Pharmacological MRI in the assessment of monoaminergic function
Schouw, M.L.J.

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CHAPTER 5

OPPOSITE EFFECTS OF METHYLPHENIDATE AND D-AMPHE TAMINE ON RESPONSE INHIBITION

M.L.J. Schouw
M.B. De Ruiter
M. Bottelier
R.J.L. Lindauer
L. Reneman

In preparation
III. IMAGING THE DOPAMINERGIC SYSTEM USING fMRI

ABSTRACT

Dopamine (DA) is involved in systems governing motor actions, motivational processes and cognitive functions. Response inhibition has been linked to DAergic and noradrenergic (NAergic) signaling and can be reliably measured with the Go/NoGo task. We compared an oral MPH and an i.v. dAMPH administration in healthy men to determine whether brain regions involved in response inhibition are affected differently by these two DAergic challenges. We therefore performed phMRI during response inhibition using two types of DAergic challenge. In experiment 1, eight healthy males, aged 22.0 years (± 3.0), received 35 mg methylphenidate (MPH), in experiment 2 twelve healthy males, aged 21.0 years (± 1.5) received an i.v. with 0.3 mg/kg dextroamphetamine (dAMPH). Baseline and post-challenge imaging data was acquired and analyzed to determine the effect of DAergic manipulation on response inhibition, and their interacting effects. As expected, response inhibition was associated with significant activation in the right inferior frontal gyrus, anterior cingulate cortex and right inferior parietal cortex. MPH challenge decreased frontostriatal activation, where dAMPH increased sensorimotor activation. The interaction between these effects was largely dominated by the dAMPH response. These results demonstrate that the effects of DAergic drugs differ considerably when performing a task testing executive function. This is most likely attributable to the difference in pharmacological action and route of administration of both drugs.
INTRODUCTION

Monoamine system deregulation is involved in several types of brain dysfunction, both neurological and psychiatric. Dopamine (DA) for instance is believed to be involved in disease processes such as Parkinson’s disease, schizophrenia and attention deficit hyperactivity disorder (ADHD) (for a review, see (Sulzer et al., 2005). Therefore, imaging of the DAergic system is important to increase our understanding of pathologies and to subsequently increase the efficacy of (pharmacological) treatment strategies. The combination of a DA challenge with functional MRI (fMRI) is a relatively new technique called pharmacological MRI (phMRI). This imaging tool allows for a minimally invasive assessment of DAergic (dys) function by depicting region-specific neurovascular responses to a DAergic challenge (Knutson et al., 2004).

Response inhibition is linked to DAergic and noradrenergic (NAergic) signaling (for review (Robbins and Arnsten, 2009) For example, children with ADHD, which is associated with impaired response inhibition as well as DAergic dysfunction, were found to have a higher frontal BOLD response during inhibition compared to controls (Vaidya et al., 1998). A reliable measure of response inhibition is the Go-NoGo task, where subjects are instructed to respond to a series of frequently presented stimuli and to inhibit their response in another category of infrequently presented stimuli.

Challenging the DAergic system is usually accomplished by either MPH or dextroamphetamine (dAMPH) administration. Both drugs increase the amount of DA in the synaptic cleft, MPH mainly by blocking its reuptake, dAMPH also causes active DA release (Kahlig et al., 2005; Kuczenski and Segal, 1997b; Segal and Kuczenski, 1997; Volkow et al., 1999). The type of drug used and manner of administration can give rise to different DAergic response patterns. For example, i.v. administration of MPH causes a fast rise in DA transporter (DAT) occupancy of MPH causing a more rapid increase in DA levels than oral administration (Spencer et al., 2006; Swanson and Volkow, 2003). In previous work, we demonstrated that oral MPH leads to a decrease in cerebral blood flow in healthy volunteers, whereas i.v. dAMPH leads to an increase in cerebral blood flow (Schouw et al., 2013a; Schouw et al., 2013b). Therefore different challenges may lead to different DAergic responses, which in turn may alter task activation in a different manner.

Oral MPH challenges have been used in several fMRI investigations involving both healthy and ADHD populations (Bush et al., 2008; Rubia et al., 2009; Schlosser et al., 2009; Shafritz et al., 2004). In a response inhibition study, MPH decreased activation in striatum of healthy controls after an oral MPH challenge, while increasing activation in children with ADHD, normalizing activation (Vaidya et al., 1998). Similar results were reported in reward-related tasks (Rubia et al., 2009; Wilkison et al., 1995). In contrast, another study showed increased putaminal activation during response inhibition after MPH challenge in healthy
volunteers (Costa et al., 2012). In addition, i.v. administration of methylphenidate (MPH), was found to increase the BOLD response during a stop-signal task in the striatum and thalamus of active cocaine users with an abstinence period of five days or more (Li et al., 2010).

Intravenous dAMPH challenges combined with fMRI have not been previously reported to our knowledge. The effects of oral dAMPH have been investigated using auditory sensorimotor tasks, showing increased activation in auditory and sensorimotor cortex after dAMPH administration (Uftring et al., 2001). Increased activation was also found during a memory load task using dAMPH challenge (Tipper et al., 2005). However, little information is available in the literature on the effects of dAMPH on fMRI activation patterns in response inhibition.

In this study, we compared an oral MPH and an i.v. dAMPH administration in healthy men to determine whether brain regions involved in response inhibition are affected differently by these two DAergic challenges. As we administered MPH orally and dAMPH intravenously, we would expect a difference in hemodynamic response. In view of the fact that response inhibition is dependent on the integrity of the fronto-striatal circuitry, and that this region contains relatively high levels of DA, we expected that both drugs would modulate frontostriatal activity. Due to the difference between the types of DAergic challenge, we expected a decrease in activation caused by oral MPH and an increase in activation by i.v. dAMPH.

**METHODS**

**Subjects**

Subjects were recruited by posting advertisements around the medical campus, on websites and in regional newspapers. For the oral MPH experiment eight male, healthy subjects were recruited; for the oral dAMPH experiment twelve male healthy subjects were recruited. Written informed consent was obtained from all subjects.

Subjects were asked to refrain from using caffeinated products on assessment days and to abstain from all psychoactive drugs for at least 2 weeks before scanning. Exclusion criteria for all participants were: any neuropsychiatric diagnosis or history of brain disease or injury, use of medication with affinity for DA (e.g., MPH) or any contra-indication to MRI such as metallic implants or claustrophobia. Subjects received a small financial compensation for their participation.

This study was approved by the medical ethics committee of the Academic Medical Centre Amsterdam.
CHAPTER 5 | OPPOSITE EFFECTS OF METHYLPHENIDATE AND D-AMPHETAMINE ON RESPONSE INHIBITION

Procedure

Two study groups were compared. The first group involved eight healthy subjects performing an fMRI version of the go-nogo task before and after the administration of an oral challenge with MPH. The second group involved twelve healthy subjects who performed the task before and after the administration of an i.v. challenge with dAMPH.

For the first study, three tasks were presented in the same order for every subject; first the go-nogo task, then a reward task followed by an emotional face recognition task. Results of the reward task and the face recognition task will be reported elsewhere. Data was also collected in a group of recreational dAMPH users. However, the aim of this paper is to investigate the difference in response to two different DAergic challenges in healthy subjects, we therefore decided to leave this subset of data out of the analyses presented.

For the oral MPH study, subjects received 35 mg MPH (approximately 0.5 mg per kg body weight) after the first scanning session, to be taken orally with water. Subjects were then free to relax for 1 ½ hours until peak plasma levels were expected (Swanson and Volkow, 2003) and then re-entered the MRI scanner for the second session which was ordered identically to the first. MPH was obtained from Sandoz B.V. (Weesp, the Netherlands). For the i.v. dAMPH study subjects performed the baseline task, after which a resting state sequence of approximately 20 minutes was started, during which (after 5 minutes) an i.v. of 0.3 mg/kg dAMPH with a saline flush was administered over a two minute interval. After this resting state sequence (15 minutes after the dAMPH challenge, when the peak of subjective drug effect has just passed (Laruelle et al., 1995)) the second Go-NoGo task was performed.

Imaging

All MR imaging was performed using a 3.0 Tesla Philips MR scanner equipped with an SENSE 8-channel head coil and body coil transmission (Philips Medical Systems, Best, The Netherlands). The session protocol consisted of a high resolution 3D T1-weighted anatomical scan for registration and segmentation purposes and a fast single shot echo planar image (EPI) sequence for BOLD analysis. For the BOLD acquisition imaging parameters were: TR/TE 2300/30 ms; FOV 220×220 mm²; 40 slices; voxel size 3 x 3 x 3 mm; no gap; 80° flip angle, SENSE 2.0.

fMRI

The Go-NoGo task was presented by a video projection system onto a white screen using E-prime software (Psychological Software Tools, USA). Subjects saw the screen via a mirror attached to the head coil. Responses were logged via a response box, instructing the individuals to press their right index finger as quickly as possible in favor of accuracy.
All subjects performed a modified version of the Go-NoGo task (Durston et al.,
2003) with go and nogo stimuli presented for 999 ms and an inter-trial interval of 2777
ms. The task consisted of three runs, with each run containing a total of 57 trials, with 75%
go trials. One run lasted approximately 2 minutes and 9 seconds. Subjects were asked to
respond as quickly as possible to go stimuli with their right index finger and not to respond
to nogo stimuli. NoGo stimuli were presented in a pseudorandom order in between 2-5 go
stimuli. Two versions of the task, as well as two practice versions of the task were available
in order to minimize learning effects. The versions were administered in a mixed, balanced
order. All subjects practiced a shortened version of the task before entering the MRI scanner.

Analysis

Continuous variables of group characteristics were analyzed using unpaired two-tailed
student’s t-tests (log transformed if necessary). All demographic and behavioral data was
analyzed in SPSS version 18.0 (SPSS Inc, Chicago, Ill) and are presented as mean ± standard
deviation unless otherwise indicated.

For the second experiment, one of the datasets was incomplete and therefore
eleven complete datasets were included for analysis. MRI scans were analyzed using FSL
5.0 (FMRIB-Software-Library, Functional Magnetic Resonance Imaging of the Brain Centre,
Non-brain structures were removed from 3DT1 anatomical scans using the Brain Extraction
Tool (Smith, 2002). Scans were analyzed using FEAT (Beckmann et al., 2003), with MCFLIRT
motion correction (Jenkinson et al., 2002), BET brain extraction, spatial smoothing set at
5 mm FWHM, high-pass filter cut-off at 100 sec. EPI scans were linearly registered to the
subject’s corresponding high resolution structural image and subsequently nonlinearly to
standard MNI space (MNI152_T1_2mm_brain from the FSL atlas library).

The general linear model (GLM) used for first-level analysis modeled go and nogo
trials and the nogo - go contrast to isolate brain regions involved in response inhibition. The
obtained first-level analysis was entered into a second-level (group effect) analysis. Main task
effect was determined by examining first level effects for all scans available, both baseline
and post-challenge scans from both experiments were used for this analysis. To this end, we
used a cluster correction to correct for multiple comparisons with Z > 2.3 and p < 0.05.
For all other comparisons cluster correction threshold was set at Z > 1.6 and p < 0.05, as
group sizes were relatively small. For both experiments a paired comparison of baseline and
post-challenge scans was performed in order to determine the effects of drug challenge on
task activation. In addition we examined the interaction effect between the two different
types of challenge.
RESULTS

Sample characteristics
Average age of the participating healthy subjects receiving a challenge with MPH was 22.0 years (± 3.0), with completed years of education 16.4 (± 2.9). Average age of those receiving a challenge with dAMPH was 21.0 years (± 1.5). The number of completed years of education in this group was 15.1 years (± 2.0).

Whole brain analysis of inhibition of response
The analysis of all scans (both studies, both drug conditions) showed activation in the right insula and inferior frontal gyrus (IFG), the dorsal anterior cingulate cortex (ACC) and parietal cortex (Figure 1 and Table 1).

Figure 1 Activation clusters overlaid on a standard brain template representing activation for the NoGo – Go condition. Activation clusters are seen in the insula/inferior frontal gyrus, the anterior cingulate cortex and the inferior parietal cortex. Results are cluster corrected with Z > 2.3 and p < 0.05.
III. IMAGING THE DOPAMINERGIC SYSTEM USING fMRI

Table 1 Activation clusters for response inhibition for both studies and for the paired results before and after challenge with MPH and dAMPH

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Activation clusters</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>Max Z</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Task effect (NoGo – Go) 1 + 2</td>
<td>Orbitofrontal/Insula</td>
<td>32</td>
<td>24</td>
<td>-22</td>
<td>4.2</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Anterior Cingulate</td>
<td>0</td>
<td>44</td>
<td>6</td>
<td>3.9</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Inferior Parietal Lobe</td>
<td>66</td>
<td>-48</td>
<td>20</td>
<td>4.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>MPH effect (pre &gt; post) 1</td>
<td>Orbitofrontal PFC</td>
<td>2</td>
<td>24</td>
<td>-10</td>
<td>2.7</td>
<td>0.004</td>
</tr>
<tr>
<td>dAMPH effect (post &gt; pre) 2</td>
<td>Somatosensory cortex</td>
<td>-56</td>
<td>-26</td>
<td>52</td>
<td>3.8</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Study 1: oral MPH challenge

When comparing baseline scans with those after the challenge with oral MPH we observed a decrease in activation during response inhibition (Figure 2). The observed cluster included the orbitofrontal cortex, mainly left putamen, bilateral thalamus, bilateral amygdala, bilateral hippocampus, ventral ACC and brainstem. The cluster maximum was located in the orbitofrontal cortex (Table 1).

Figure 2 Effect of oral MPH (35 mg) on task-activation for the NoGo-Go contrast in healthy subjects on brain areas that show decreased activation after the MPH challenge including the orbitofrontal cortex, left putamen and left and right thalamus, left and right amygdala and hippocampus and ventral ACC, analyzed with paired t-testing with cluster-correction (Z > 1.6, p < 0.05). No increases in activation were observed (left panel). Right panel shows percentage signal change across activating voxels pre and post challenge.
Study 2: Intravenous dAMPH challenge

Analysis of the baseline and post-infusion scans revealed that there was an increase in activation after infusion with dAMPH (Figure 3). This increase was located in the left sensorimotor cortex (Table 1).

![Figure 3](image.png)

**Figure 3** Effect of i.v. dAMPH infusion (0.3 mg/kg) on task-activation of the NoGo-Go contrast in healthy subjects. In voxels in yellow-red color coding indicate the area of increased activation after infusion in the left somatosensory cortex, medial visual cortices and cerebellum, analyzed with paired t-testing with cluster-correction ($Z > 1.6, p < 0.05$). No decreases in activation were observed.

Interaction effect

In figure 4 the interaction between task and challenge is presented. The i.v. dAMPH infusion is the main contributor to the interaction effects that were observed. Again the sensorimotor cortex extending into the superior part of the temporal cortex, as well as several areas in the lateral visual cortices and superior parts of the cerebellum (bilaterally) show a statistically significant difference between the two groups of scans. Results are more or less identical to that of the analysis of just the dAMPH effect and the effect of MPH, when compared to dAMPH, is much less pronounced.
Figure 4 Effect of interaction between the MPH and dAMPH effects on task-activation of the NoGo-Go contrast in healthy subjects. Activating voxels indicate the area that is statistically different between the decrease in signal after MPH and increase in signal after dAMPH infusion. Again the left somatosensory cortex, medial visual cortices and cerebellum are involved, as analyzed with paired t-testing with cluster-correction ($Z > 1.6$, $p < 0.05$).

Figure 5 Cluster activation of the dAMPH effect from figure 2 with the interaction effect overlaid in blue, demonstrating the small difference between the interaction and dAMPH effects. The main difference is a small cluster in the sensorimotor cortex in addition to small differences in cluster size of the other activating areas.
DISCUSSION

Main findings
In this study we showed that a pharmacological challenge with MPH or dAMPH affects brain function during response inhibition when performing a Go/NoGo task. We demonstrated that an oral challenge with MPH (35 mg) caused a decrease in frontostriatal activation, as previously reported in the literature. Interestingly, our second experiment showed that a challenge with i.v. dAMPH (0.3 mg/kg) induced a much stronger and opposite effect from MPH, in that it significantly increased the response to inhibition in the somatosensory cortex in healthy human subjects.

Task activation
Response inhibition was associated with activation in the right IFG, right insula, the ACC and the right inferior parietal cortex (collapsed for baseline and post MPH/dAMPH scans). This is in agreement with earlier literature (Verbruggen et al., 2008; Pliszka et al., 2006). The supplementary motor cortex (SMC) has also been implicated in executive function, having a role in the alteration or suppression of an already initiated response (Nachev et al., 2008). By suppressing the Go response in a NoGo trial, the SMC activates in order to inhibit the button press. In our experiment, this response appears to be augmented after dAMPH infusion.

MPH and inhibition of response
We found a decrease in activation after an oral MPH challenge during response inhibition in the orbitofrontal cortex, left putamen and left and right thalamus, ventral ACC and amygdala. Costa and co-investigators found increased activation in the putamen in detected failed inhibitions during a Go/NoGo task in healthy subjects receiving an oral dose of MPH. They interpreted this as an effect of striatal-mediated performance monitoring, part of involvement of the basal ganglia in the executive function network used during the task (Costa et al., 2012). The effects of MPH on a stop-signal task have also been investigated, showing reduced activation in the IFG in response to an oral MPH challenge, but increased activation for failed inhibition, but only when controlling for attentional capture (Pauls et al., 2012). However, the stop-signal and Go/NoGo tasks seem to activate slightly different functional networks as the first is a purely reactive form of inhibition where the second can be proactive (Aron, 2011). Error in inhibition during go-nogo tasks was also the object of a study by Hester and colleagues, showing decreased activation in the ACC and insula after MPH administration during unaware errors of inhibition and increased activation in aware errors (Hester et al., 2004). In conclusion, MPH seems to diminish activation used during inhibition of response,
III. IMAGING THE DOPAMINERGIC SYSTEM USING fMRI

but to increase activation during error detection. This could tie in with a hypothesis that MPH decreases the amount of effort that is needed to inhibit the response and that more brain activity is allocated to error detection.

**dAMPH and inhibition of response**

To our knowledge dAMPH has not been previously used in fMRI investigations of response inhibition. Previous work in healthy human subjects has shown that oral dAMPH administration leads to quicker response times in a stop-signal task, with no improvement on other performance measures (Fillmore et al., 2005). Levodopa administration also did not affect performance in a Go/NoGo task, while reducing the magnitude of BOLD signal activation in the cerebellum and right parietal cortex (Hershey et al., 2004). In that light, our finding of increased activation in the sensorimotor area in reaction to i.v. administration of dAMPH could be explained to represent increased recruitment of this area in inhibitory processes. Poor motor response inhibition is observed in ADHD on the Go/NoGo and stop-signal tasks (Rubia et al., 2007; Willcutt et al., 2005). Therefore dAMPH might improve performance in these groups by increasing sensorimotor activation, in particular the supplementary motor cortex, which is involved in initiating inhibition in a planned motor response (Nachev 2008).

**Differences between MPH and dAMPH**

The main difference between the two drugs, when looking at the interaction effect, is dAMPH mediated. The effect of i.v. dAMPH administration is significantly larger than that of oral MPH, thereby overshadowing the MPH effect. Here we found that two drugs that both increase the levels of DA in the synaptic cleft (Fleckenstein et al., 2007; Swanson and Volkow, 2002), seem to affect the same task in very different manners, most likely caused by the difference in rise of DA levels in regards of amount of DA and the timing of the release (a quick and large effect in i.v. dAMPH versus a slower and smaller rise in DA after oral MPH). Administration of levodopa, a DA precursor, was found to reduce the magnitude of BOLD signal activation in the cerebellum and right parietal cortex in both healthy controls and patients with tic disorders during inhibition of response, again providing a different result of increasing DA levels during inhibition (Hershey 2004). It would be beneficial to obtain data in large patient groups, preferably while objectifying monoaminergic modulation, for example by obtaining PET or SPECT data on DA release in the same group so that it can be determined how the timing and size of DA release influences task function.
Response inhibition in DAnergic dysfunction

Executive function is very vulnerable to neurotoxic damage and all drugs with abuse potential, excepting cannabis, have been linked to poorer executive functioning, inhibition in particular (for review (van Holst and Schilt, 2011). For example cocaine users (cocaine is a DAnergic drug) have been shown to have a larger activation pattern during inhibition in comparison to control subjects (Connolly et al., 2012). When a memory component was added to the Go/NoGo paradigm, decreased activation in ACC and cerebellum were observed in cocaine users (Hester et al., 2004). Interestingly, adolescents with ADHD have also been shown to have a larger activation pattern during inhibitory processes (Schulz et al., 2004). This may lead to hypothesizing about similar DAnergic deficits in patients suffering from ADHD and those (ab)using drugs acting on the DAnergic system, and indeed similarities in the area of craving have been demonstrated before (Frodl, 2010). Our results may be used to further investigate the effects of DAnergic dysfunction on response inhibition.

Limitations

First, the number of participants in this study was rather small. The study was designed as explorative involving a limited number of subjects. However, even with this relatively small sample size, effects were considerable and significant even when using strict statistical thresholding.

Second, unfortunately we were unable to obtain behavioral data in this group, although performance was monitored during scanning to ensure compliance with the task administered. Therefore, we were not able to determine the effects of DAnergic challenge on inhibitory performance and to tease out brain regions involved in successful and unsuccessful response inhibition. However, the general task effect was in agreement with the current literature (Verbruggen et al., 2008; Pliszka et al., 2006).

Finally, because we did not include a placebo challenge we cannot completely rule out the possibility that an expectation of drug effect, or a volume effect, may have affected our results. The two DAnergic challenges did not modulate any overlapping brain region, however, which makes it unlikely that the observed effects are due to expectation only. Second, none of the groups had previous experience with MPH or dAMPH and did not know (exactly) what to expect. Finally, a previous study only found a small expectancy effect on brain hemodynamics with i.v. administration of MPH, whereas in the current study MPH was given orally (probably resulting in an even smaller expectancy effect) (Volkow et al., 2006). In addition, this expectancy effect during i.v. MPH administration was observed only on resting state MRI and not on task-related brain hemodynamics. These observations suggest that in the current study drug expectancy may have affected the results only minimally, if at all.
Conclusions

Challenging the DAergic system with either MPH or dAMPH, both increasing DA levels, affects activation during inhibition of response. However, where oral MPH (35 mg) caused decreases in activation in frontostrial areas, i.v. dAMPH (0.3 mg/kg) caused an increase in activation in the sensorimotor area. This demonstrates that type of drug and route of administration can have profound effects on parameters of functional activation.