews, 3*, 201 - 215.

Coull, J. T., Nobre, A. C., & Frith, C. D. (2001). The noradrenergic alpha2 agonist clonidine modulates behavioural and neuroanatomical correlates of human attentional orienting and alerting. *Cereb Cortex, 11*(1), 73-84.

De Jong, R., Coles, M. G., & Logan, G. D. (1995). Strategies and mechanisms in nonselective and selective inhibitory motor control. *J Exp Psychol Hum Percept Perform, 21*(3), 498-511.

De Jong, R., Coles, M. G., Logan, G. D., & Gratton, G. (1990). In search of the point of no return: the control of response processes. *J Exp Psychol Hum Percept Perform, 16*(1), 164-182.

Floden, D., & Stuss, D. T. (2006). Inhibitory control is slowed in patients with right superior medial frontal damage. *J Cogn Neurosci, 18*(11), 1843-1849.

Kapur, S., Zipursky, R., Jones, C., Remington, G., & Houle, S. (2000). Relationship between dopamine D(2) occupancy, clinical response, and side effects: a double-blind PET study of first-episode schizophrenia. *Am J Psychiatry, 157*(4), 514-520.

Kenemans, J. L., Bekker, E. M., Lijffijt, M., Overtoom, C. C., Jonkman, L. M., & Verbaten, M. N. (2005). Attention deficit and impulsivity: selecting, shifting, and stopping. *Int J Psychophysiol, 58*(1), 59-70.

Kenemans, J. L., & Kähkönen, S. (2011). How human electrophysiology informs psychopharmacology: from bottom-up driven processing to top-down control. *Neuropsychopharmacology, 36*(1), 26-51.

Lijffijt, M., Kenemans, J. L., ter Wal, A., Quik, E. H., Kemner, C., Westenberg, H., et al. (2006). Dose-related effect of methylphenidate on stopping and changing in children with attention-deficit/hyperactivity disorder. *Eur Psychiatry, 21*(8), 544-547.

Logan, G. D., Cowan, W. B., & Davis, K. A. (1984). On the ability to inhibit simple and choice reaction time responses: a model and a method. *J Exp Psychol Hum Percept Perform, 10*(2), 276-291.

Mangun, G. R., & Hillyard, S. A. (1991). Modulations of sensory-evoked brain potentials indicate changes in perceptual processing during visual-spatial priming. *J Exp Psychol Hum Percept Perform, 17*(4), 1057-1074.

Meinke, A., Thiel, C. M., & Fink, G. R. (2006). Effects of nicotine on visuo-spatial selective attention as indexed by event-related potentials. *Neuroscience, 141*(1), 201-212.

Nyberg, S., Nordstrom, A. L., Halldin, C., & Farde, L. (1995). Positron emission tomography studies on D2 dopamine receptor occupancy and plasma antipsychotic drug levels in man. *Int Clin Psychopharmacol, 10 Suppl 3*, 81-85.

Overtoom, C. C., Bekker, E. M., van der Molen, M. W., Verbaten, M. N., Kooij, J. J., Buitelaar, J. K., et al. (2009). Methylphenidate restores link between stop-signal sensory impact and successful stopping in adults with attention-deficit/hyperactivity disorder. *Biol Psychiatry, 65*(7), 614-619.

Pliszka, S. R., Liotti, M., Bailey, B. Y., Perez, R., 3rd, Glahn, D., & Semrud-Clikeman, M. (2007). Electrophysiological effects of stimulant treatment on inhibitory control in children with attention-deficit/hyperactivity disorder. *J Child Adolesc Psychopharmacol, 17*(3), 356-366.

Posner, M. I., Snyder, C. R., & Davidson, B. J. (1980). Attention and the detection of signals. *J Exp Psychol, 109*(2), 160-174.

Potter, A. S., Bucci, D. J., & Newhouse, P. A. (2012). Manipulation of nicotinic acetylcholine receptors differentially affects behavioral inhibition in human subjects with and without disordered baseline impulsivity. *Psychopharmacology (Berl), 220*(2), 331-340.

Schmajuk, M., Liotti, M., Busse, L., & Woldorff, M. G. (2006). Electrophysiological activity underlying inhibitory control processes in normal adults. *Neuropsychologia, 44*(3), 384-395.

Schulz, K. P., Fan, J., Bedard, A. C., Clerkin, S. M., Ivanov, I., Tang, C. Y., et al. (2012). Common and unique therapeutic mechanisms of stimulant and nonstimulant treatments for attention-deficit/hyperactivity disorder. *Arch Gen Psychiatry, 69*(9), 952-961.

Swanson, J. M., Sergeant, J. A., Taylor, E., Sonuga-Barke, E. J., Jensen, P. S., & Cantwell, D. P. (1998). Attention-deficit hyperactivity disorder and hyperkinetic disorder. *Lancet, 351*(9100), 429-433.

Tannock, R., Schachar, R. J., Carr, R. P., Chajczyk, D., & Logan, G. D. (1989). Effects of methylphenidate on inhibitory control in hyperactive children. *J Abnorm Child Psychol, 17*(5), 473-491.

Thiel, C. M., & Fink, G. R. (2008). Effects of the cholinergic agonist nicotine on reorienting of visual spatial attention and top-down attentional control. *Neuroscience, 152*(2), 381-390.

Thiel, C. M., Zilles, K., & Fink, G. R. (2005). Nicotine modulates reorienting of visuospatial attention and neural activity in human parietal cortex. *Neuropsychopharmacology, 30*(4), 810-820.

van der Lubbe, R. H. J., Neggers, S. F. W., Verleger, R., & Kenemans, J. L. (2006). Spatiotemporal overlap between brain activation related to saccade preparation and attentional orienting. *Brain Research, 1072*(1), 133-152.

Vossel, S., Thiel, C. M., & Fink, G. R. (2008). Behavioral and neural effects of nicotine on visuospatial attentional reorienting in non-smoking subjects. *Neuropsychopharmacology, 33*(4), 731-738.

Wald, F. D. M. (1984). *De verkorte POMS*. *Master's Thesis.*, University of Amsterdam., Amsterdam.

Wald, F. D. M., & Mellenbergh, G. J. (1990). Instrumenteel onderzoek. *Nederlands tijdschrift voor de psychologie, 45*, 86-90.

Witte, E. A., Davidson, M. C., & Marrocco, R. T. (1997). Effects of altering brain cholinergic activity on covert orienting of attention: comparison of monkey and human performance. *Psychopharmacology (Berl), 132*(4), 324-334.

CHAPTER 8

GENERAL DISCUSSION

The goal of this thesis was to elucidate the specific roles of the noradrenergic, cholinergic and dopaminergic neurotransmitter system in terms of visuospatial attention (bias and disengagement) and inhibition. These neurotransmitter systems were manipulated using clonidine, nicotine, and haloperidol, respectively. The effects of these manipulations were assessed on performance measures of visuospatial attention (validity effect on reaction time) and inhibition (SSRT). Furthermore we assessed brain activity (ERPs) specifically related to mechanisms of visuospatial attention and inhibition. Secondary parameters were drug-induced effects on motor activity (in the haloperidol study) and on cardiovascular variables (in the clonidine and nicotine study). These secondary parameters were used as a proxy for individual responsivity to the drug. All experiments followed a randomized placebo-controlled repeated measures design, and both the clonidine and haloperidol studies were performed double blind.

8.1. Summary of results pertaining to manipulating the noradrenergic, cholinergic, and dopaminergic neurotransmitter system

An overview of results is depicted in table 8.1, and summarized in sections 8.1.2 through 8.1.4.

8.1.2. Noradrenaline

In chapter 2 and 3 we report on the effect of attenuating noradrenergic signaling on visuospatial attention and inhibition, respectively. With respect to visuospatial attention, it was expected that clonidine would diminish bias related activity, without affecting disengagement. Hence, with respect to performance measures, a decrease of the validity effect on reaction time was expected. With respect to electrophysiological variables, we expected that clonidine would diminish the onset of bias (EDAN, LDAP), and result of bias (P1/N1 effect), without affecting disengagement (LPD). Results showed that clonidine specifically attenuated the N1 effect, indicating a reducing effect on the result of bias. This effect, however, was restricted to the sample for which the validity effect on reaction time during the pretest indicated sufficient room for a possible drug effect.

The validity effect on reaction time was not affected and neither were the ERPs related to the onset of bias, nor the disengagement related ERP. However, the clonidine-

induced effect on diastolic blood pressure was associated with the clonidine-induced effect on the LPD modulation, suggesting a modulating effect of clonidine on disengagement.

In line with the latter result, it was expected in chapter 3 that clonidine would lengthen SSRT, the performance measure of inhibitory motor control. Secondly, it was expected that brain activity correlates of inhibitory activity (stop N2, reflecting activity in the right Inferior Frontal Gyrus and stop P3, reflecting activity in the Superior Frontal Gyrus) would be reduced under clonidine. Results pertaining to performance variables indicated that global attention was affected as reflected in a clonidine-induced increase in omission rate and response variability. The behavioural measure of inhibition (SSRT) was not affected, but electrophysiological results showed a specific reduction of the stop P3 in response to clonidine. The stop N2 was not affected. In sum, results suggest that clonidine negatively affected attention, and negatively affected the dorsal part of the inhibition system but not the ventral part.

8.1.3. Acetylcholine

Chapters 4 and 5 report on the effect of enhancing cholinergic neurotransmission by nicotine on visuospatial attention and inhibition in healthy non-smokers. Chapter 6 reports on baseline differences between nicotine abstinent smokers and non-smokers and differences with respect to the acute effect of nicotine. With respect to the effect of nicotine on visuospatial attention in non-smokers, it was expected that cholinergic facilitation would result in facilitated disengagement as reflected in a reduced validity effect on reaction time (performance) and an enhanced LPD effect (electrophysiology). Results showed some indication of a reduction of the validity effect on reaction time, but only for post-treatment data (excluding baseline data) and the effect was restricted to half of the sample in which room for a drug effect was most pronounced. With respect to electrophysiology, nicotine did not affect disengagement as evidenced in the lack of effect on the LPD effect. However, (the result of) bias as reflected in the P1 effect, was reduced under nicotine.

With respect to inhibition, we expected that nicotine would augment inhibitory functioning as reflected in a reduced SSRT (performance) and enhanced stop N2 and stop P3. Overall, nicotine did not seem to affect any of the parameters. However, separate analyses

excluding pretest-data, indicated a significant nicotine induced reduction of SSRT. Furthermore, in half of the sample in which the drug effect on diastolic blood pressure was most pronounced (diastolic responders), nicotine specifically enhanced the stop P3. In other words, nicotine enhanced inhibitory functioning, but the effect was limited to strong responders, and restricted to the frontal dorsal part of the inhibitory system.

In chapter 6 performance differences between nicotine abstinent smokers and non-smokers with respect to visuospatial and inhibitory functioning were addressed. It was expected that nicotine abstinent smokers would exhibit poor inhibitory control as reflected in relatively longer SSRTs in comparison to non-smokers. In a similar vein, we hypothesized that the validity effect on reaction time would be enhanced for smokers as opposed to non-smokers. It was expected that the acute reducing effect of nicotine on SSRT and on the validity effect on RT would be more pronounced in nicotine abstinent-smokers as opposed to non-smokers. Results indicated no baseline differences between groups. Post-hoc analyses showed that nicotine reduced SSRT more in non-smokers as compared to smokers.

8.1.4. Dopamine

In chapter 7 the effect of dopaminergic antagonism by 2 mg haloperidol on performance variables in a limited sample (n=10), dose-finding study is described, as well as the effects on cardiovascular functioning and motor activity. With respect to the primary parameters, we expected a haloperidol-induced reduction of the validity effect on reaction time in the VSC task, and an increase in SSRT in the stop task. Results suggest a possible reduced validity effect on reaction time under haloperidol in the VSC task. Furthermore, in the SST, haloperidol increased response variability without affecting SSRT. Pertaining to the secondary parameters, haloperidol did not affect cardiovascular variables. With respect to motor activity variables, bradykinesia (as reflected in velocity scaling measures), but not dyskinesia (as reflected in spontaneous motor activity), was enhanced following haloperidol. In sum, results suggest that haloperidol may affect bias without affecting disengagement and inhibition. Furthermore, drug-induced effects on velocity scaling may be a useful proxy to assess individual differences in drug responsivity.

Table 8.1. Overview of results pertaining to relevant cognitive and electrophysiological variables.

Manipulation	Task	Output: behaviour	Output: EEG
Clonidine	SST	SSRT - , STDRT ↑, omission rate ↑	Stop N2 -, Stop P3 ↓
	VSC	Validity effect on RT -	N1 effect ↓[1], possible modulation of LPD effect
Nicotine	SST	SSRT ↓[2]	Stop N2 -, Stop P3 ↑[1]
	VSC	Validity effect on RT ↓[1,2]	LPD effect -, P1 effect ↓
Baseline differences non smokers - nicotine abstinent smokers	SST	SSRT -	
	VSC	validity effect on RT -	
Nicotine effect in non smokers - nicotine abstinent smokers	SST	SSRT ↓[1,2]	
	VSC	Validity effect on RT -	
Haloperidol	SST	SSRT -, SDRT ↑	
	VSC	Validity effect on RT ↓[2,3]	

[1] Restricted to selected sample; [2] Restricted to post-treatment data; [3] Effect size implies the effect may be detected in the final complete sample (30 participants).

8.2. Important issues and methodological considerations

Some noteworthy challenges arose during the projects pertaining to unexpected side effects (even after careful scrutiny of the literature), and individual variability. I think it is important to reflect on these issues in relation to future studies.

8.2.1. Unexpected adverse events during the clonidine study

Based on previous literature, it was expected that participants could experience some side effects following 200 microgram clonidine. Furthermore, a clonidine-induced blood pressure drop was expected. (Bitsios, Langley, Szabadi, & Bradshaw, 1996; Coull, Nobre, & Frith, 2001; Kennedy, Gnam, Ralevski, & Brown, 1995; Morley, Bradshaw, & Szabadi, 1991; Smith, Brice, Nash, Rich, & Nutt, 2003; Turetsky & Fein, 2002). Subjective side effects (also related to the hypotensive effects) to be expected were sedation and fatigue, and a feeling of a dry mouth (Johnson, Blackwell, & Smith, 1995; Kennedy et al., 1995; Smith et al., 2003; Turetsky & Fein, 2002). However, side effects in the first two participants in our study were more marked than previously reported, necessitating a lowering of the dosage to 100 microgram after correspondence with the local medical ethics committee. Systolic blood pressure dropped

approximately 40 mmHg for both participants. One participant complained about sedation/fatigue, a sensation of warmth, dry mouth, diminished perception and auditory and visual hallucinations and nausea. However, symptoms were relatively transient and subsided after 4 hours (with the exception of fatigue, which lasted longer). The other participant responded stronger to the drug, and reported a feeling of sedation and a dry mouth, and, approximately 150 minutes after ingestion of the capsules, about not feeling well; shortly after, he likely experienced (although very transiently) a syncope. About 30 minutes after the syncope, all side effects subsided.

It is surprising that these marked side-effects have not (yet) been reported in the literature; only one study reports on the implementation of a clonidine dosage lower than the initial 300 microgram which had induced sleep in a number of participants (Kennedy *et al.*, 1995). One possibility is that the present combination of tasks was relatively taxing and stressful for participants and that the combination with clonidine enhanced the chance of a vasovagal collapse (i.e. "freeze reaction"). Another possibility is that side effects are in general underreported in the literature.

In order to prevent unexpected side effects in participants and related time delays in the projects, we devised a different approach for the haloperidol study. After a literature review, we started our study with the smallest dose reported to have a possible effect relevant (to our study) outcome variables, with reported minimal side effects. We planned on performing an interim analysis on a preliminary sample of eight participants. Based on this sample, the burden for participants in terms of side effects on the one hand and the effect size on the dependent variables for final sample estimation (yielding 80 percent power) on the other hand was estimated. Only if the effect size was insufficient (i.e. resulting in a sample size of over 38 participants), we would consider to continue the study with a higher dosage of maximally 3mg, otherwise the study would be continued with 2 mg.

8.2.2. Analyses on restricted samples: ensuring room for drug-effects and accounting for individual variability in drug responses

8.2.2.1. Ensuring room for a drug-effect, restricting the sample for secondary analysis

As discussed, the most relevant output parameter of the VSC task is the validity effect on reaction time. In general participants respond faster to targets presented in the attended visual hemifield as opposed to targets presented in the unattended hemifield. Obviously, the size of the validity effect on reaction time determines the room for detecting a possible effect of some manipulation, such as a drug challenge, on this attentional modulation by cueing. One issue is that some participants do not show such clear validity effect (Thiel & Fink, 2008). One solution is to exclude participants who do not show this clear attentional modulation (validity effect) (Thiel & Fink, 2008). In a similar vein, our strategy was to restrict secondary analyses to the sample in which the effect size (Cohen's d) pertaining to the effect of validity on reaction time during the pre-test ensured room for detecting a possible drug effect. After the implementation of this strategy, firstly, a reduction of the validity effect on reaction time was detected under nicotine (albeit restricted to post-treatment data). Secondly, a bias reducing effect after noradrenergic antagonism by clonidine was evident (as reflected in the reduction of the N1 modulation).

For the primary readout measure of the SST in the nicotine study a related issue pertaining to possible lack of room for a drug-effect, was expected. Results of Potter et al. (Potter, Bucci, & Newhouse, 2012) suggested that the effect of nicotine on SSRT may be restricted to subjects for which SSRTs are relatively long (reflecting poor inhibitory motor control). We acknowledged the possibility that in our group of healthy subjects the effect of nicotine may be undetectable as a result of a ceiling effect. Therefore, we performed secondary analyses on half of the sample for which SSRTs were longest. These analyses showed that nicotine resulted in a relatively enhanced shortening of SSRT for non-smokers as opposed to smokers (albeit restricted to post-treatment data).

Ascertaining sufficient room for improvement is important to enable detecting a potential drug effect on dependent variables. Hence, the aforementioned strategy is highly recommendable, especially when individual variance pertaining to performance is sizable.

8.2.2.2. Accounting for individual variability in drug-responses

Receptor occupancy may vary significantly across subjects, even at the same dosage (de Haan *et al.*, 2003). To account for possible related interindividual variance in central drug responses, we used two types of proxies of drug responsivity. Firstly, both clonidine and nicotine have been shown to significantly affect cardiovascular variables, albeit in opposite ways (Turetsky & Fein (2002) and Fisher et al. (2012), respectively). Hence, cardiovascular variables as a proxy for drug responsivity were included under the assumption that a drug response on peripheral (cardiovascular) variables is correlated with a drug response on central parameters. The inclusion of a proxy for drug response provided evidence for a modulation of disengagement related activity after clonidine. Furthermore, for strong diastolic responders to nicotine, a nicotine induced reduction of bias related activity and enhancement of dorsal inhibitory related activity was evident.

Secondly, as is evident from chapter 7, 2 mg haloperidol seems to have negligible effects on cardiovascular variables. However, it is known that haloperidol may affect motor activity at D2 receptor occupancy exceeding 78 - 80 percent (de Haan et al., 2003; Kapur, Zipursky, Jones, Remington, & Houle, 2000). Hence, we assessed indices of bradykinesia (velocity scaling) and dyskinesia (spontaneous motor activity), for use as possible proxies for individual central responsivity to haloperidol.

As responsiveness to a specific drug may vary markedly across individuals, using a proxy for individual responsivity may be a necessity and is highly recommendable for detecting a potential drug effect, especially if overall drug effects are relatively subtle.

8.3. The role of noradrenaline, acetylcholine and dopamine in visuospatial attention and inhibition

8.3.1. Noradrenaline

Results of chapter 3 indicate that clonidine, albeit in a restricted sample with enough room for an effect, reduces the result of bias as reflected in a reduction of the N1 modulation by cueing.

Furthermore, results suggest that clonidine has a detrimental effect on disengagement. This last interpretation is a rather complicated one. As mentioned in section 8.1.2, cardiovascular measures were used as a proxy for individual responsivity in which a positive correlation was assumed between cardiovascular measures on the one hand, and central effects on the other. Results indicated that the LPD effect, reflecting disengagement, was enhanced in strong systolic responders as opposed to low systolic responders. In other words, disengagement seemed facilitated in participants with relatively strong peripheral response as compared to participants with a relatively weak peripheral response. This contrasts with the performance data. No effect of clonidine was evident on the validity effect on reaction time. A combination of diminished bias (which is evident in ERP data) and enhanced disengagement would result in a reduction of the validity effect on reaction time, which is not evident in the behavioural data. As mentioned in chapter 2, an alternative possibility is that, at least with respect to clonidine, the peripheral response on the one hand and central effect on the other are negatively correlated. In other words, a stronger peripheral response may result in reduced central bioavailability of the agent. Hence, disengagement may be reduced after a strong central effect, which may be the case in weak systolic responders. This last interpretation fits with the performance data, i.e. a reduction of bias and reduction of disengagement results in a netto nill effect in terms of validity effect, while reducing overall reaction time. Results of chapter 4 in which the effect of clonidine in the SST is reported, seem in line with results reported in chapter 3. In chapter 1 we argued that disengagement and inhibition are tightly related, and importantly, dorsal inhibitory processing (presumably in the Superior Frontal Gyrus) as reflected in the stop P3 component, is significantly reduced following noradrenergic attenuation by clonidine, as reported in chapter 3. It is interesting to note that we did not find a reduction of the stop N2, reflecting inhibitory related activity in the right Inferior Frontal Gyrus. It may be that the LPD is (at least in part) generated by the same brain region as the stop P3. Somewhat surprisingly, we did not find an effect of clonidine on behavioural inhibition as indexed by the SSRT. It might be that the stop P3 reflects a more general behavioral-interrupt process that may not specifically relate to speed of inhibition as indexed by SSRT.

In sum, and partly in contrast to what was expected, the present results are consistent with the notion that the NE system is involved in bias implementation at the level of visual

cortex, but possibly also in disengagement. Furthermore NE plays a role in what was presently termed the dorsal inhibition system, not the ventral one.

8.3.2. Acetylcholine

As summarized in 8.1.3., nicotine did not seem to affect performance measures of bias, disengagement or inhibition. The only exception was the enhancing effect of nicotine on SSRT in non-smokers, although restricted to post-test data. The lack of differences between smokers and non-smokers remains puzzling in the light of recent reports on inhibitory dysfunctions reported in smokers (de Ruiter, Oosterlaan, Veltman, van den Brink, & Goudriaan, ; Luijten et al., ; Nestor, McCabe, Jones, Clancy, & Garavan). Furthermore, it has been suggested that ADHD patients smoke in order to alleviate symptoms related to the pathology (Potter, Newhouse, & Bucci, 2006), and hence, smokers may smoke to normalize anomalous inhibitory processing.

Nicotine did affect electrophysiological reflections of these parameters, and it might be that –at least for our paradigms- EEG is a more sensitive measure as compared to performance measures, or reflect a less subtle (i.e. more general) cognitive component. Indeed, nicotine did seem to positively affect inhibition as reflected in an enhanced stop P3. It must be noted though, that this effect was restricted to the split-half sample in which the nicotine induced peripheral effect, as reflected in diastolic blood pressure change, was largest. We argued that, although speculative, the LPD effect (reflecting disengagement) and stop P3 (reflecting inhibition) might be subserved by the same neuroanatomical brain region, namely the Superior Frontal Gyrus. Since nicotine did not seem to affect disengagement, it might be argued that the results of chapter 5 contradict the results in chapter 4, in terms of a nicotine induced effect on superior frontal gyrus activity. However, the LPD may very well consist of other generators as well, such as the Temporal Parietal Junction (Bruin, Kenemans, Verbaten, & Van der Heijden, 2000), also crucial in the process of disengagement (M. Corbetta, Patel, & Shulman, 2008; Maurizio Corbetta & Shulman, 2002). Interestingly, with regard to inhibition, we did not find an effect on the stop N2, which presumably reflects activity within the right inferior frontal gyrus (Schmajuk, Liotti, Busse, & Woldorff, 2006). Hence, it may be that nicotine specifically affects dorsal inhibitory related activity.

Nicotine had a trend-level negative effect on the result of bias (P1 modulation). Further analyses indicated that this effect was significant in participants showing the strongest peripheral drug effect as reflected in diastolic blood pressure (it was assumed that a stronger peripheral effect would be positively correlated with a stronger central effect). Nicotine did not seem to affect the onset of bias (EDAN, LDAP).

In sum, the nicotinic ACh system may be involved in (the result of) bias, and in functioning of the dorsal inhibition system. Contrary to expectations, there was no sign of ACh involvement in disengagement.

8.3.3. Dopamine

As summarized in 8.1.4., performance data suggest that attenuation of dopaminergic signaling results in a decrease in bias (as reflected in the reduction of the validity effect), but not in affected disengagement or in inhibition (as reflected in SSRT). Again, the effect on bias was restricted to post-treatment data. Furthermore, the reduction of the validity effect under haloperidol was at trend-level significance, underscoring the preliminary nature of these data as obtained from a small sample. This pattern of attenuated bias combined with intact disengagement and inhibition may be linked with results from previous studies using methylphenidate and atomoxetine. These two pharmacological agents that affect both DA as well as NE neurotransmission, both seem to positively affect the right inferior frontal gyrus, implicated in inhibition. It has been reported that methylphenidate results in an enhancement of the stop N2 in ADHD patients (Pliszka et al., 2007) and atomoxetine has been shown to increase inhibition related activity within the right inferior frontal gyrus (Chamberlain et al., 2009). Our results suggest that these effects may be specifically related to the effect of the facilitation of the noradrenergic system (chapter 2,3), and not the dopaminergic system (chapter 7).

However, the lack of an effect on SSRT may also be related to the specific implemented paradigm. More specifically, as argued in chapter 7, the SST as implemented in the current studies, may prompt a more reactive strategy as opposed to a variant of the SST in which stop stimuli are presented in the auditory domain, in which case stopping is more proactive. Importantly, both methylphenidate (Overtoom et al. (2009) and Pliszka et al. (2007),

in patients) and atomoxetine (Chamberlain et al. (2009); Chamberlain et al. (2006) , healthy volunteers) have been shown to affect inhibition in both contexts. Now, as described in detail in chapter 7, it may be speculated that inhibitory mechanisms triggered by proactive stopping, but not reactive stopping, are (mostly) subserved by subcortical dopamine. Reactive stopping, on the other hand, may depend more on noradrenergic neurotransmission.

However, the possibility cannot be excluded that haloperidol does affect inhibition in reactive stopping. Specifically, we previously reported effects on the stop P3 in the absence of effects on SSRT. Hence, it may be possible that haloperidol does affect the stop N2, or the stop P3 in the absence of an effect on SSRT. In sum, the current preliminary behavioural data suggest that haloperidol primarily affects bias, without an effect on disengagement or inhibition. However, EEG indices of inhibition may yield more conclusive data.

8.4. New insights with respect to the (electro)psychopharmacology of visuospatial attention and inhibition

Our results indicate that stimulants do not necessarily augment processes pertaining to visuospatial attention. Clonidine (a sedator) negatively affected the result of bias as evidenced in the reducing effect on the N1 modulation, while nicotine (a stimulant) also impaired processing related to the result of bias as evidenced by a drug-induced attenuation of the P1 modulation. It should also be noted that these pharmacological effects were restricted to the result of bias (effects on the P1/N1 complex), without any effect on ERPs related to the directing of attention (EDAN, LDAP). It may be the case that the occipital attentional modulation, as evidenced in the P1/N1 complex, is specifically dependent on noradrenergic and cholinergic neurotransmission. In this vein, biochemical manipulation may affect a rather specific aspect of a brain mechanism that was initially presumed to be unitary; for example, NE may be instrumental in the processing of top-down signals in visual cortex (and therefore affect modulation of the P1/N1 complex), but not in the production of the top-down signals themselves. It must be noted though, that previous literature suggests ERPs related to the directing of attention do not explicitly predict ERPs related to the result of biasing signals (Hopf & Mangun, 2000). Hence, other mechanisms involved with the directing of attention, but not probed in our experimental paradigm may well be affected by our drug challenges.

Interestingly, while nicotine reduced bias, inhibitory processing (as reflected in an enhanced stop P3 under nicotine) was augmented. In this vein, smoking may alleviate inhibitory deficiencies, but at the expense of bias related processing. Alternatively, it may be speculated that the P1 reduction following a nicotine challenge, may actually promote disengagement, and in this way the P1 reduction under nicotine may fit an account in terms of facilitated disengagement. Interestingly, performance data suggests that the effect of nicotine on inhibition may be stronger in non-smokers as opposed to non-smokers. This is plausibly attributable to drug-tolerance in smokers, since no baseline differences were evident comparing smokers to non-smokers in terms of SSRT.

Pharmacological effects on the stop P3 were, however, not mirrored by effects on SSRT. Both are reported to index inhibitory processing, so the question is why pharmacological effects do not overlap. One possibility is that although both indices reflect inhibitory processing, they may reflect different components of inhibition. Firstly, as SSRT is thought to reflect the speed of the inhibition process, the stop P3 may reflect a more general interrupt signal. Secondly, each parameter and neuroanatomical correlate may differ in terms of underlying neurotransmitter systems.

To the extent that clonidine affects (the result of) bias and dorsal inhibition, and haloperidol effects seem limited more to aspects of attention, this could be taken as indicating that beneficial effects of methylphenidate on inhibitory control are more NE based than DA based. However, there are problems in relation to this idea. Firstly, the NE manipulation by clonidine attenuated the dorsally distributed stop P3, which in a previous though isolated study was found to also be reduced under methylphenidate (Overtoom *et al.*, 2009). Furthermore, we did not find any evidence of an (attenuating) effect of clonidine on right frontal gyrus activity as reflected in the stop N2, while it has been reported that methylphenidate enhances the stop N2 in ADHD patients (Pliszka *et al.*, 2007). Secondly, atomoxetine seems to mirror this latter effect of methylphenidate. More specifically, it has been shown that NE facilitation by atomoxetine results in increased inhibition related activity within the right inferior frontal gyrus, but not in more superior frontal regions (Chamberlain *et al.*, 2009). Furthermore, plasma levels correlated positively with activity within the right inferior frontal gyrus (Chamberlain *et al.*, 2009). With respect to DA antagonism, it remains to be seen whether haloperidol does or does not affect stop P3, in the possible absence of an effect on SSRT. For the time being, the

possibility should be acknowledged that NE and DA receptor-specific antagonists and agonists do not provide good models for the effect of reuptake inhibitors on these same neurotransmitter systems.

To wrap up, the expectations that were formulated at the outset of this project were confirmed only to very limited extent. It was expected that attenuation of noradrenergic neurotransmission by clonidine would have a negative impact on orienting of attention and the result of bias, without any clear effect on disengagement. Indeed, the result of bias was reduced after clonidine. However, contrary to the expectation, orienting of attention did not seem to be affected, while clonidine seemed to modulate disengagement. In line with the latter finding, it was hypothesized that clonidine would negatively impact mechanisms of inhibition, which was partly confirmed as clonidine affected the dorsal inhibitory mechanism, but not the ventral system. With respect to the cholinergic system, it was expected that facilitation of this system by nicotine would result in enhanced disengagement and related inhibition, in the absence of an effect on bias. Nicotine specifically augmented the dorsal inhibitory system, without affecting the ventral inhibitory system. Furthermore, nicotine actually had a negative impact on the result of bias in the absence of an effect on the orienting of attention or disengagement. With respect to dopamine, it was hypothesized that dopaminergic antagonism by haloperidol would have a negative effect on disengagement and inhibition. However, preliminary results of performance data, suggest that haloperidol negatively affects bias, without affecting inhibition or disengagement. EEG data may provide more conclusive results.

8.5. Recommendations

With respect to whether effects of methylphenidate and atomoxetine on central parameters are attributable to dopaminergic and/or noradrenergic manipulation, there is ambiguity. Hence, it would be recommendable to assess whether other potential pharmaceutical candidates provide a possible better fit for disentangling effects of neurotransmitter functioning (dopaminergic versus noradrenergic) on central parameters.

Furthermore, with respect to future pharmacological endeavors aimed at elucidating the effects, of the manipulation of neurotransmitter signaling, on central parameters, it would be recommendable to take into account individual variability in responsivity and to ascertain sufficient room for improvement for a possible drug effect to be assessed, as outlined in section 8.2.2.1 and 8.2.2.2.

REFERENCES

Barkley, R. A. (1998). *Attention deficit hyperactivity disorder: A handbook for diagnosis and treatment* (2 ed.). New York: Guildford.

Bitsios, P., Langley, R. W., Szabadi, E., & Bradshaw, C. M. (1996). Comparison of the effects of clonidine on tyramine- and methoxamine-evoked mydriasis in man. *Br J Clin Pharmacol, 41*(4), 269-275.

Bruin, K. J., Kenemans, J. L., Verbaten, M. N., & Van der Heijden, A. H. (2000). Habituation: an event-related potential and dipole source analysis study. *Int J Psychophysiol, 36*(3), 199-209.

Chamberlain, S. R., Hampshire, A., Muller, U., Rubia, K., Del Campo, N., Craig, K., et al. (2009). Atomoxetine modulates right inferior frontal activation during inhibitory control: a pharmacological functional magnetic resonance imaging study. *Biol Psychiatry, 65*(7), 550-555.

Chamberlain, S. R., Muller, U., Blackwell, A. D., Clark, L., Robbins, T. W., & Sahakian, B. J. (2006). Neurochemical modulation of response inhibition and probabilistic learning in humans. *Science, 311*(5762), 861-863.

Corbetta, M., Patel, G., & Shulman, G. L. (2008). The reorienting system of the human brain: from environment to theory of mind. *Neuron, 58*(3), 306-324.

Corbetta, M., & Shulman, G. L. (2002). Control of goal-directed and stimulus-driven attention in the brain. *Nature Reviews, 3*, 201 - 215.

Coull, J. T., Nobre, A. C., & Frith, C. D. (2001). The noradrenergic alpha2 agonist clonidine modulates behavioural and neuroanatomical correlates of human attentional orienting and alerting. *Cereb Cortex, 11*(1), 73-84.

de Haan, L., van Bruggen, M., Lavalaye, J., Booij, J., Dingemans, P. M., & Linszen, D. (2003). Subjective experience and D2 receptor occupancy in patients with recent-onset schizophrenia treated with low-dose olanzapine or haloperidol: a randomized, double-blind study. *Am J Psychiatry, 160*(2), 303-309.

de Ruiter, M. B., Oosterlaan, J., Veltman, D. J., van den Brink, W., & Goudriaan, A. E. (2012). Similar hyporesponsiveness of the dorsomedial prefrontal cortex in problem gamblers and heavy smokers during an inhibitory control task. *Drug Alcohol Depend, 121*(1-2), 81-89.

Fisher, D. J., Daniels, R., Jaworska, N., Knobelsdorf, A., & Knott, V. J. (2012). Effects of acute nicotine administration on behavioral and neural (EEG) correlates of working memory in non-smokers. *Brain Res, 1429*, 72-81.

Hopf, J. M., & Mangun, G. R. (2000). Shifting visual attention in space: an electrophysiological analysis using high spatial resolution mapping. *Clin Neurophysiol, 111*(7), 1241-1257.

Johnson, M. A., Blackwell, C. P., & Smith, J. (1995). Antagonism of the effects of clonidine by the alpha 2-adrenoceptor antagonist, fluparoxan. *Br J Clin Pharmacol, 39*(5), 477-483.

Kapur, S., Zipursky, R., Jones, C., Remington, G., & Houle, S. (2000). Relationship between dopamine D(2) occupancy, clinical response, and side effects: a double-blind PET study of first-episode schizophrenia. *Am J Psychiatry, 157*(4), 514-520.

Kenemans, J. L., Bekker, E. M., Lijffijt, M., Overtoom, C. C., Jonkman, L. M., & Verbaten, M. N. (2005). Attention deficit and impulsivity: selecting, shifting, and stopping. *Int J Psychophysiol, 58*(1), 59-70.

Kennedy, S. H., Gnam, W., Ralevski, E., & Brown, G. M. (1995). Melatonin responses to clonidine and yohimbine challenges. *J Psychiatry Neurosci, 20*(4), 297-304.

Luijten, M., Veltman, D. J., Hester, R., Smits, M., Nijs, I. M., Pepplinkhuizen, L., et al. (2012). The role of dopamine in inhibitory control in smokers and non-smokers: A pharmacological fMRI study. *Eur Neuropsychopharmacol.*

Morley, M. J., Bradshaw, C. M., & Szabadi, E. (1991). Effects of clonidine and yohimbine on the pupillary light reflex and carbachol-evoked sweating in healthy volunteers. *Br J Clin Pharmacol, 31*(1), 99-101.

Nestor, L., McCabe, E., Jones, J., Clancy, L., & Garavan, H. (2011). Differences in "bottom-up" and "top-down" neural activity in current and former cigarette smokers: Evidence for neural substrates which may promote nicotine abstinence through increased cognitive control. *Neuroimage, 56*(4), 2258-2275.

Overtoom, C. C., Bekker, E. M., van der Molen, M. W., Verbaten, M. N., Kooij, J. J., Buitelaar, J. K., et al. (2009). Methylphenidate restores link between stop-signal sensory impact and successful stopping in adults with attention-deficit/hyperactivity disorder. *Biol Psychiatry, 65*(7), 614-619.

Pliszka, S. R., Liotti, M., Bailey, B. Y., Perez, R., 3rd, Glahn, D., & Semrud-Clikeman, M. (2007). Electrophysiological effects of stimulant treatment on inhibitory control in children with attention-deficit/hyperactivity disorder. *J Child Adolesc Psychopharmacol, 17*(3), 356-366.

Potter, A. S., Bucci, D. J., & Newhouse, P. A. (2012). Manipulation of nicotinic acetylcholine receptors differentially affects behavioral inhibition in human subjects with and without disordered baseline impulsivity. *Psychopharmacology (Berl), 220*(2), 331-340.

Potter, A. S., Newhouse, P. A., & Bucci, D. J. (2006). Central nicotinic cholinergic systems: a role in the cognitive dysfunction in attention-deficit/hyperactivity disorder? *Behav Brain Res, 175*(2), 201-211.

Schmajuk, M., Liotti, M., Busse, L., & Woldorff, M. G. (2006). Electrophysiological activity underlying inhibitory control processes in normal adults. *Neuropsychologia, 44*(3), 384-395.

Smith, A., Brice, C., Nash, J., Rich, N., & Nutt, D. J. (2003). Caffeine and central noradrenaline: effects on mood, cognitive performance, eye movements and cardiovascular function. *J Psychopharmacol, 17*(3), 283-292.

Swanson, J. M., Sergeant, J. A., Taylor, E., Sonuga-Barke, E. J. S., Jensen, P. S., & Cantwell, D. P. (1998). Attention-deficit hyperactivity disorder and hyperkinetic disorder. *The Lancet, 351*(9100), 429-433.

Thiel, C. M., & Fink, G. R. (2008). Effects of the cholinergic agonist nicotine on reorienting of visual spatial attention and top-down attentional control. *Neuroscience, 152*(2), 381-390.

Turetsky, B. I., & Fein, G. (2002). Alpha2-noradrenergic effects on ERP and behavioral indices of auditory information processing. *Psychophysiology, 39*(2), 147-157.

Zetterstrom, T., Sharp, T., Collin, A. K., & Ungerstedt, U. (1988). In vivo measurement of extracellular dopamine and DOPAC in rat striatum after various dopamine-releasing drugs; implications for the origin of extracellular DOPAC. *Eur J Pharmacol, 148*(3), 327-334.

CHAPTER 9

DUTCH SUMMARY

(Visuospatiale) aandacht is van groot belang in ons dagelijks functioneren. Twee kerncomponenten van visuospatiële aandacht zijn "bias" en "disengagement". Bias wordt gedefinieerd als het resultaat van het richten van de aandacht dat vervolgens leidt tot versnelde verwerking van stimuli die binnen het aandachtsveld vallen. Disengagement wordt gedefinieerd als de loskoppeling van de focus van aandacht. Dit maakt het mogelijk om te reageren op onverwachte, maar relevante stimuli buiten het directe aandachtsveld. De heersende theorie stelt dat cholinerge neurotransmissie van belang is bij bias, en disengagement afhangt van het noradrenerge system (o.a. Corbetta & Shulman (2002)). Resultaten uit psychofarmacologisch onderzoek lijken echter tegenstrijdig met deze theorie. In dit project werd een alternatieve theorie getoetst die het tegenovergestelde van de heersende theorie stelt, en die resultaten uit voorgaand onderzoek beter kan verklaren.

In dit proefschrift wordt tevens de farmacologie van inhibitie besproken, gezien de sterke conceptuele relatie met disengagement. Evenals (visuospatiële) aandacht, is ook inhibitie van belang bij ons functioneren. Zo staan problemen met aandacht alsmede inhibitie centraal bij ADHD (Kenemans *et al.*, 2005). Methylfenidaat is (het medicijn van) eerste keus bij de behandeling hiervan en resulteert in verbetering op het domein van zowel aandacht als inhibitie (Kenemans *et al.*, 2005). Methylfenidaat faciliteert zowel noradrenerge als dopaminerge neurotransmissie (Zetterstrom, Sharp, Collin, & Ungerstedt, 1988) en de rol van deze systemen bij aandacht en inhibitie is nog onduidelijk. Dit is een belangrijk probleem, aangezien een substantieel percentage patiënten niet goed reageert op de standaard medicatie (Barkley, 1998; Swanson et al., 1998) . Bij deze groep is de balans tussen klinisch-effect en bijwerkingen dus niet optimaal.

In de projecten beschreven in dit proefschrift, is de rol van drie neurotransmitter systemen (noradrenaline, acetylcholine en dopamine) in relatie tot visuospatiële aandacht en inhibitie onderzocht. In drie afzonderlijke studies zijn deze systemen gemanipuleerd met medicatie, waarbij is gekeken naar het effect hiervan op gedrags- en electrofysiologische parameters gerelateerd aan bias, disengagement en inhibitie. Dit laatste is bewerkstelligd door computertaken die deze componenten activeren in de hersenen, te combineren met EEG metingen. Om te controleren voor interindividuele variabiliteit in drug-responsiviteit is tevens een index voor deze responsiviteit gebruikt.

Noradrenaline

In hoofdstuk 2 is de rol van het noradrenerge systeem bij visuospatiële aandacht onderzocht. Het noradrenerge systeem werd gedempt door middel van clonidine en het effect werd vergeleken met een placebo conditie. De verwachting was dat het dempen van noradrenerge neurotransmissie zou leiden tot een specifieke afname van bias. Gedragsdata leken geen indicatie voor een reductie van bias te impliceren, maar electrofysiologische data wezen uit dat het resultaat van bias inderdaad gereduceerd was na clonidine. Het effect was wel beperkt tot een selecte groep met genoeg ruimte om een drug effect te kunnen detecteren. Daarnaast was het effect specifiek voor het resultaat van bias, het richten van aandacht werd niet beïnvloed. Verder bleek er een associatie te zijn tussen het effect van clonidine op diastole bloeddruk en het effect van clonidine op aan disengagement gerelateerde activiteit (of elektrische hersenresponsen).

In hoofdstuk 3 werd gekeken naar het effect van clonidine op inhibitie. Hierbij werd in het verlengde van de resultaten betreffende disengagement uit hoofdstuk 2, verwacht dat clonidine een negatief effect zou hebben op inhibitie. Resultaten wezen hier uit dat clonidine leidde tot een afname van inhibitie gerelateerde hersenactiviteit. Dit effect was beperkt tot een specifiek deel (het dorsale gedeelte) van het inhibitie systeem. In tegenstelling tot de electrofysiologische data lieten de aan inhibitie gerelateerde gedragsparameters geen effect van clonidine zien. De gedragsdata suggereerden dat aandacht negatief werd beïnvloed door clonidine (toename respons variabiliteit en omissies).

Acetylcholine

In hoofdstuk 4 en 5 is bij gezonde niet-rokers gekeken naar het effect van de facilitatie van cholinerge neurotransmissie door middel van nicotine, op respectievelijk visuospatiële aandacht en inhibitie. In hoofdstuk 6 werden potentiële baseline verschillen alsmede verschillen betreffende het acute effect van nicotine op visuospatiële aandacht en inhibitie tussen nicotine-abstinente rokers en niet-rokers geëxploreerd. Bij hoofdstuk 4 was de verwachting dat facilitatie van cholinerge neurotransmissie zou resulteren in een toename van disengagement. Resultaten wezen uit dat nicotine resulteerde in een afname van activiteit

gerelateerd aan het resultaat van bias. Noch het richten van aandacht noch het loskoppelen van aandacht (disengagement) werd beïnvloed door nicotine.

In het verlengde van onze hypothese bij hoofdstuk 4, verwachtten we in hoofdstuk 5 dat nicotine inhibitie verbeterd. Op basis van de gedragsdata leek nicotine inhibitie positief te beïnvloeden. Uit electrofysiologische data bleek een specifieke toename van inhibitie-gerelateerde activiteit in het dorsale inhibitie systeem na nicotine. Dit effect was wel beperkt tot de helft van de groep deelnemers met de sterkste drug response op diastole bloeddruk.

Wat betreft baseline verschillen en verschillen betreffende het acute effect van nicotine tussen nicotine-abstinente rokers en niet-rokers, was de verwachting dat nicotine abstinente rokers relatief slechtere disengagement en inhibitie zouden vertonen vergeleken met niet-rokers. Daarnaast verwachtten we dat nicotine zou leiden tot een sterkere facilitatie van disengagement en inhibitie bij nicotine-abstinente rokers in vergelijking met niet-rokers. Resultaten wezen uit dat er geen baseline verschillen waren tussen de rokers en niet-rokers. Post-hoc analyses suggereerde dat nicotine mogelijk een sterkere faciliterende werking op inhibitie heeft bij niet-rokers vergeleken met rokers.

Dopamine

In hoofdstuk 7 is gekeken naar het effect van het dempen van dopaminerge neurotransmissie, door middel van 2mg haloperidol, op gedragsvariabelen. Dit betrof een studie met een kleine sample met als doel het bepalen van de optimale dosering voor het detecteren van een effect bij maximaal 30 deelnemers. De verwachting was dat haloperidol zou resulteren in een afname van zowel bias, als (in mindere mate) disengagement en inhibitie. Resultaten ondersteunen onze verwachting betreffende visuospatiële aandacht (reductie van het validiteits effect op reactie tijd na haloperidol). De verwachting is dat bij een complete sample van 30 deelnemers, dit (geschatte) effect te detecteren is. Echter, haloperidol lijkt geen (of een verwaarloosbaar) effect te hebben op de gedragsmaat van inhibitie. Haloperidol bleek reeds in onze beperkte sample te leiden tot een significante toename in bradykinesie.

Conclusie

Demping van noradrenerge neurotransmissie door middel van clonidine resulteerde in een reductie van activiteit gerelateerd aan het effect van bias (zonder dat de signalen verantwoordelijk voor biasing werden beïnvloed) en in een reductie van disengagement, alsmede in een reductie van inhibitie gerelateerde activiteit. Facilitatie van cholinerge neurotransmissie door middel van nicotine resulteerde in een reductie van activiteit gerelateerd aan het effect van bias, alsmede in een toename van inhibitie gerelateerde activiteit. Ten slotte, voorlopige resultaten (gedragsdata) laten zien dat demping van dopaminerge activiteit door middel van haloperidol, bias mogelijk negatief beïnvloedt en geen effect heeft op inhibitie.

Aanbevelingen

Ten eerste, voorzichtigheid is geboden betreffende het generaliseren van de effecten van antagonisten naar de inverse van agonisten betreffende hetzelfde systeem en vice versa. Ten tweede, met het oog op toekomstige farmacologische studies gericht op het ontrafelen van specifieke effecten van neurotransmitter manipulaties op centrale parameters, zou het aan te bevelen zijn om rekening te houden met individuele variabiliteit in termen van drug-responsiviteit.

ACKNOWLEDGEMENTS

First of all I would like to thank my supervisor, Leon Kenemans, for offering me this very interesting and challenging project outlined in the current thesis. I learned a great deal from him with respect to many aspects of research. He was also a very accessible supervisor, and always available for discussion and support. Although I am quite optimistic, and content with how the projects proceeded, I do not think I have ever met anyone who is as optimistic as Leon, this also fueled my motivation. I am also most grateful for all support from Koen Böcker. He always showed a very clear and thoughtful way of commenting on my work, and was always open for discussion. Also, like Leon, he was always available for support. Gert, Camfferman, the first thing that comes to mind is: perseverance. You helped me a lot with many technical aspects, mostly pertaining to EEG. For example, I remember working on the port-conflict and adjar issues until late in the evening. I was at home using my laptop, you were in the Went building and we were discussing many steps over the phone while my wife suggested we really should eat at some point... Peter Deschamps was involved in many of my projects as medical advisory investigator and standby in case I had questions regarding the medical screening of participants or in case participants presented with side effects. This resulted in calls during easter, queensday, weekends etc. I also vividly remember our discussion about extrapyramidal side effects pertaining to the haloperidol study, and I will never forget the imitation of an acute dystonia. I also thank Peter van Harten and Jeroen Koning for the collaboration in the haloperidol study, thank you for sharing your expertise. Chantal Kemner: thank you for allowing me to work in your lab and for your critical remarks to the various chapters. I thank Stijn and Ivo for their substantial help with my project, for the nice campfire discussions at Achterveld and "whisky and steak" evenings! Peter Bos, it is nice that you were willing to take up the role of monitor in my nicotine and haloperidol studies, sorry for the massive amount of paperwork... Johanna Baas, I was happy to be able to assist you with several EEG-demo's, I learned a lot. You were of great help with my projects. Iris, thank you for your help with regard to drug-storage. Ans, Veronica, Renée and Eveline I appreciated your day-to-day help at the department. Rudmer, Alyanne, and Nathan, thank you for the friendly and welcome atmosphere at our office. Many thanks go out to all of my students,

especially with respect to the careful acquisition of EEG data, which is responsible work and helped to "fine tune" all parameters.

Bart en Marieke, I am very grateful you are my friends! Bart, we are friends for about 29 years now, thank you for all the support (and the multiplayer games!). Since we now recently own the yacht "The Variant", I look forward to much (sea?)sailing in the future! Evan and Eszter, my friends; it seems that every time I meet Evan, something completely crazy happens, from keys to your house falling in the elevator shaft, to accidently transporting an exotic butterfly from the botanical gardens to the Educatorium. Maciek and Nora, we made some nice trips, and I enjoyed playing lots of Fifa! Marcel and Gyönyvér, I always feel very welcome at your place, and it is always gezellig (and always good food!!). Guno, without any specific reason we see each other just around once a year, but we always have much fun and lots to tell. I still remember very vividly the last time sailing from Hoorn to Spakenburg, wow! Greetje and Richard, no dinner without nice (and very funny!!) conversations. Sorry for the food last time... Monique, Paul, Douwe and Puck, always nice talks during the (very!) extensive and tasteful "borrels"!

Thank you, Joke and Frits, mom and dad, for all your support and wise lessons from life, which I take close at heart. I also always considered you as very close friends and our many discussions and travels enriched me to great extent. Lidia and Jozsef, my second (Hungarian) mom and dad, and of course Gergely (my "black brother") thank you for everything; you always make me feel part of the Hungarian family. I wish to thank Onne and Werner, my sister and my brother-in-law, for their unrelenting hospitality and for our many nice talks, I learned a lot from you. Arthur my brother, and Sylvia: thank you for your support when our family suffered a relatively difficult time.

I want to thank my wife, Zsófia Molnár for moving to The Netherlands for me (I did warn you about the rain...) about nine years ago, and for providing me a life exceeding my most wonderful dreams. Nagyon, nagyon szeretlek. Last, but certainly not least, I would like to thank our kids, Zsófia Logemann jr. and Alexander Logemann jr.. You always make me laugh and, importantly, you keep me aware that there is so much to marvel and wonder about in life.

CV

H.N.A. (Alexander) Logemann was born on the 25th of September 1979 in Leusden, The Netherlands. In 2002 he enrolled at Utrecht University, The Netherlands and attained the Bachelor of Science degree in Psychology. In 2005 he enrolled in the research master Neuroscience & Cognition at Utrecht University and in 2006 started, in parallel, in the master Clinical- and Health psychology at the same university, but withdrew from that master, shortly after the start of his internship, since he developed more interest in research. In 2008 he attained the Master of Science degree and was employed as PhD student by Prof. dr. J.L. Kenemans at the department of Psychopharmacology at Utrecht University. Currently he is employed by Prof. dr. J.L. Kenemans as researcher at the department of Experimental Psychology at Utrecht University.