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The effects of the phosphodiesterase type 5 inhibitor vardenafil on cognitive performance in healthy adults: a behavioral-electroencephalography study

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Abstract

Phosphodiesterase type 5 inhibitors (PDE5-Is) improve cognitive performance of rodents, but the few human studies investigating their effects did not systematically investigate cognitive effects and the results have been quite contradictory. Therefore, we examined whether the PDE5-I vardenafil improves memory and executive functioning and affect electroencephalography (EEG) in healthy young adults. Participants were selected out of a group of volunteers, based on their performance on a memory screening and they were orally treated with vardenafil (10–20 mg or placebo). Memory and executive functioning were tested while EEG activity was recorded. Additionally, a simple reaction time task and questionnaires addressing various complaints were presented. No prominent effects of vardenafil on cognition were found: participants only made more mistakes on a reaction time task after 20 mg vardenafil. During encoding of words, the P300 was generally smaller after vardenafil treatment. Furthermore, the N400 was larger after vardenafil 10 mg than placebo treatment in a spatial memory task at Fz. Finally, headache and feeling weak were reported more after vardenafil treatment. Vardenafil did not affect cognitive performance of healthy adults and showed only some incidental effects on ERPs. These findings in humans do not corroborate the cognition-enhancing effects of PDE5-Is in healthy animals.

Keywords

Cognition, memory, executive functioning, phosphodiesterase, learning, human

Introduction

Phosphodiesterase type 5 inhibitors (PDE5-Is) such as sildenafil (also known as Viagra), tadalafil (Cialis) and vardenafil (Levitra) are often used as drug treatments for erectile dysfunction (ED) (Langtry and Markham, 1999; Setter et al., 2005). This is accomplished by the selective inhibition of phosphodiesterase type 5 (PDE5), an enzyme that inactivates cyclic guanosine monophosphate (cGMP) (Bender and Beavo, 2006). Due to the presence of PDE5 in the brain it is not inconceivable that when a PDE5 inhibitor enters the brain it could have cognitive effects as well (Lakics et al., 2010). It has indeed been demonstrated that these drugs can enhance cognition in a variety of behavioral tasks in animals (for review see Reneerkens et al., 2009). For example, PDE5 inhibition did not only improve learning and memory performance in healthy rodents (e.g. Baratti and Boccia, 1999; Prickaerts et al., 1997; Prickaerts et al., 2002; Rutten et al., 2005), but also enhanced executive functioning (Rodefer et al., 2012) and memory performance in animals impaired by age (Domek-Lopacinska and Strosznajder, 2008), pharmacological intervention (Devan et al., 2007; Reneerkens et al., 2012) or a model of the amyloid deposition of Alzheimer's disease (Puzzo et al., 2009). In addition, treatment with the PDE5-I sildenafil increased response inhibition and executive functioning in healthy cynomolgus macaque monkeys (Rutten et al., 2008).

In contrast to the extensive report of the positive effects of PDE5 inhibition on cognition in animals, relatively little is known about the effects in humans. Schultheiss and colleagues (2001) showed that sildenafil treatment did not affect the behavioral

response of healthy adults, but appeared to have a positive influence on event-related potential (ERP) measurements related to selective attention. Another study by Grass et al. (2001) demonstrated that sildenafil decreased motor reaction time (RT) and showed a weak tendency of psychomotor improvement but also failed to find a positive effect of PDE5 inhibition on cognition in healthy volunteers. In a recent study using the PDE5-I vardenafil we did not observe any effect on information processing as measured with sensory gating (Reneerkens et al., 2013). It has also been shown that sildenafil treatment did not affect cognition in patients with schizophrenia (Goff et al., 2009). However, another study investigating the effects of repeated dosing of the PDE5-I udenafil in patients suffering from ED, demonstrated that this treatment improved performance of these patients on the Korean version of the mini-mental state examination (MMSE) and on an

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assessment battery measuring frontal executive function (Shim et al., 2011).

In general, the results of the animal and human studies seem to be rather contradictory and we therefore examined the influence of PDE5-Is more specifically on memory functioning, since it has already been established extensively that PDE5 inhibition has memory-enhancing effects in animals in a variety of behavioral models (e.g. Domek-Lopacinska and Strosznajder, 2008; Patil et al., 2004; Prickaerts et al., 2002; Reneerkens et al., 2012; Rutten et al., 2007; Rutten et al., 2005; Van Donkelaar et al., 2008). Therefore, we included multiple memory tasks in our study. Furthermore, since sildenafil improved executive functioning in rodents (Rodefer et al., 2012) and monkeys (Rutten et al., 2008), we incorporated executive functioning tasks as well. In addition, instead of the PDE5-I sildenafil, which was used in most human cognition studies so far, we used vardenafil because we previously found that, compared to sildenafil, a lower dose of vardenafil is needed to obtain memory improving effects in rats (Prickaerts et al., 2002). Thus, vardenafil appears to be more potent. Given the fact that sildenafil appeared to affect ERPs during attention-related tasks (Schultheiss et al., 2001), we decided to include electroencephalography (EEG) in the present study as well of which the sensory gating data has already been reported (Reneerkens et al., 2013).

The aim of this study was to examine the effects of vardenafil on cognition, in particular memory as well as executive function and attention, and the electrophysiological correlates, i.e. ERPs, of information processing in healthy volunteers. Cognitive performances were assessed while recording brain activity simultaneously. The results will provide further information on the potential of vardenafil as cognitive enhancer and will increase our knowledge on the role of PDE5 in human cognition in general.

Methods and materials

Participants

All experimental procedures were approved by the independent Ethics Committee of Maastricht University and the Academic Hospital Maastricht (The Netherlands). The study was conducted according to the code of ethics on human experimentation established by the Declaration of Helsinki (1964) and amended in Edinburgh (2000) and in accordance with the Medical Research Involving Human Subjects Act (WMO) [law on medical scientific research in humans]. The participants were recruited through advertisements at Maastricht University. Participants had to be willing to sign an informed consent form and were paid for their participation.

The present study started with a screening of 40 university students in which they were asked to complete the memory tasks used in our main study. Based on their performance we invited the volunteers within the 25th and 75th percentile to participate in our study. The reason for this distinction was as follows. Participants with the highest scores were not likely to benefit from the treatment (ceiling effect). However, participants with the lowest scores are (a) likely to show better performance on the test the next time (regression to the mean) and (b) may have scored not very high because of motivation problems.

Based on this screening, 18 out of the 40 participants were included in our study because we needed a multiple of six for our

randomization conditions. The volunteers (21 ± 0.7 years old, five males) were screened by a physician by means of a standard medical questionnaire and a medical examination. Participants were excluded if they suffered from, or had a history of, cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal, haematological or psychiatric illness. Other exclusion criteria were excessive drinking (>20 glasses of alcohol-containing beverages a week), pregnancy or lactation, use of medication other than oral contraceptives, use of recreational drugs from two weeks before, and until the end of, the experiment, and any sensory or motor deficit which could reasonably be expected to affect test performance. In addition, participants who had a first-degree relative with a (history of) psychiatric disorder were excluded as well. The participants could leave the study at any given time without any consequence.

Design and treatment

The study was conducted according to a double-blind, placebo-controlled, three-way cross-over design. Order of treatments was balanced over three test days and separated by a washout period of at least seven days. The balancing of the treatment order was accomplished by counterbalancing.

Treatment consisted of a placebo, or 10 mg vardenafil HCl (Levitra), or 20 mg vardenafil HCl (Levitra) and was within the range of dosages (5–20 mg) approved for human use (EMA, 2008). Previous studies have shown that peak plasma levels of vardenafil were reached 30–120 minutes (median 60 min) after a single dose of 20 mg vardenafil: the terminal half-life was around 4–5 hours (EMA, 2008). Therefore, we started the measurement 45 min after drug intake. The drugs were ingested orally and combined with a low-fat breakfast because fatty food might affect the absorption of vardenafil. The experimenter and participants were blind to the compound and doses tested.

Assessments

After enrolment in the study, the participants first underwent a training session. During this session, all tests were practiced to familiarize the participants with the study procedures and minimize procedural learning effects.

Each test day started with assessment of the general status of the participants and filling in questionnaires. Next, they received the capsules either containing vardenafil (10 or 20 mg) or a placebo. Forty-five min later the test battery started with the immediate recall of a verbal learning task (VLT), followed by the continuous recognition memory task (CRMT), the immediate recognition of a spatial memory task (SMT) and sensory gating, of which the last has already been reported as part of a translational study (Reneerkens et al., 2013). Next, the participants had a short break during which they filled in the questionnaires and had a glass of water if they wanted. After 5–10 min testing was started again; first the participants performed the Tower of London (TOL), then the Stroop task, a RT task, the delayed recall and recognition of the VLT, and finally the delayed recognition of the SMT.

Questionnaires

- Profile of Mood States (POMS): The POMS (McNair et al., 1971) is a self-evaluation scale for short, alternating states. It consists of 64 adjectives comprising five bipolar mood

factors (depression, anger, fatigue, vigor and tension) paired at 32 visual analogue scales (100 mm). In this way, the participant could indicate to what extent these items are appropriate to his/her mood.

- Bond and Lader Visual Analogue Scale (VAS): Subjective evaluations of alertness were assessed by using an adjusted series of nine visual analogue scales (100 mm), which provided summary scores for alertness (Bond and Lader, 1974).
- Questionnaire on medical complaints: This questionnaire addressed 31 potential physical complaints, including headache, nausea, dry mouth, blurred vision and dizziness. Next to each complaint was a four-point scale. In this way, the participant could indicate to what extent these items were appropriate to his/her physical well being (0 = not present; 3=extremely present).

VLT. The VLT is an adapted version of the Rey auditory verbal learning test (Lezak, 1995), which assesses immediate and delayed memory for verbal information. This task, modified by Riedel et al. (1999), was developed to maximize the possibility of measuring enhancement rather than impairment only by prolonging the list words to be learned. The list consisted of 30 monosyllabic words (18 nouns and 12 adjectives) in Dutch. The words were shown on a computer screen for 1 s; the total intertrial interval (ITI) was 3 s. Three trials with the same item sequence were presented. Each trial ended with a free recall of the words (immediate recall). Eighty minutes after the third trial, the participant was asked to recall as many words as possible without the words being presented (delayed recall). Subsequently, a recognition test was presented, consisting of 15 familiar words and 15 new but comparable words (distracters). The words were shown on a computer screen for 2 s (total ITI of 3 s) and participants were asked to rate whether they were presented in the learning trials by a 'yes/no' response. Different versions of this test were balanced over test days. For immediate and delayed recall, the total words correct, incorrect and double (named twice) were calculated for the analyses: for the recognition test, RTs and correct responses were used. For the EEG analysis, the ERPs of the three encoding trials were averaged.

CRMT. This task assessed recognition memory and was used as an immediate recognition test (based on a task used by e.g. Curran et al., 1998; Van Strien et al., 2007). A series of pictures (black and white line drawings) was presented on a computer screen with an ITI of 3 s. Five pictures were presented five times at the beginning of the task and occurred randomly in the series as fillers. Sixty pictures were only repeated once in the series 1, 3 or 10 stimuli after they were presented the first time (20 pictures in each condition). The participants had to rate each of the pictures as 'old' (I have seen it before) or 'new' (I have not seen it before). Different versions of this test were balanced over test days. The variables used for the analyses were the RT and the number of correct responses in general. In addition, these variables were calculated for the 'old' pictures presented 1, 3 or 10 stimuli after they were presented for the first time.

SMT. The SMT is a spatial memory task (based on the object relocation test, see Kessels et al., 1999; Sambeth et al., 2009) that consisted of two parts; immediate and delayed recognition. The immediate recognition comprises six trials in which ten pictures

(total of 60 pictures) were presented one by one on a computer screen (encoding phase) with an ITI of 3 s. The participants had to remember the location of the pictures. After each trial, the objects disappeared from the screen and reappeared one by one in the middle of the screen (repetition phase), followed by the presentation of a '1' and a '2' in different locations (relocation phase). The participants had to determine whether the picture had been presented on the location indicated by 1 or 2 consecutively for each picture. During the delayed recognition procedure 60 min after the initial presentation of the pictures, the subject had to decide again what the location of the pictures had been. Different versions of this test were balanced over test days. The measures used are the RT and the number of correct responses.

TOL. The TOL is used to assess executive functioning, including frontal planning abilities (Schmitt et al., 2005). This comprises the ability to think ahead and to evaluate the consequences of one's actions. The original version of the TOL consisted of three colored balls which had to be arranged on three sticks to match the target configuration on a picture while only one ball could be moved at a time (Shallice, 1982). In our study, we used a digitalized version that consisted of computer-generated images of the begin- and end-arrangements of the balls. The subject had to decide as fast as possible, whether the end-arrangement could be accomplished in 2, 3, 4, 5 or 6 steps from the begin arrangement by pushing the corresponding number coded button (the arrangement with six steps was excluded from the analysis). Each condition was randomly presented 10 times, except for the six steps condition which only occurred four times. Different versions of this test were balanced over test days. RTs and correct responses were the main performance measures.

Stroop. The Stroop task is well known for its ability to induce interference, and assesses response inhibition and focused attention. In this task, color names (in Dutch) were printed in colored ink and presented with an ITI between 2.5–3.5 s: in the congruent category, the color name and the color of the ink were the same, whereas in the incongruent category they were not. The participants had to name the color of the ink, not the words themselves. Due to the urge to read the printed words (even if one is asked to ignore them) interference occurs. Since the printed words and ink color differed in the incongruent category, interference was stronger in this category than in the congruent category: this is called the 'Stroop effect' and is known to remain even after extended practice (Gazzaniga et al., 2002). The colors used in this task were blue, red, green and yellow. The color of the ink had to be named by pressing one out of four buttons, each representing one of the colors. Each color was randomly presented 20 times in the congruent, as well as in the incongruent, condition which brings the total number of stimuli in the congruent as well as in the incongruent condition to 80. The main performance measures were the RTs and the number of correct responses.

RT task. The RT task (modified version of the CANTAB® choice reaction task e.g. Dassanayake et al., 2012) assessed motor speed and it was used to assess whether the drugs administered in the current experiment impaired vigilance. Participants were presented with an arrow that was either pointing to the left or right side of the screen. Depending on whether the arrow pointed to the left or the right, the subject had to push the left or right button

respectively. Dependent variables are the RT and number of correct responses.

EEG recordings

An EEG cap was used to place a set of 32 EEG electrodes according to the international 10–20 system (Jasper, 1958) but only the midline electrodes (Fz, FCz, Cz, CPz, Pz) were used in the statistical analysis. A reference and a ground were placed at the linked mastoids and at the forehead, respectively. Eye movements were detected by horizontal and vertical electro-oculogram (EOG) recordings. Before electrode attachment, the positions were cleaned with alcohol and slightly scrubbed with a gel in order to provide a good measurement. Both EEG and EOG were filtered between 0.01–100 Hz and sampled at 1000 Hz. The EEG responses were recorded during the VLT, CRMT, SMT and Stroop task.

The EEG data was analyzed using Vision Analyzer 2 (Brain Products, Gilching, Germany) software. Epoch files were made from 100 ms before stimulus onset until 1000 ms after onset, using the last 100 ms before stimulus onset as baseline. High pass (1 Hz) and low pass (30 Hz) filters were applied offline. The segments were checked for EOG activity (visually and by using the Gratton and Coles method in Vision Analyzer) and other artifacts, and excluded if an artifact occurred during the first 1000 ms after stimulus presentation. Next, averages were calculated for each stimulus type and treatment. The grand average was used to determine the ERP components. Although the time windows for peak detection varied for each task, generally taken the N100 (not detected for the SMT) was defined as the most negative value between 70–140 ms after stimulus onset, P150 as the most positive value between 130–210 ms, N200 as the most negative value between 140–310 ms, P300 as the most positive value between 255–380 ms, N400 as the most negative value between 365–520 ms (not detected in the CRMT and Stroop) and P600 (not detected in the SMT encoding phase) as the most positive value between 380–700 ms.

Statistical analysis

General linear model (GLM) repeated measures were used to analyze the effects of vardenafil treatment on the outcome variables of the cognitive tasks, the subjective mood scales, and the peak amplitudes of the ERP measurements. The results from the questionnaires during the baseline measurement (directly before ingesting the compound/placebo) were subtracted from the treatment measurement (approximately 100 min later) for further analysis. Treatment (three levels: placebo, vardenafil 10 mg, vardenafil 20 mg) was used as a within-subjects factor, as were the different stimulus and/or response types within a task if applicable (type). In the analyses of the ERP components, the factor channel (five levels: Fz, FCz, Cz, CPz, Pz) was included as well. In case of a statistically reliable effect, comparisons between means of the different conditions were analyzed in more detail using post-hoc *t*-tests ($p < 0.05$) with Bonferroni correction. One participant was excluded from the entire analysis and another one from the analysis of the behavioral response to the Stroop and the RT task because of an incomplete data set. Additionally, one subject had to be excluded from the analyses of the P300 peak of the CRMT, based on the results on the outlier test.

Results

A wide variety of channel effects was found for the ERP components across the tasks. It is beyond the scope of this paper to report all of them separately but, in general, the N200 was less negative at the parietal compared to the frontal part of the midline, whereas the P300 and the P600 grew more positive across the midline from the frontal to the parietal area. These are common effects.

VLT behavior

Immediate recall: No effects of vardenafil treatment were found with regard to words recalled correctly ($F_{2,32}=0.38$, not significant (n.s.)), incorrectly ($F_{2,32}=0.05$, n.s.) or mentioned twice ($F_{2,32}=1.13$, n.s.) (see also Table 1).

Delayed recall: With regards to the delayed recall, no effects of vardenafil were found for the words correctly recalled ($F_{2,32}=0.23$, n.s.), incorrectly recalled ($F_{2,32}=0.32$, n.s.) or double ($F_{2,32}=0.08$, n.s.).

Delayed recognition: An effect for the RT of old versus new words was found, with the response to the new words being slower ($F_{1,16}=14.00$, $p < 0.01$). However, no main effect of vardenafil treatment or interaction with stimulus type (new/old words) was found on RT ($F_{2,32}=1.72$, n.s. and $F_{2,32}=0.69$, n.s., respectively) or correct responses ($F_{2,32}=0.25$, n.s. and $F_{2,32}=0.71$, n.s., respectively).

VLT ERP

Only those analyses that revealed effects are reported (also for the other tasks).

EEG during encoding: For the P300 an interaction effect of treatment and channel was found ($F_{8,128}=5.11$, $p < 0.001$) during the presentation of the words (see Figure 1). Further analyses showed that the P300 was decreased (a) after vardenafil 20 mg compared to placebo at the Fz, Cz and CPz, (b) after both vardenafil conditions compared to placebo treatment at the FCz and Pz, and (c) after vardenafil 20 mg compared to 10 mg treatment at the FCz and Pz.

EEG during recognition: The N400 was less negative after the presentation of old stimuli than after new ones ($F_{1,16}=5.21$, $p < 0.05$). Additionally, the P600 was larger after the presentation of old than new stimuli ($F_{1,16}=27.57$, $p < 0.001$). Treatment interacted with electrode location ($F_{8,128}=2.05$, $p < 0.05$), however further analyses revealed no effects.

CRMT behavior

The overall RT was faster and the number of correct responses was higher for the old than for the new pictures ($F_{1,16}=6.11$, $p < 0.05$ and $F_{1,16}=7.40$, $p < 0.05$, respectively). Administration of vardenafil did not affect these RTs ($F_{2,32}=1.51$, n.s.) and the correct responses ($F_{2,32}=3.28$, n.s.) in this immediate picture recognition task (see also Table 1). Furthermore, when subdividing the responses to the old pictures presented 1, 3 or 10 stimuli after they had been presented for the first time, neither an effect of vardenafil for correct responses ($F_{2,32}=1.45$, n.s.) nor for RT ($F_{2,32}=1.49$, n.s.) was found. However, the interval between the pictures had an effect on RT ($F_{2,32}=5.41$, $p < 0.05$) with the interval of 10 stimuli causing a slower RT than the interval of three stimuli.

Table 1. No effects of vardenafil treatment on the behavioral performance on the memory tasks were found. Reaction times are presented in ms, $n=17$. Data are mean values (standard error of the mean (SEM)).

			Placebo	Vardenafil 10 mg	Vardenafil 20 mg
VLT	Immediate recall	Correct	48.47 (2.92)	46.24 (2.88)	47.00 (3.31)
		Incorrect	1.12 (0.28)	1.00 (0.37)	1.00 (0.28)
		Double	1.24 (0.37)	1.12 (0.27)	1.76 (0.48)
	Delayed recall	Correct	15.77 (1.36)	15.88 (1.21)	15.18 (1.20)
		Incorrect	0.47 (0.15)	0.47 (0.19)	0.29 (0.14)
		Double	0.29 (0.11)	0.29 (0.11)	0.24 (0.16)
	Delayed recognition old	Reaction time	846.16 (28.82)	796.67 (24.06)	848.50 (31.40)
		Correct	12.94 (0.49)	13.59 (0.40)	13.35 (0.35)
	Delayed recognition new	Reaction time	907.69 (36.20)	862.45 (26.83)	875.58 (26.06)
		Correct	13.47 (0.45)	13.35 (0.42)	13.41 (0.41)
CRMT	Immediate recognition new	Reaction time	703.46 (19.33)	719.71 (16.65)	721.33 (19.22)
		Correct	56.94 (0.75)	56.24 (0.94)	56.94 (0.73)
	Immediate recognition old	Reaction time	668.70 (20.46)	690.65 (21.61)	692.12 (21.56)
		Correct	58.53 (0.43)	57.65 (0.47)	58.24 (0.38)
	Interval 1	Reaction time	661.12 (28.79)	691.86 (23.73)	697.42 (24.14)
		Correct	19.18 (0.26)	19.47 (0.23)	19.29 (0.25)
	Interval 3	Reaction time	664.62 (18.46)	662.91 (22.72)	676.19 (21.69)
		Correct	19.65 (0.12)	19.00 (0.33)	19.47 (0.21)
	Interval 10	Reaction time	680.55 (18.30)	716.77 (23.29)	703.78 (22.94)
		Correct	19.65 (0.17)	19.18 (0.20)	19.35 (0.15)
SMT	Immediate recognition	Reaction time	853.40 (42.71)	820.03 (46.31)	816.81 (51.85)
		Correct	52.06 (1.03)	51.06 (1.13)	50.18 (1.14)
	Delayed recognition	Reaction time	899.56 (62.37)	863.99 (67.18)	894.55 (59.51)
		Correct	44.29 (1.22)	45.47 (1.10)	45.35 (1.23)

CRMT: continuous recognition memory task; SMT: spatial memory task; VLT: verbal learning task.

CRMT ERP

The P150 and the P300 were more positive after the presentation of the old than new picture ($F_{1,16}=10.94$, $p<0.01$ and $F_{1,15}=21.77$, $p<0.001$ respectively). In addition, the N200 was more negative after the new than the old pictures ($F_{1,16}=58.79$, $p<0.001$). However, no treatment effects were found for any of the ERP components.

SMT behavior

Immediate recognition: No effects of vardenafil treatment were found with regard to the RT ($F_{2,32}=0.32$, n.s.) or number of correct responses ($F_{2,32}=1.70$, n.s.) in the immediate recognition (see also Table 1).

Delayed recognition: No effects of vardenafil treatment were found on RT ($F_{2,32}=0.31$, n.s.) and number of correct responses ($F_{2,32}=0.69$, n.s.) in the delayed recognition.

SMT ERP

During the encoding phase, there was an interaction between treatment and channel at the N400 ($F_{8,128}=3.93$, $p<0.001$). However, further analyses revealed no effects. In the immediate repetition phase, treatment and channel interacted at the P150 ($F_{8,128}=2.22$, $p<0.05$) and N400 ($F_{8,128}=2.52$, $p<0.05$). Further analysis showed no effect for the P150, but the N400 response was more negative after the vardenafil 10 mg condition than after the placebo or vardenafil 20 mg condition at the Fz.

TOL

GLM repeated measures showed that the RT increased ($F_{3,48}=135.39$, $p<0.001$) and the number of correct responses decreased ($F_{3,48}=20.46$, $p<0.001$) as the number of steps in this executive function task that had to be taken to reach the end-arrangement increased. However, no main effect of vardenafil treatment or interaction with number of steps was found for RT ($F_{2,32}=0.29$, n.s. and $F_{6,96}=0.86$, n.s., respectively) or correct responses ($F_{2,32}=0.69$, n.s. and $F_{6,96}=0.34$, n.s.) (see Table 2).

Stroop behavior

The RT was slower in the incongruent than in the congruent condition of this attention/response inhibition task ($F_{1,15}=83.50$, $p<0.001$). In addition, the number of correct responses was higher in the congruent condition ($F_{1,15}=11.79$, $p<0.01$). No main effect of vardenafil or interaction with stimulus type (congruent/incongruent) on Stroop performance was found (RT ($F_{2,30}=0.27$, n.s. and $F_{2,30}=1.20$, n.s., respectively), correct responses ($F_{2,30}=0.52$, n.s. and $F_{2,30}=0.22$, n.s., respectively)) (see also Table 2).

Stroop ERP

The P150 was more positive in the congruent than in the incongruent condition ($F_{1,16}=4.50$, $p=0.05$). Additionally, a main treatment effect was found for the P300 ($F_{2,32}=3.66$, $p<0.05$), however post-hoc analyses showed no further effects.

Figure 1. Effects of vardenafil treatment on grand average event-related potentials (ERPs) during encoding in the verbal learning task (VLT). Generally the P300 was decreased after vardenafil treatment compared to placebo. Latencies are shown on the x-axis in ms, amplitudes on the y-axis in μV .

Table 2. No effects of vardenafil treatment on the behavioral performance on the TOL and Stroop were found. Reaction times are presented in seconds (s) for the Tower of London (TOL) and ms for the Stroop. $n_{\text{TOL}}=17$, $n_{\text{Stroop}}=16$. Data are mean values (standard error of the mean (SEM)).

			Placebo	Vardenafil 10 mg	Vardenafil 20 mg
TOL	2 steps	Reaction time	4.57 (0.24)	4.27 (0.21)	4.36 (0.25)
		Correct	9.71 (0.19)	9.24 (0.18)	9.41 (0.24)
	3 steps	Reaction time	5.77 (0.38)	5.91 (0.37)	5.68 (0.27)
		Correct	9.53 (0.12)	9.53 (0.21)	9.35 (0.21)
	4 steps	Reaction time	8.76 (0.75)	8.89 (0.53)	9.16 (0.67)
		Correct	8.82 (0.33)	8.76 (0.25)	8.82 (0.23)
Stroop	5 steps	Reaction time	14.78 (1.10)	13.44 (1.13)	14.51 (1.19)
		Correct	8.06 (0.42)	7.82 (0.36)	7.65 (0.42)
	Congruent	Reaction time	594.45 (10.90)	596.58 (11.77)	596.47 (13.95)
		Correct	70.44 (0.36)	70.52 (0.40)	70.80 (0.32)
	Incongruent	Reaction time	695.62 (19.67)	679.15 (16.02)	692.15 (14.52)
		Correct	68.76 (0.92)	69.32 (0.52)	69.12 (0.44)

RT task

GLM repeated measures showed an effect of hand side on the RT ($F_{1,15}=4.98$, $p<0.05$). More specifically, the RT was higher when the participants had to respond with their right hand compared to the left hand. Furthermore, there was a treatment $\times$ side interaction for the correct responses ($F_{2,30}=6.84$, $p<0.01$). This effect was further analyzed by examining the correct responses for both hands separately. No effect of treatment was found for the left hand ($F_{2,30}=0.82$, n.s.), whereas an effect of treatment was found for the right hand ($F_{2,30}=7.11$, $p<0.01$). Post-hoc analyses revealed that more errors were made in the vardenafil 20 mg condition than in the placebo condition.

Questionnaires

Profile of Mood States (POMS): No effects of vardenafil treatment on depression ($F_{2,32}=1.89$, n.s.), anger ($F_{2,32}=0.63$, n.s.), fatigue ($F_{2,32}=1.61$, n.s.), vigor ($F_{2,32}=0.90$, n.s.) or tension ($F_{2,32}=0.40$, n.s.) were found.

Bond and Lader Visual Analogue Scale: The participants reported no effect of vardenafil treatment on alertness ($F_{2,32}=2.71$, n.s.).

Questionnaire on medical complaints: An effect of treatment was found on the report of headache ($F_{2,32}=6.34$, $p<0.01$) and feeling weak ($F_{2,32}=7.43$, $p<0.01$). Bonferroni post-hoc analysis revealed that there was an increased report of both complaints after administration of vardenafil 10 mg or 20 mg compared to the placebo condition.

Discussion

The aim of this study was to investigate the effects of a PDE5-I, vardenafil, on cognition and ERP measurements in healthy volunteers. No effects of vardenafil treatment were found on any of the behavioral performances in the cognitive tasks measuring memory (VLT, CRMT and SMT) and executive functioning (TOL and Stroop task). However, a small effect was found on the RT task: the volunteers made more errors during this task with their right hand after vardenafil 20 mg treatment than placebo. For the VLT immediate recall, the P300 was in general decreased after vardenafil treatment during the encoding of the words. In addition, the N400 was increased after vardenafil 10 mg than after placebo treatment in the SMT immediate repetition phase at the Fz electrode. No other effects on ERPs after vardenafil were found, i.e. on the VLT recall and recognition, the CRMT, the SMT acquisition and delayed repetition, and the Stroop task. Finally, there was an increased report of headache and feeling weak after vardenafil treatment (10 mg and 20 mg) compared to the placebo condition.

The lack of effect of PDE5 inhibition on cognitive performance is in line with previous studies investigating the effects of PDE5 inhibition in healthy volunteers. Grass et al. (2001) demonstrated that a PDE5-I, sildenafil, did not affect the performance of their participants on a variety of psychophysical tasks including a short term memory task. In addition, they did find an effect on RT as we did in our current study. However, they found an improvement in performance after sildenafil treatment, while we found an impairment after vardenafil treatment. Furthermore, another study by Schultheiss et al. (2001) showed that sildenafil treatment did not affect the behavioral response on a word recognition task. Additionally, it was found that the sildenafil had an effect on the

EEG measurements during this task: a reduction of their negativity was found between 150–250 ms after stimulus presentation. However, the authors mentioned that the meaning of this increased responsiveness remains to be determined. We did not find an effect of PDE5 inhibition on ERP measurements on the recognition part of the VLT but did find a decrease of the P300 after vardenafil treatment during the encoding of the words whereas behavioral performance remained unaffected. Schultheiss et al. (2001) also found an effect of PDE5 inhibition on the P300: they detected an increase in P300 during an auditory attention task after sildenafil treatment. This seems to be in contrast with our finding of a decrease in P300 during word encoding in the VLT after vardenafil treatment: however it has to be noted that their auditory attention task is completely different from our VLT which might also affect a change in the P300.

In our current study, we did not demonstrate any effects of vardenafil on the early and middle phase ERP components related to e.g. basic sensory processing, attentive manipulations and auditory oddball paradigms, such as the N100, P150 and N200 (Cacioppo et al., 2000; Luck, 2005). In contrast, there were effects on late-phase ERP components P300 in the VLT and N400 in the SMT which are generally related to higher cognitive functioning such as (semantic) memory (Cacioppo et al., 2000; Federmeier and Laszlo, 2009). The effect of vardenafil 10 mg on the N400 might be a spurious finding, since the effect seems to be quite random as it was only found at one frontal midline electrode while the N400 is normally predominantly generated at the left temporal lobe (Luck, 2005). Additionally, behavioral performance remained unaffected. The decrease of the P300 during word presentation after vardenafil treatment seems to be a more robust finding demonstrated at several electrode locations and at a task in which changes in P300 could be expected. However, vardenafil treatment did not affect the behavioral response in this task which makes it difficult to pinpoint the meaning of this effect.

It is not uncommon to find task and/or treatment effects on EEG or fMRI measurements in pharmacological studies whereas no effect on behavioral performance can be found (e.g. Bossong et al., 2012; Linssen et al., 2011). In our current study, this apparent discrepancy could be explained by the fact that the decreased P300 was elicited while participants watched words on a screen which they had to remember but that they did not have to execute an explicit behavioral response at the same time. So the vardenafil treatment might have affected encoding without eliciting an effect on the free recall of the learned words, possibly due to the fact that the EEG is more sensitive to pick up changes as compared to behavior.

Although no effects of vardenafil administration on the POMS and the Bond and Lader assessments were found, the questionnaire addressing medical complaints showed that vardenafil 10 mg and 20 mg increased reports of feeling weak and headache. The latter is one of the most commonly reported side effects after vardenafil treatment being reported by more than 10% of the participants from clinical trials (EMA, 2008). Together with the fact that maximum plasma concentrations of vardenafil after oral administration are reached within 30–120 minutes (EMA, 2008) this indicates that vardenafil was very likely bioactive during our testing period.

It could be argued that the doses of vardenafil used in this study may be not be in the optimal dose range for cognition-enhancing effects. In order to compare the effective doses in

animals with the doses used in this study, we used the formula of Reagan-Shaw et al. (2008) to extrapolate the dosages across species. When taking into account the body surface area and body weight, it was shown that the doses of 1–3 mg/kg (by mouth) which have been found to enhance memory function in rats (e.g. Reneerkens et al., 2012; Rutten et al., 2007) should be equivalent to 10–31 mg in humans (given the average weight of 64 kg of our participants). This indicates that although we did not find an effect of vardenafil treatment on cognitive performance in healthy adults, we used the translational dosages, time point and route of administration.

In rats we have shown that vardenafil crosses the blood brain barrier (Reneerkens et al., 2012). Since vardenafil was presently found to affect ERPs it might have entered the brain and could be biologically active there. Nevertheless, it cannot be ruled out that there may not be sufficient vardenafil that entered the brain or that PDE5 levels in the human brain are not high enough to have a clear cognitive effect. Along similar lines, the levels of PDE5 are relatively low compared to other PDEs (Lakics et al., 2010; Loughney et al., 1998) and show a strong decrease with aging (Reyes-Irisarri et al., 2007). Thus, PDE5-Is may not be effective in humans because the target may no longer be sufficiently available. Of note, it could be argued that the tasks we used were not translational enough to find an effect. However, previous animal studies provide ample evidence to expect effects of PDE5 inhibition on memory and executive functioning (for review see Reneerkens et al., 2009) and the tasks we used, such as the VLT, TOL and Stroop, are well-established tasks for studying these cognitive processes. Therefore, the battery of cognitive tasks used in this study should be sensitive enough to pick up any relevant proof in principle for the putative cognition-enhancing effects of PDE5-Is.

The effects of PDE5 inhibition on cognition were not only investigated in healthy volunteers: Goff et al. (2009) showed that acute sildenafil treatment did not affect cognition, positive or negative symptoms in patients with schizophrenia. This in contrast to another study (Akhondzadeh et al., 2011) in which sildenafil combined with the atypical antipsychotic risperidone reduced the negative symptoms in these patients. Importantly, the latter study used chronic sildenafil treatment as an adjunctive therapy while the former study used acute sildenafil treatment only. Interestingly, Shim et al. (2011) demonstrated that the performance of patients with ED on an assessment battery addressing frontal executive function and a mini-mental state examination was improved after repeated dosing of the PDE5-I udenafil. Furthermore, in a mouse model of Alzheimer's disease, it was found that chronic treatment with sildenafil reduced amyloid-beta load and memory decline (Puzzo et al., 2009). These findings suggest that although the cognition-enhancing effects of a single dose of a PDE5-I in healthy volunteers seem limited, there are interesting options in studying (sub)chronic PDE5-I treatment using a patient population and/or PDE5 inhibition as an adjunctive therapy. In addition, since the high level of education of our test subjects might have resulted in a ceiling effect, it would also be interesting to use deficit models or use low-cognitive performers in future studies.

To summarize, the PDE5-I vardenafil did not affect behavioral performance in different cognitive tasks. However, vardenafil treatment decreased the P300 in a memory task which implies that PDE5 inhibition might even affect cognitive processing in

humans, although the exact meaning of this effect is not clear yet. This indicates that the effects of PDE5 inhibition on cognition in healthy young adults needs further investigation in the future, e.g. by using deficit models and additional tasks as well. Taken together, the PDE5-I vardenafil did not affect the cognitive performance of healthy adults and showed only some incidental and rather contradictory effects on the electrophysiological correlates of cognition.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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References

- Akhondzadeh S, Ghayyoumi R, Rezaei F, et al. (2011) Sildenafil adjunctive therapy to risperidone in the treatment of the negative symptoms of schizophrenia: A double-blind randomized placebo-controlled trial. *Psychopharmacology (Berl)* 213: 809–815.
- Baratti CM and Boccia MM (1999) Effects of sildenafil on long-term retention of an inhibitory avoidance response in mice. *Behav Pharmacol* 10: 731–737.
- Bender AT and Beavo JA (2006) Cyclic nucleotide phosphodiesterases: Molecular regulation to clinical use. *Pharmacol Rev* 58: 488–520.
- Bond A and Lader M (1974) The use of visual analogue scales in rating subjective feelings. *Br J Med Psychol* 80: 1–16.
- Bossong MG, Jager G, van Hell HH, et al. (2012) Effects of Delta9-tetrahydrocannabinol administration on human encoding and recall memory function: A pharmacological fMRI study. *J Cogn Neurosci* 24: 588–599.
- Cacioppo JT, Tassinary LG and Bertson GG (2000) *Handbook of Psychophysiology*, Cambridge: Cambridge University Press.
- Curran HV, Pooviboonsuk P, Dalton JA, et al. (1998) Differentiating the effects of centrally acting drugs on arousal and memory: An event-related potential study of scopolamine, lorazepam and diphenhydramine. *Psychopharmacology (Berl)* 135: 27–36.
- Dassanayake TL, Michie PT, Jones A, et al. (2012) Cognitive impairment in patients clinically recovered from central nervous system depressant drug overdose. *J Clin Pharmacol* 32: 503–510.
- Devan BD, Pistell PJ, Daffin LW Jr, et al. (2007) Sildenafil citrate attenuates a complex maze impairment induced by intracerebroventricular infusion of the NOS inhibitor N(omega)-nitro-L-arginine methyl ester. *Eur J Pharmacol* 563: 134–140.
- Domek-Lopacinska K and Strosznajder JB (2008) The effect of selective inhibition of cyclic GMP hydrolyzing phosphodiesterases 2 and 5 on learning and memory processes and nitric oxide synthase activity in brain during aging. *Brain Res* 1216: 68–77.
- EMA (2008) European Public Assessment Report; Revision 6. www.emea.europa.eu.
- Federmeier KD and Laszlo S (2009) Time for meaning: Electrophysiology provides insights into the dynamics of representation and processing in semantic memory. In: Ross BH (ed) *Psychology of Learning and Motivation*. Burlington: Academic Press, pp.1–44.
- Gazzaniga MS, Ivry RB and Mangun GR (2002) *Cognitive Neuroscience: The Biology of the Mind*, New York: WW Norton & Company, Inc.

- Goff DC, Cather C, Freudenreich O, et al. (2009) A placebo-controlled study of sildenafil effects on cognition in schizophrenia. *Psychopharmacology (Berl)* 202: 411–417.
- Grass H, Klotz T, Fathian-Sabet B, et al. (2001) Sildenafil (Viagra): Is there an influence on psychological performance? *Int Urol Nephrol* 32: 409–412.
- Jasper H (1958) The ten-twenty electrode system of the international federation. *Electroencephalogr Clin Neurophysiol* 10: 371–375.
- Kessels RP, Postma A and de Haan EH (1999) Object Relocation: A program for setting up, running, and analyzing experiments on memory for object locations. *Behav Res Methods Instrum Comput* 31: 423–428.
- Lakics V, Karran EH and Boess FG (2010) Quantitative comparison of phosphodiesterase mRNA distribution in human brain and peripheral tissues. *Neuropharmacology* 59: 367–374.
- Langtry HD and Markham A (1999) Sildenafil: a review of its use in erectile dysfunction. *Drugs* 57: 967–989.
- Lezak MD (1995) *Neuropsychological Assessment*. Oxford: Oxford University Press.
- Linssen AM, Riedel WJ and Sambeth A (2011) Effects of tyrosine/phenylalanine depletion on electrophysiological correlates of memory in healthy volunteers. *J Psychopharmacol* 25: 230–238.
- Loughney K, Hill TR, Florio VA, et al. (1998) Isolation and characterization of cDNAs encoding PDE5A, a human cGMP-binding, cGMP-specific 3',5'-cyclic nucleotide phosphodiesterase. *Gene* 216: 139–147.
- Luck SJ (2005) *An Introduction to the Event-Related Potential Technique*. Cambridge, MA: MIT Press.
- McNair DM, Lorr M and Droppleman LF (1971) *Manual for the Profile of Mood States*. San Diego, CA: Educational and Industrial Testing Service.
- Patil CS, Jain NK, Singh VP, et al. (2004) Differential effect of the PDE5 inhibitors, sildenafil and zaprinast, in aging- and lippoly-saccharide-induced cognitive dysfunction in mice. *Drug Dev Res* 63: 66–75.
- Prickaerts J, Steinbusch HW, Smits JF, et al. (1997) Possible role of nitric oxide-cyclic GMP pathway in object recognition memory: Effects of 7-nitroindazole and zaprinast. *Eur J Pharmacol* 337: 125–136.
- Prickaerts J, van Staveren WC, Sik A, et al. (2002) Effects of two selective phosphodiesterase type 5 inhibitors, sildenafil and vardenafil, on object recognition memory and hippocampal cyclic GMP levels in the rat. *Neuroscience* 113: 351–361.
- Puzzo D, Staniszewski A, Deng SX, et al. (2009) Phosphodiesterase 5 inhibition improves synaptic function, memory, and amyloid-beta load in an Alzheimer's disease mouse model. *J Neurosci* 29: 8075–8086.
- Reagan-Shaw S, Nihal M and Ahmad N (2008) Dose translation from animal to human studies revisited. *FASEB J* 22: 659–661.
- Reneerkens OA, Rutten K, Akkerman S, et al. (2012) Phosphodiesterase type 5 (PDE5) inhibition improves object recognition memory: Indications for central and peripheral mechanisms. *Neurobiol Learn Mem* 97: 370–379.
- Reneerkens OA, Rutten K, Steinbusch HW, et al. (2009) Selective phosphodiesterase inhibitors: a promising target for cognition enhancement. *Psychopharmacology (Berl)* 202: 419–443.
- Reneerkens OA, Sambeth A, Van Duinen MA, et al. (2013) The PDE5 inhibitor vardenafil does not affect auditory sensory gating in rats and humans. *Psychopharmacology (Berl)* 225: 303–312.
- Reyes-Irisarri E, Markerink-Van Ittersum M, Mengod G, et al. (2007) Expression of the cGMP-specific phosphodiesterases 2 and 9 in normal and Alzheimer's disease human brains. *Eur J Neurosci* 25: 3332–3338.
- Riedel WJ, Klaassen T, Deutz NE, et al. (1999) Tryptophan depletion in normal volunteers produces selective impairment in memory consolidation. *Psychopharmacology (Berl)* 141: 362–369.
- Rodefer JS, Saland SK and Eckrich SJ (2012) Selective phosphodiesterase inhibitors improve performance on the ED/ID cognitive task in rats. *Neuropharmacology* 62: 1182–1190.
- Rutten K, Basile JL, Prickaerts J, et al. (2008) Selective PDE inhibitors rolipram and sildenafil improve object retrieval performance in adult cynomolgus macaques. *Psychopharmacology (Berl)* 196: 643–648.
- Rutten K, Prickaerts J, Hendrix M, et al. (2007) Time-dependent involvement of cAMP and cGMP in consolidation of object memory: Studies using selective phosphodiesterase type 2, 4 and 5 inhibitors. *Eur J Pharmacol* 558: 107–112.
- Rutten K, Vente JD, Sik A, et al. (2005) The selective PDE5 inhibitor, sildenafil, improves object memory in Swiss mice and increases cGMP levels in hippocampal slices. *Behav Brain Res* 164: 11–16.
- Sambeth A, Riedel WJ, Tillie DE, et al. (2009) Memory impairments in humans after acute tryptophan depletion using a novel gelatin-based protein drink. *J Psychopharmacol* 23: 56–64.
- Schmitt JA, Jorissen BL, Dye L, et al. (2005) Memory function in women with premenstrual complaints and the effect of serotonergic stimulation by acute administration of an alpha-lactalbumin protein. *J Psychopharmacol* 19: 375–384.
- Schultheiss D, Muller SV, Nager W, et al. (2001) Central effects of sildenafil (Viagra) on auditory selective attention and verbal recognition memory in humans: a study with event-related brain potentials. *World J Urol* 19: 46–50.
- Setter SM, Iltz JL, Fincham JE, et al. (2005) Phosphodiesterase 5 inhibitors for erectile dysfunction. *Ann Pharmacother* 39: 1286–1295.
- Shallice T (1982) Specific impairments of planning. *Philos Trans R Soc Lond B Biol Sci* 298: 199–209.
- Shim YS, Pae CU, Kim SW, et al. (2011) Effects of repeated dosing with Udenafil (Zydena) on cognition, somatization and erection in patients with erectile dysfunction: A pilot study. *Int J Impot Res* 23: 109–114.
- Van Donkelaar EL, Rutten K, Blokland A, et al. (2008) Phosphodiesterase 2 and 5 inhibition attenuates the object memory deficit induced by acute tryptophan depletion. *Eur J Pharmacol* 600: 98–104.
- Van Strien JW, Verkoeijen PP, Van der Meer N, et al. (2007) Electrophysiological correlates of word repetition spacing: ERP and induced band power old/new effects with massed and spaced repetitions. *Int J Psychophysiol* 66: 205–214.