Can PDE inhibition improve cognition? : Translational insights

Can PDE inhibition improve cognition? Translational insights

ISBN: 978 94 91602 08 5

Can PDE inhibition improve cognition? Translational insights

Cover illustration by Hans Klaasse
Typesetting and layout by Olga A. H. Reneerkens
Printed by Print Service Ede

Financial support for the publication of this dissertation was kindly provided by the Van Leersumfonds (Koninklijke Nederlandse Akademie van Wetenschappen) and Internationale Stichting Alzheimer Onderzoek

Additional financial support for this publication was granted by Alzheimer Nederland (Amersfoort)

Can PDE inhibition improve cognition? Translational insights

Proefschrift

ter verkrijging van de graad van doctor
aan de Universiteit Maastricht,
op gezag van de Rector Magnificus,
Prof. Dr. L.L.G. Soete,
volgens het besluit van het College van Decanen,
in het openbaar te verdedigen
op donderdag 18 april 2013 om 14.00 uur

door

Olga Antonia Hendrika Reneerkens
Geboren op 29 juni 1982 te Maastricht

Promotor:
Prof. Dr. H.W.M. Steinbusch

Copromotor:
Dr. J. Prickaerts

Beoordelingscommissie:
Prof. Dr. F. Verhey (voorzitter)
Dr. M. Meeter (Vrije Universiteit Amsterdam, Nederland)
Prof. Dr. J.G. Ramaekers
Dr. T. Steckler (Janssen Pharmaceutica, Johnson & Johnson, België)

Contents

CHAPTER 1

General introduction

GENERAL INTRODUCTION

Cognitive impairment is one of the major complaints people suffering from neurodegenerative and psychiatric disorders such as Alzheimer's disease or other types of dementia and schizophrenia have to face (Keller 2006; O'Carroll 2000). This impairment has a large, negative impact on the quality of the daily life of these patients and their family and friends. In the US, the prevalence of Alzheimer's disease among people of 71 years or older is 9.7%, but the total of people suffering from dementia is as high as 13.9%, which leads us to a number of approximately 3.4 million individuals (Plassman et al. 2007). The Alzheimer's association (2009) estimated the cost implications related to Alzheimer's disease and other dementias at 148 billion dollars in the United States (US) alone and did not include 94 billion dollars of unpaid services of an estimated 10 million caregivers. In Europe, the total prevalence of dementia in the population aged 65 and older was 4.9 million in 2004 and the total costs were estimated at 55 billion euros (Andlin-Sobocki et al. 2005). There are several drugs which have shown to be effective in improving symptoms of mild-to-moderate Alzheimer's disease, such as donepezil and rivastigmine, but for more severe Alzheimer's and other types of dementia, the options are very limited or non-existent so far (Burns 2003). The lifetime prevalence of schizophrenia is about 4 per 1,000 (McGrath et al. 2008; Saha et al. 2005) and the total societal costs in the United Kingdom (UK) in 2004/2005 were estimated 6.7 billion pounds (Mangalore and Knapp 2007) and 62.7 billion dollars in the US in 2002 (McEvoy 2007). In Europe the costs were estimated at 35 billion euros in 2004, however, it was noted by the authors that indirect costs were not included in this estimation, but were expected to make up a substantial amount of the total costs (Andlin-Sobocki et al. 2005). Despite the increased attention for cognitive deficits in schizophrenia and the wide range of pharmalogical targets, the results are generally disappointing (Goff et al. 2011).

Because of the high social as well as economical costs of cognitive impairments, it is of utmost importance to continue the search for cognitive enhancers. Recently, phosphodiesterases (PDEs) gained increased attention as a target for cognition enhancement (for review see e.g. Blokland et al. 2006; Menniti et al. 2006). PDEs are enzymes that degrade the second messenger molecules cyclic adenosine monophosphate (cAMP) and/or cyclic guanosine monophosphate (cGMP). In total, there are eleven classes of PDEs identified based on several criteria including molecular properties, substrate specifity, and regulation (Bender and Beavo 2006). PDEs are expressed throughout the body and in the central nervous system (CNS) (Lakics et al. 2010). One fundamental distinction between the PDE classes is made on the basis of the difference in affinity for the two distinct cyclic nucleotides. A differentiation is possible between cAMP-specific enzymes (PDE4, 7, and 8), cGMP-specific enzymes (PDE5, 6, and 9), and the so-called dual-substrate PDEs, which have affinity for both cyclic nucleotides (PDE1, 2, 3, 10, and 11) (Bender and Beavo 2006).

The second messengers cAMP and cGMP play an important role in intracellular signaling and in processes such as neuroplasticity including long-term potentiation (LTP) (Chien et al. 2003; Frey et al. 1993; Son et al. 1998) that form the neurophysiological origin of learning and memory (Bliss and Collingridge 1993). It has indeed been demonstrated that central administration of analogues of these second messengers can improve memory function in animals (Bernabeu et al. 1996; Matsumoto et al. 2006; Prickaerts et al. 2002).

Furthermore, it has been found that specific PDE inhibitors (PDE-Is) facilitate for example LTP (e.g. Boess et al. 2004) and increase neuronal excitability (e.g. Threlfell et al. 2009). Consequently, the inhibition of PDEs could be a tool to modulate second messenger signaling and subsequently influence pathways involved in learning and memory.

AIM AND OUTLINE OF THIS THESIS

In this thesis we investigated whether PDE inhibition can improve cognition. This was done by using a translational approach in which we studied the effects of PDE inhibition on memory function and sensory gating in rats as well as on cognition and sensory gating in humans.

First, we provide an overview of the literature on the effects of PDE-Is on cognition across species (**Chapter 2**). In this chapter we also discuss the possible underlying mechanisms of these effects, such as blood flow, emotional arousal and LTP.

Next, we start the description of our behavioural studies with **Chapter 3** in which we examined the effects of PDE5 inhibition on memory function in rats. We used two different phosphodiesterase type 5 inhibitors (PDE5-Is): vardenafil, which is assumed to cross the blood-brain barrier (BBB), and UK-343,664 as a negative control as it is assumed not to cross the BBB. We examined the efficacy of these compounds in three variants of the object recognition task (ORT): a 1 h delay interval in which memory was disrupted by either the muscarinic antagonist scopolamine or the N-methyl-D-aspartate (NMDA) receptor agonist MK-801, and a 24 h delay interval where memory degrades over time. In addition, we investigated whether vardenafil and UK-343,664 were indeed able to penetrate the BBB or not, respectively.

In **Chapter 4**, we aim to further characterize the effects of PDE inhibition on memory function by studying the effects of a phosphodiesterase type 2 inhibitor (PDE2-I) and a phosphodiesterase type 10 inhibitor (PDE10-I) on object memory in rats. We investigated the effects of the PDE2-I BAY 60-7750 and PDE10-I PQ-10 in a 1 h delay interval in the ORT. We again used the scopolamine- and MK-801-induced deficit models, which are both commonly used preclinical models to assess cognitive deficits related to Alzheimer's disease and schizophrenia, respectively. We also determined the concentrations of BAY 60-7550 and PQ-10 in blood plasma and brain tissue after treatment to gain insight into their brain penetrating properties.

Chapter 5 describes a translational study in which we investigated the effects of PDE5 inhibition on sensory gating, which is an automatic process involved in information processing that can be compromised in various clinical disorders including schizophrenia and Alzheimer's disease. Rats were included because of the extensive learning and memory enhancing effects that have already been reported in rodents while to our knowledge basic auditory information processing has not been studied yet after PDE5 inhibition. Likewise, the effects on sensory gating in humans were studied to gain further insight into the effects of PDE5 inhibition on information processing in humans, but also to

see whether the drug effects found in rodents can be translated to the human situation and vice versa.

In **Chapter 6** we studied the effects of the PDE2-I BAY 60-7550 and PDE10-I PQ-10 on sensory gating in rats. Since both phosphodiesterase type 2 (PDE2) and type 10 (PDE10) are possible targets for cognition enhancement, it is important to gain further insight into the nature of the effects of PDE inhibition. In our case, we want to know as in line with Chapter 5 whether putative positive effects on cognition are limited to higher cognitive processes or whether lower cognitive processes such as information processes are affected as well.

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. Therefore, in our final study presented in **Chapter 7**, we examined the effects of vardenafil on cognition, in particular memory and executive function, and its electrophysiological correlates in healthy human volunteers. Cognitive performances were assessed while simultaneously recording brain activity. 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.

Lastly, in **Chapter 8** we summarize and discuss our experimental findings. In addition, we address several recommendations for future research.

REFERENCES

Andlin-Sobocki P, Jonsson B, Wittchen HU, Olesen J (2005) Cost of disorders of the brain in Europe. *Eur J Neurol* 12 Suppl 1: 1-27

Association As (2009) 2009 Alzheimer's disease facts and figures. *Alzheimers Dement* 5: 234-70

Bender AT, Beavo JA (2006) Cyclic nucleotide phosphodiesterases: molecular regulation to clinical use. *Pharmacol Rev* 58: 488-520

Bernabeu R, Schmitz P, Faillace MP, Izquierdo I, Medina JH (1996) Hippocampal cGMP and cAMP are differentially involved in memory processing of inhibitory avoidance learning. *Neuroreport* 7: 585-8

Bliss TV, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 361: 31-9

Blokland A, Schreiber R, Prickaerts J (2006) Improving memory: a role for phosphodiesterases. *Curr Pharm Des* 12: 2511-23

Boess FG, Hendrix M, van der Staay FJ, Erb C, Schreiber R, van Staveren W, de Vente J, Prickaerts J, Blokland A, Koenig G (2004) Inhibition of phosphodiesterase 2 increases neuronal cGMP, synaptic plasticity and memory performance. *Neuropharmacology* 47: 1081-92

Burns A (2003) Treatment of cognitive impairment in Alzheimer's disease. *Dialogues Clin Neurosci* 5: 35-43

Chien WL, Liang KC, Teng CM, Kuo SC, Lee FY, Fu WM (2003) Enhancement of long-term potentiation by a potent nitric oxide-guanylyl cyclase activator, 3-(5-hydroxymethyl-2-furyl)-1-benzyl-indazole. *Mol Pharmacol* 63: 1322-8

Frey U, Huang YY, Kandel ER (1993) Effects of cAMP simulate a late stage of LTP in hippocampal CA1 neurons. *Science* 260: 1661-4

Goff DC, Hill M, Barch D (2011) The treatment of cognitive impairment in schizophrenia. *Pharmacol Biochem Behav* 99: 245-53

Keller JN (2006) Age-related neuropathology, cognitive decline, and Alzheimer's disease. *Ageing Res Rev* 5: 1-13

Lakics V, Karran EH, Boess FG (2010) Quantitative comparison of phosphodiesterase mRNA distribution in human brain and peripheral tissues. *Neuropharmacology* 59: 367-74

Mangalore R, Knapp M (2007) Cost of schizophrenia in England. *J Ment Health Policy Econ* 10: 23-41

Matsumoto Y, Unoki S, Aonuma H, Mizunami M (2006) Critical role of nitric oxide-cGMP cascade in the formation of cAMP-dependent long-term memory. *Learn Mem* 13: 35-44

McEvoy JP (2007) The costs of schizophrenia. *J Clin Psychiatry* 68 Suppl 14: 4-7

McGrath J, Saha S, Chant D, Welham J (2008) Schizophrenia: a concise overview of incidence, prevalence, and mortality. *Epidemiol Rev* 30: 67-76

Menniti FS, Faraci WS, Schmidt CJ (2006) Phosphodiesterases in the CNS: targets for drug development. *Nat Rev Drug Discov* 5: 660-70

O'Carroll R (2000) Cognitive impairment in schizophrenia. *APT* 6: 161-8

Plassman BL, Langa KM, Fisher GG, Heeringa SG, Weir DR, Ofstedal MB, Burke JR, Hurd MD, Potter GG, Rodgers WL, Steffens DC, Willis RJ, Wallace RB (2007) Prevalence of dementia in the United States: the aging, demographics, and memory study. *Neuroepidemiology* 29: 125-32

Prickaerts J, de Vente J, Honig W, Steinbusch HW, Blokland A (2002) cGMP, but not cAMP, in rat hippocampus is involved in early stages of object memory consolidation. *Eur J Pharmacol* 436: 83-7

Saha S, Chant D, Welham J, McGrath J (2005) A systematic review of the prevalence of schizophrenia. *PLOS Med* 2: e141

Son H, Lu YF, Zhuo M, Arancio O, Kandel ER, Hawkins RD (1998) The specific role of cGMP in hippocampal LTP. *Learn Mem* 5: 231-45

Threlfell S, Sammut S, Menniti FS, Schmidt CJ, West AR (2009) Inhibition of Phosphodiesterase 10A Increases the Responsiveness of Striatal Projection Neurons to Cortical Stimulation. *J Pharmacol Exp Ther* 328: 785-95

CHAPTER 2

Selective phosphodiesterase inhibitors: a promising target for cognition enhancement

Olga A. H. Reneerkens, Kris Rutten, Harry W. M. Steinbusch, Arjan Blokland & Jos Prickaerts

Published in Psychopharmacology (2009), 202, 419-443

ABSTRACT

One of the major complaints most people face during aging, is an impairment in cognitive functioning. This has a negative impact on the quality of daily life and is even more prominent in patients suffering from neurodegenerative and psychiatric disorders including Alzheimer's disease, schizophrenia and depression. So far, the majority of cognition enhancers are generally targetting one particular neurotransmitter system. However, recently phosphodiesterases (PDEs) have gained increased attention as a potential new target for cognition enhancement. Inhibition of PDEs increases the intracellular availability of the second messengers cGMP and/or cAMP. The aim of this review was to provide an overview of the effects of phosphodiesterase inhibitors (PDE-Is) on cognition, the possible underlying mechanisms and the relationship to current theories about memory formation. Studies of the effects of inhibitors of different PDE families (2, 4, 5, 9, and 10) on cognition were reviewed. In addition, studies related to PDE-Is and blood flow, emotional arousal and long-term potentiation (LTP) were described. PDE-Is have a positive effect on several aspects of cognition, including information processing, attention, memory and executive functioning. At present, these data are likely to be explained in terms of an LTP related mechanism of action. PDE-Is are a promising target for cognition enhancement; the most suitable candidates appear to be PDE2-Is or PDE9-Is. The future for PDE-Is as cognition enhancers lies in the development of isoform specific PDE-Is that have limited aversive side effects.

One of the problems many people come to face as they age, is a decline in cognitive functions, which has a negative impact on their daily activities and quality of life (Mattson et al. 2002). The loss of cognitive functioning is even more serious in patients suffering from pathological conditions such as Alzheimer's disease or other types of dementia. Also in depressed and schizophrenic patients, prominent cognitive deficits are present (Blaney 1986; Frith 1996). Since these deficits have a major impact on the quality life of these patients, it is of utmost importance to develop strategies or drugs that counteract cognitive decline. So far, several preventive strategies have been described which could ameliorate or slow down the cognitive decline resulting from brain aging. Research has focused on avoiding genetic and environmental factors that cause neuronal dysfunction and death or by enhancement of the ability of neurons to adapt to the aging process (Mattson et al. 2002). Examples of avoiding genetic factors are genetic counseling or germ line gene therapy and examples of avoiding environmental factors are dietary restrictions or behavioral modification. These strategies can induce successful ageing and can reduce the risk of cognitive decline and dementia (for a review see Mattson et al. 2002). Despite these strategies, there is a great need for drugs that counteract the processes involved in ageing and more specifically the decline of cognitive functions and memory.

For cognition enhancement or reversal of cognitive deficits different drug targets have been suggested based on neurotransmitter systems. Serotonergic, cholinergic and monoaminergic neurotransmitter systems have been shown to be involved in cognition. Furthermore, cognitive performance, including memory, can be improved by numerous biological factors such as neuromodulators, hormones, intracellular molecules, plant extracts, and nutritional ingredients, which enhance neurotransmission, blood flow, glucose metabolism or have free radical scavenging properties (Cahill et al. 1994; Davis and Squire 1984; DeZazzo and Tully 1995; Izquierdo et al. 1998; McGaugh 1989; Messier 2004; Parrott et al. 2004).

SECOND MESSENGERS CAMP AND CGMP

A relatively novel and promising field in cognition research focuses on the involvement of second messenger systems. Neurotransmitter receptors can be divided into two main groups according to the way in which receptor and effector function are coupled. One group consists of ionotropic (ion channel) receptors and the other consists of the GTP-binding protein (G-protein) coupled receptor. G-protein activation engages second messenger cascades (Shah and Catt 2004). Traditionally, the cyclic adenosine monophosphate (cAMP) second messenger system (Gs and Gi linked) and the phosphoinositol second messenger system (Gq linked) received the most attention. The second messenger cAMP is synthesised by adenylate cyclase (AC), which is stimulated or inhibited by Gs or Gi, respectively. The second messenger complex inositol-1,4,5,triphosphate/diacylglycerol (IP3/DAG) is formed out of the hydrolysis of phosphatidylinositol 4,5-biphosphate (PIP2) by phospholipase C (PLC) after activation by Gq. cAMP activates cAMP-dependent protein kinase (PKA), which phosphorylates cAMP response element binding protein (CREB). P-CREB is an activated transcription factor, which initiates transcription of specific genes. DAG activates calcium–dependent protein kinase (PKC) in the presence of calcium (Ca2+),

which is mobilized by IP3. PKC has an effect on CREB via the MAP kinase pathway. Of note, Ca2+ can also bind to calmodulin. This so-called Ca2+/CaM complex activates Ca2+/CaM protein kinase (CaMK), which can activate calium-dependent protein kinase (PKC) as well, but also PKA. On the other hand, PKA can also activate the MAP kinase pathway. Thus, interplay exists between the cAMP second messenger system and the phosphoinositol second messenger system. Recently, the cyclic guanosine monophosphate (cGMP) second messenger system receives more and more attention. cGMP is produced by guanylate cyclase (GC) which is stimulated by nitric oxide (NO) (Murad et al. 1978). cGMP activates cGMP-dependent protein kinase (PKG), which in turn phosphorylates certain proteins which influence the synthesis and/or release of other neurotransmitters, and thus signal transduction (Schmidt et al. 1993).

Cyclic nucleotide phosphodiesterases (PDEs) are enzymes which play an important role in the above mentioned intracellular signal transduction pathways. This is because these enzymes hydrolyze the second messengers cAMP and cGMP by breaking their phosphodiester bond with the corresponding monophosphate (Bender and Beavo 2006). There are eleven families of PDEs (PDE1-PDE11) and most of these families have more than one gene product (e.g. PDE4A, PDE4B, PDE4C, PDE4D). In addition, each gene product may have multiple splice variants (e.g. PDE4D1-PDE4D9). In total there are more than 100 specific human PDEs (Bender and Beavo 2006).

LOCALIZATION OF PDES

PDE1 is predominantly localized in the brain, heart, smooth muscles and lungs (Dent et al. 1998; Sonnenburg et al. 1998; Yan et al. 1994). In addition, PDE2 can be found in the brain, heart, adrenal cortex and platelets (Ito et al. 1996; Martins et al. 1982; Van Staveren et al. 2003). Furthermore, the localization of PDE3 includes the brain, heart, smooth muscles, kidneys and platelets (Reinhardt et al. 1995; Shakur et al. 2001). PDE4 is expressed in a wide variety of tissues, e.g. brain, lungs and testes (Perez-Torres et al. 2000; Reyes-Irisarri et al. 2008; Richter et al. 2005; Salanova et al. 1999). PDE5 has been detected in the brain, lungs, smooth and skeletal muscles, kidneys and platelets (Giordano et al. 2001; Hotston et al. 2007; Kotera et al. 2000; Yanaka et al. 1998). In contrast, PDE6 has been found in the pineal gland and the rod and cone cells of the photoreceptor layer of the retina (Holthues and Vollrath 2004; Morin et al. 2001; Stearns et al. 2007). PDE7 was identified in the brain, heart, liver, skeletal muscles, kidneys, testes and pancreas (Hetman et al. 2000; Miro et al. 2001), while the localization of PDE8 includes the brain, liver, kidneys, colon, testes, ovary, spleen and thyroid (Fisher et al. 1998a; Gamanuma et al. 2003; Hayashi et al. 1998; Hayashi et al. 2002; Kobayashi et al. 2003; Soderling et al. 1998; Wang et al. 2001). Also, PDE9 is located in the brain, kidneys, spleen, prostate and various gastrointestinal tissues (Andreeva et al. 2001; Fisher et al. 1998b; Rentero et al. 2003; Soderling et al. 1998; van Staveren and Markerink-van Ittersum 2005; Van Staveren et al. 2003; Wang et al. 2003). The localization of PDE10 comprises the brain, heart, muscles, testes and thyroid (Fujishige et al. 1999; Loughney et al. 1999; Soderling et al. 1999). And finally, it has been shown that PDE11 is primary located in the brain (pituitary), liver, skeletal muscles, kidneys, testes, prostate and thyroid (Fawcett et al. 2000).

Table 1 Localization of the different PDE isoforms in the adult brain of rodents and humans. Note that this table does not provide information with respect to the level of expression of the different isoforms in the brain. In addition, expression can implicate mRNA levels or protein levels dependent on the study referred to

Isoform	Localization in Brain	Species	Reference
PDE1A	Hippocampus, cortex, olfactory bulb, striatum, thalamus, cerebellum	Human, rat, mouse	(Billingsley et al. 1990; Cho et al. 2000; Lal et al. 1999; Yan et al. 1994)
PDE1B	Hippocampus, cortex, olfactory bulb, striatum	Mouse, rat	(Cho et al. 2000; Polli and Kincaid 1994; Reed et al. 1998)
PDE1C	Hippocampus, cortex, amygdala, cerebellum	Mouse	(Yan et al. 1996)
PDE2A	Hippocampus, cortex, striatum, amygdala, hypothalamus, midbrain	Human, rat, mouse	(Bolger et al. 1994; Repaske et al. 1993; Reyes-Irisarri et al. 2007; van Staveren et al. 2004; Van Staveren et al. 2003)
PDE3	Throughout brain	Rat	(Bolger et al. 1994)
PDE4A	Hippocampus, cortex, olfactory bulb, striatum, thalamus, hypothalamus, amygdala, midbrain, cerebellum	Human, rat, mouse	(Braun et al. 2007; Cherry and Davis 1999; Cho et al. 2000; D'Sa et al. 2005; Fujita et al. 2007)
PDE4B	Hippocampus, cortex, striatum, hypothalamus, midbrain, cerebellum	Human, rat, mouse	(Braun et al. 2007; Cherry and Davis 1999; Cho et al. 2000; Fujita et al. 2007)
PDE4D	Hippocampus, cortex, striatum, hypothalamus, midbrain, cerebellum	Human, rat, mouse	(Cherry and Davis 1999; Cho et al. 2000; Fujita et al. 2007; McLachlan et al. 2007; Richter et al. 2005)
PDE5A	Hippocampus, cortex, cerebellum	Human, rat, mouse	(Reyes-Irisarri et al. 2007; van Staveren et al. 2004; Van Staveren et al. 2003)
PDE7A	Hippocampus, cortex, olfactory bulb, striatum	Human, rat	(Miro et al. 2001; Perez-Torres et al. 2003)
PDE7B	Hippocampus, cortex, striatum, midbrain	Human, rat	(Perez-Torres et al. 2003; Sasaki et al. 2002)
PDE8B	Hippocampus, cortex, olfactory bulb, striatum, midbrain	Human, rat	(Kobayashi et al. 2003; Perez-Torres et al. 2003)
PDE9A	Hippocampus, cortex, olfactory bulb, striatum, thalamus, hypothalamus, amygdala, midbrain, cerebellum	Human, rat, mouse	(Reyes-Irisarri et al. 2007; van Staveren et al. 2004; Van Staveren et al. 2003)
PDE10	Hippocampus, cortex, striatum, midbrain, cerebellum	Rat	(Seeger et al. 2003)

The localizations of the different PDE isoforms differ between specific brain areas as is illustrated in detail in Table 1. Since PDEs are involved in the regulation of second

messenger signaling in numerous important body and brain structures, specific inhibitors of the PDE families have been generated. PDE inhibitors (PDE-Is) increase the intracellular amount of cAMP and/or cGMP by inhibiting the enzymatic degradation of these second messengers, dependent on the substrate specificity of the corresponding PDE (see also Table 2). Several selective PDE-Is and the substrate, i.e. cAMP and/or cGMP, of their target PDEs are classified in Table 2.

Table 2 Overview of PDEs. The properties and substrate specificity are depicted (Bender and Beavo 2006). In addition, commonly used selective PDE inhibitors are mentioned. PDE: phosphodiesterase; cAMP: cyclic adenosine monophosphate; cGMP: cyclic guanosine monophosphate

Type	Number of Genes	Property	Substrate	Selective Inhibitors
PDE1	3	Ca2+ -CaM stimulated	cAMP/cGMP	IBMX, calimidazolium, phenethiazines, vinpocetine, SCH51866
PDE2	1	cGMP stimulated	cAMP/cGMP	EHNA, BAY 60-7550, aptosyn
PDE3	2	cGMP inhibited	cAMP	Cilostamide, milrinone, SK&F 95654
PDE4	4	cAMP specific	cAMP	Rolipram, rofluminast, Ariflo, HT0712, Ibudilast, Mesembrine
PDE5	1	cGMP specific	cGMP	Zaprinast, sildenafil, vardenafil, tadelafil, SK&F 96231, udenafil, avanafil, DA-8159
PDE6	4	Photoreceptor	cGMP	(Sildenafil)
PDE7	2	cAMP high affinity	cAMP	BRL 50481
PDE8	2	cAMP high affinity	cAMP	?
PDE9	1	cGMP high affinity	cGMP	SCH 81566, BAY 73-6691
PDE10	1	cAMP-inhibited	cGMP	Papaverine, TP-10, PQ-10
PDE11	1	Dual substrate	cAMP/cGMP	(Tadelafil)

By far, not all classes of PDEs have selective inhibitors. In addition, these inhibitors might have poor penetration properties concerning the blood-brain barrier. In the literature only five PDE-Is have been used in behavioral cognition studies, namely PDE 2, 4, 5, 9 and 10 inhibitors, as will become evident in this review. These inhibitors are widely available, can be administered peripherally and show central effects. The existing literature on PDE-Is and cognition is rapidly emerging and pro-cognitive effects of PDE-Is have been described in fish, rodents, monkeys and man (e.g. Best et al. 2008; Rutten et al. 2008a; Rutten et al. 2007b; Schultheiss et al. 2001). Studies were conducted to asses the effects of PDE-Is on intact cognition as well as in cognitive deficit models. In addition, knockout-models have been developed to study the role of PDEs in cognition processes. This review provides a comprehensive overview of the currently available literature on the effects of selective PDE-Is on cognition in pre-clinical models. Furthermore, possible implications for human studies are discussed. Finally the underlying mechanisms of action for the pro-cognitive effects of

PDE-Is are discussed and a concomitantly novel theory describing the relationship between different stages of memory consolidation and different types of long-term potentiation (LTP) is proposed.

EFFECTS OF SELECTIVE PDE-IS ON COGNITION

PDE2

Table 3 Overview of effects of PDE2-Is on cognition. T1: trial 1; T2: trial 2; po: per os; ip: intraperitoneal

Task (cognitive process, area involved)	Model (species)	Treatment	Results	Reference
Object recognition task (object memory, hippocampus and rhinal cortex)	Unimpaired (rat)	BAY 60-7550 (3mg/kg, po) immediately after, 1h, 3h or 6h after first trial. (24h interval T1-T2)	BAY 60-7550 (3 mg/kg, immediately after T1 or 3h after T1) improved memory consolidation	(Rutten et al. 2007b)
	Unimpaired (rat)	BAY 60-7550 (0.3, 1 or 3 mg/kg, po) immediately after first trial. (24h interval T1-T2)	BAY 60-7550 (1 or 3 mg/kg, immediately after T1) improved memory consolidation	(Boess et al. 2004)
	Impaired by age, 3, 12 and 24 months old (rat)	BAY 60-7550 (0.3 mg/kg, sc) 1h before first trial or immediately after first trial. (2h interval T1-T2)	BAY 60-7550 1h before T1 improved acquisition in all age groups. In addition, it improved consolidation in animals of 3 and 12 months when given immediately after T1.	(Domek-Lopacinska and Strosznajder 2008)
	Unimpaired (mouse)	BAY 60-7550 (0.3, 1 or 3 mg/kg, po) immediately after first trial. (24h interval T1-T2)	BAY 60-7550 (0.3 or 1 mg/kg, immediately after T1) improved memory consolidation	(Boess et al. 2004)
Social recognition (social memory, hippocampus and amygdale)	Unimpaired (rat)	BAY 60-7550 (0.3, 0.6, 1, 2, 3 or 6 mg/kg, po) immediately after first trial. (24h interval T1-T2)	BAY 60-7550 (1, 2, 3 or 6 mg/kg, immediately after T1) improved memory consolidation	(Boess et al. 2004)
T-maze (working memory, hippocampus)	Impaired by MK-801, 0.125 mg/kg, ip, 30 min before test session (mouse)	BAY 60-7550 (0.3, 1 or 3 mg/kg, po) 30 min before test session.	BAY 60-7550 (3 mg/kg) reversed MK-801 induced deficit	(Boess et al. 2004)

So far, only a couple studies have been published that investigated the effects of PDE2 inhibition in behavioral models. To our knowledge, BAY 60-7550 is the only selective PDE2-I which has been tested in animal models of cognition (Boess et al. 2004; Domek-Lopacinska and Strosznajder 2008; Rutten et al. 2007b). It has been shown that BAY 60-7550 improved memory acquisition and consolidation in the object recognition task in both rats and mice, and consolidation in the social recognition task in rats (Boess et al. 2004; Domek-Lopacinska and Strosznajder 2008; Rutten et al. 2007b). In addition, this PDE2-I improved acquisition and consolidation in the object recognition task in age-impaired rats (Domek-Lopacinska and Strosznajder 2008).

Furthermore, BAY 60-7550 reversed the MK-801 induced working memory deficit in the T-maze in mice (Boess et al. 2004). A more detailed overview of these studies is provided in Table 3.

PDE4

The next section provides a general summary of the available literature on PDE4-Is and cognition. A more detailed overview is provided in Table 4.

It has been shown in several studies that acute as well as subchronic administration of the PDE4-I rolipram improved memory consolidation in unimpaired rats in the object recognition task (Rutten et al. 2007a; Rutten et al. 2007b; Rutten et al. 2008c). In addition, memory deficits caused by scopolamine or acute tryptophan depletion were reversed by rolipram in this task (Rutten et al. 2007a; Rutten et al. 2006). Several spatial memory tasks (e.g. water escape task and radial arm maze) showed that PDE4-Is did not only improve spatial memory in unimpaired rats and mice (Bach et al. 1999; Huang et al. 2007), but also in rats of which spatial memory was impaired by age or microsphere embolism-induced cerebral ischemia (Nagakura et al. 2002). An impairment of spatial reference memory caused by scopolamine, MK-801 or MEK (MAPK/ERK kinase) inhibition was also reversed by various PDE4-Is (Egawa et al. 1997; Zhang et al. 2000; Zhang et al. 2005; Zhang and O'Donnell 2000; Zhang et al. 2004).

In addition, various studies investigated the effects of PDE4-Is on passive avoidance learning and PDE4-Is reversed impairments caused by scopolamine, MK-801, anisomycin, and MEK inhibition in this task (Egawa et al. 1997; Ghelardini et al. 2002; Imanishi et al. 1997; Randt et al. 1982; Zhang et al. 2005; Zhang and O'Donnell 2000; Zhang et al. 2004). Furthermore, it was shown that acute as well as chronic treatment of rolipram improved the performance of unimpaired rats and mice in contextual fear conditioning (Barad et al. 1998; Comery et al. 2005; Monti et al. 2006).

The effects of PDE4-Is on working memory in rats have been studied in various deficit models. It was shown that working memory deficits caused by scopolamine, MK-801, cerebral ischemia or electro convulsive shocks (ECS) were reversed by the administration of PDE4-Is in the radial arm maze and the 3-panel runway task (Egawa et al. 1997; Imanishi et al. 1997; Zhang et al. 2000; Zhang et al. 2005; Zhang et al. 2004). Of note, the effects of rolipram on spatial working memory are twofold; on one hand rolipram tended to improve working memory in young rhesus monkeys in a delayed responding task (Ramos et al. 2003). However, on the other hand rolipram had a negative effect on working memory in aged monkeys in this task (Ramos et al. 2003; Ramos et al. 2006).

The effects of rolipram on information processing have been studied in several behavioral setups in the prepulse inhibition and startle response task. Rolipram did not only facilitate information processing in unimpaired mice and zebrafish, but also reversed deficits caused by D-amphetamine in mice (Best et al. 2008; Kanes et al. 2007). In contrast, the PDE4-I RO-20-1724 did not reverse prepulse inhibition deficit caused by D-amphetamine (Halene and Siegel 2008). In another model of information processing, sensory gating, this PDE-I increased the amplitudes of P20 and N40 in the CA3 area during the first stimulus, and reversed the N40 deficit in the first click caused by D-amphetamine (Halene and Siegel 2008). Additionally, executive functioning was improved in an object retrieval task in cynomolgus macaques after administration of rolipram (Rutten et al. 2008a). In this task monkeys try retrieve a food reward from a transparent box with one open side that alternates between trials. This is a prefrontal cortical mediated task likely to capture attention and response inhibition, and rolipram treatment significantly dose dependently enhanced performance, as measured by an increased percentage correct first reaches.

Besides deficit models based on pharmacological or surgical interventions, the use of transgenic animals, i.e. isoform specific knock-out models of PDE4B or PDE4D, have been recently introduced to study the role of PDE4 in the central nervous system (CNS). It was shown that PDE4B knock-out (KO) in mice had no effect on spatial memory performance in the water escape task and the passive avoidance task (Siuciak et al. 2008a). Furthermore, these mice showed an impairment in information processing in the prepulse inhibition task (Siuciak et al. 2008a), although they performed similar to wild-type animals on conditioned avoidance responding (Siuciak et al. 2007). A recent study showed more controversial data demonstrating enhanced LTP but impaired fear conditioning in PDE4D knock-out mice (Rutten et al. 2008b).

In addition, a variety of transgenic mice models was used in combination with the administration of PDE4-Is. It has been shown that acute as well as chronic treatment of PDE4-Is improved long-term memory functioning in a Rubenstein-Taybi syndrome and two Alzheimer's disease KO mouse models for cognitive impairment in the fear conditioning and object recognition task (Bourtchouladze et al. 2003; Comery et al. 2005; Gong et al. 2004). Also, the PDE4-I rolipram improved working memory and spatial memory in a transgenic model of Alzheimer's disease, i.e. PS1/PDAPP KO mice in the radial arm water maze (Costa et al. 2007; Gong et al. 2004).

To our knowledge, no studies have been published in which the effects of PDE4-Is on cognition in humans are described. However, the PDE4-I MK 0952 is now entering phase 2 clinical trials for cognition enhancement (Merck & Co. 2006).

Table 4 Overview of effects of PDE4-Is on cognition. KO: knock-out; im: intramuscular; ip: intraperitoneal; po: per os; sc: subcutaneous; MEK: MAPK/ERK kinase; T1: trial 1; T2: trial 2; ECS: electro convulsive shocks; ATD: acute tryptophan depletion; ORT: object recognition task

Task (cognitive process, area involved)	Model (species)	Treatment	Results	Reference
Water escape task (spatial memory, hippocampus)	Impaired by microsphere embolism-induced cerebral ischemia (rat)	Rolipram (3 mg/kg, ip) 10 days, after embolism	Rolipram attenuates acquisition deficit measured at days 7-9	(Nagakura et al. 2002)
	Impaired by PDE4B KO (mouse)	-	No effect	(Siuciak et al. 2008a)
Delayed matching to position watermaze (spatial memory, hippocampus)	Unimpaired (rat)	L-454,560 (0, 0.1, 0.3 or 1 mg/kg, po) 30 min before testing	L-454,560 (0.3 and 1 mg/kg) improved performance	(Huang et al. 2007)
Radial arm water maze (spatial memory, hippocampus)	Impaired by APP-PS1 Alzheimer KO (mouse)	Rolipram (0.03 mg/kg, sc) for 3 weeks	Improvement when tested at 2 months after 3-week treatment	(Gong et al. 2004)
	Impaired by PS1/PDAPP KO (mouse)	Rolipram (0.03 mg/kg, s.c.) once a day for 2 weeks before testing	Rolipram improved working memory	(Costa et al. 2007)
Barnes circular maze (spatial memory, hippocampus)	Impaired by age, 18 months old (mouse)	Rolipram (0.016 mg/kg, ip) 40 min before training	More mice acquire the task and number of errors is reduced	(Bach et al. 1999)
Radial arm maze (working & reference memory, hippocampus)	Impaired by scopolamine 0.5/1.0mg/kg, ip, 30 min before test (rat)	Rolipram (0.01 – 1 mg/kg, ip) 45 min before test	MED: 0.1 (working memory) and >0.1 mg/kg (reference memory)	(Zhang and O'Donnell 2000)
	Impaired by scopolamine, 0.5 mg/kg, ip, 30 min before test (rat)	(±)-rolipram 0.01 – 1 mg/kg, po (-)-rolipram 0.005-1mg/kg, po (+)-rolipram 0.1-50 mg/kg, po	MED (working memory): (±)-rolipram 0.02-0.2 mg/kg (-)-rolipram 0.01-0.02 and 0.2/0.5 mg/kg (bi phasic) (+)-rolipram 20/50 mg/kg	(Egawa et al. 1997)
	Impaired by glutamate MK-801, 0.l mg/kg, ip, 60 min before test (rat)	Rolipram (0.01 – 0.1 mg/kg, ip) 30 min before test	MED: 0.05 (working memory) and 0.1 mg/kg (reference memory)	(Zhang et al. 2000)

Task (cognitive process, area involved)	Model (species)	Treatment	Results	Reference
	Impaired by MK-801, 0.1 mg/kg, ip, 60 min before testing (rat)	Rolipram (0.1 mg/kg, ip), MEM 1018 or MEM 1091 (0.1 – 2.5 mg/kg, ip) 45 min before test	MED: 0.1 mg/kg rolipram working memory, MED: 2.5 mg/kg MEM 1018 working and reference memory MED:2.5 mg/kg MEM 1091 on reference memory.	(Zhang et al. 2005)
	Impaired by MEK inhibitor UO126, 8ug/rat into hippocampus, given twice: 60 and 30 min before test (rat)	Rolipram (0.05, 0.1, mg/kg, ip) 30 min before test	MED: 0.1 mg/kg (reference memory)	(Zhang et al. 2004)
Passive avoidance learning (learning, hippocampus and amygdala)	Impaired by 1. Protein synthesis inhibitor anisomycin, 150 mg/kg, sc, 30 min before training 2. Low baseline (mouse)	Rolipram (3 or 10 mg/kg, ip, immediately after training or 3 hours after training	MED 10 mg/kg, given immediately after training (1 + 2)	(Randt et al. 1982)
	Impaired by scopolamine, 1 mg/kg, ip, 30 min before acquisition (mouse)	Rolipram (1-30 mg/kg, ip) 30 min before acquisition	MED: 10 mg/kg	(Imanishi et al. 1997)
	Impaired by scopolamine, 1.5 mg/kg, ip, immediately after training (mouse)	Rolipram (10 or 30 mg/kg, po) 30 min before training	MED: 30 mg/kg	(Ghelardini et al. 2002)
	Impaired by scopolamine, 3 mg/kg, ip, 30 min before retention test (rat)	Given 60 min before retention test. (±)-rolipram 0.01 – 0.1 mg/kg, po (-)-rolipram 0.005-0.02 mg/kg, po (+)-rolipram 0.3-10 mg/kg, po	MED: (±)-rolipram 0.02-0.1 mg/kg (-)-rolipram 0.01-0.02 mg/kg (+)-rolipram 2 mg/kg; no effect at 10 mg/kg	(Egawa et al. 1997)

Task (cognitive process, area involved)	Model (species)	Treatment	Results	Reference
	Impaired by glutamate antagonist MK-801 0.l mg/kg, ip, 60 min before test (rat)	Rolipram (0.1 mg/kg, ip) 30 min before test	MED: ≤ 0.1 mg/kg	(Zhang et al. 2000)
	Impaired by MK-801, 0.1 mg/kg, ip, 60 min before testing (rat)	Rolipram (0.1mg/kg, ip), MEM 1018 or MEM 1091 (0.1 – 2.5 mg/kg, ip) 45 min before test	MED: Rolipram 0.1mg/kg, MEM1018 0.1-2.5mg/kg and MEM 1091 0.5-2.5 mg/kg on reversal latency	(Zhang et al. 2005)
	Impaired by MEK inhibitor UO126, 8ug/rat into hippocampus, given twice: 60 and 30 min before test (rat)	Rolipram (0.1, mg/kg, ip) 30 min before test or 30 ug/rat into hippocampus, 20 min before test	Reversal retention deficit 48-h post training	(Zhang et al. 2004)
	Impaired by PDE4B KO (mouse)	-	No effect	(Siuciak et al. 2008a)
3-panel runway task (working memory, hippocampus and prefrontal cortex)	Impaired by scopolamine, 0.56 mg/kg, ip, 15 min before first trial (rat)	Rolipram (0.032 or 0.1 mg/kg, ip) 30 min before first trial	MED: 0.1 mg/kg for decrease errors	(Imanishi et al. 1997)
	Impaired by cerebral ischemia by four-vessel occlusion (rat)	Rolipram (0.032 or 0.1 mg/kg, ip) 30 min before first trial (immediately after reperfusion)	MED: 0.1 mg/kg for decrease errors	(Imanishi et al. 1997)
	Impaired by ECS immediately after training (rat)	Rolipram (0.1 or 0.32 mg/kg, ip) just before ECS	MED: 0.32 mg/kg for decrease errors	(Imanishi et al. 1997)
Inhibitory avoidance learning (learning, hippocampus and amygdala)	Impaired by 1. Protein synthesis inhibitor anisomycin, 150 mg/kg, sc, 30 min before training 2. Low baseline (mouse)	Rolipram (3 or 10 mg/kg, ip, immediately after training or 3 hours after training	MED 10 mg/kg, given immediately after training (1 + 2)	(Randt et al. 1982)
Contextual fear-conditioning (learning, hippocampus and amygdala)	Unimpaired (mouse)	Rolipram (0.03 mg/kg, sc) 30 min before training	Improved retention 24 h after training	(Barad et al. 1998)

Task (cognitive process, area involved)	Model (species)	Treatment	Results	Reference
	Unimpaired (rat)	Rolipram 0.5 mg/kg per day for 7 days chronic delivery by osmotic mini-pumps	Improved memory consolidation and slower extinction of conditioned fear	(Monti et al. 2006)
	Impaired by TG2576 KO Alzheimer mice (mouse)	Rolipram (0.1 mg/kg, ip) 30 min prior to training	Improvement in mutants and wild type	(Comery et al. 2005)
	Impaired by APP-PS KO Alzheimer mice (mouse)	Rolipram 0.1 uM/kg for 3 weeks	Improvement when tested 2 months following 3-week treatment	(Gong et al. 2004)
	Impaired by PDE4D KO (mouse)	-	Impairment long-term memory for context and cued fear	(Rutten et al. 2008b)
Object recognition task (object memory, hippocampus and rhinal cortex)	Unimpaired young (rat)	Rolipram (0.01, 0.03 or 0.1 mg/kg, ip) given: 1. 30 min before training 2. directly after training 3. 3h after training.	Rolipram (0.03 mg/kg 3h after T1) improved memory consolidation in ORT	(Rutten et al. 2006)
	Unimpaired young (rat)	Rolipram (0.03 mg/kg ip) given: 1. directly after training 2. 1h after training 3. 3h after training 4. 6h after training	Rolipram (0.03 mg/kg 3h after T1) improved memory consolidation in ORT	(Rutten et al. 2007b)
	Impaired by scopolamine, 0.1 mg/kg, ip, 30 min before training (rat)	Rolipram (0.03, 0.1 or 0.3 mg/kg, i.p.) 30 min before training.	Rolipram (0.1 mg/kg) reversed the scopolamine induced short-term memory deficit	(Rutten et al. 2006)
	Impaired by acute tryptophan depletion, 3h before training (rat)	Rolipram (0.01, 0.03 or 0.1 mg/kg, ip) 30 min before training	Rolipram (0.1 mg/kg) reversed ATD induced short-term memory deficit	(Rutten et al. 2007a)

Task (cognitive process, area involved)	Model (species)	Treatment	Results	Reference
	Unimpaired (rat)	Subchronic treatment of rolipram (0.5 mg/kg, po) for 5 days. Testing before, during (day 2-3) and after treatment (T1-T2 24h)	Subchronic rolipram treatment improved object recognition memory. Timing of final dose did not affect performance	(Rutten et al. 2008c)
	Impaired by heterozygous CBP mutation (mouse)	Rolipram (0.1 mg/kg, ip) or HT0712 (0.001 – 0.5 mg/kg, ip) 20 min before training	MED: 0.1 mg/kg for both drugs. Improved object recognition at 24 hours	(Bourtchouladze et al. 2003)
Delayed responding (spatial working memory, prefrontal cortex)	Unimpaired young and aged-impaired (rhesus monkey)	Rolipram (0.01-100 ug/kg, im) 1 hour before testing	At 0.1 ug/kg, trend for improvement in young subjects. Aged subjects impaired by 10 ug/kg.	(Ramos et al. 2003)
	Impaired by age (rhesus monkey)	Rolipram (0, 0.001-0.05 µg/kg, im) 2h before testing and guanfacine (0, 0.0001-0.01 mg/kg, im (one animal 0.5 mg/kg))	Rolipram alone no effect. Rolipram reversed beneficial effect of guanfacine on working memory	(Ramos et al. 2006)
Object retrieval (executive functioning and response inhibition, prefrontal cortex)	Unimpaired (cynomolgus macaque)	Rolipram (0.003, 0,01 or 0.03 mg/kg, im) 30min before testing	Rolipram (0.01, 0.33 mg/kg) improved object retrieval performance.	(Rutten et al. 2008a)
Prepulse inhibition (information processing, frontal cortex)	Unimpaired (mouse)	Rolipram (0.1, 0.66, 1 or 10 mg/kg, ip) 15 min before testing	Rolipram (0.66, 1, 10 mg/kg) increased PPI and decreased startle response	(Kanes et al. 2007)
	Impaired by D-amphetamine, 10 mg/kg, ip, 15 min before testing (mouse)	D-amphetamine (10 mg/kg, ip) and rolipram (0.66 mg/kg, ip) 15 min before testing	Rolipram attenuated the PPI deficit caused by D-amphetamine, but had no effect on startle response	(Kanes et al. 2007)
	Impaired by PDE4B KO (mouse)	-	Increased startle response and decreased PPI (independent of startle response)	(Siuciak et al. 2008a)

Task (cognitive process, area involved)	Model (species)	Treatment	Results	Reference
	Impaired by D-amphetamine, 5 mg/kg (mouse)	RO-20-1724 (0.25, 2.5 or 4 mg/kg, sc) or rolipram (mg/kg, sc), 5 min before testing	RO-20-1724 did not reverse PPI deficit caused by D-amphetamine	(Halene and Siegel 2008)
Startle response (non-associative learning)	Unimpaired (zebrafish)	Rolipram (3, 10 or 30 μM)	Rolipram (3 μM) enhanced startle response	(Best et al. 2008)
Acquisition of conditioned avoidance responding (learning, hippocampus)	Impaired by PDE4B KO (mouse)	-	No effect	(Siuciak et al. 2007)
Auditory event-related potentials (information processing, frontal cortex)	Unimpaired (mouse)	RO-20-1724 (0.1, 0.25, 0.5, 1, 2.5 mg/kg, sc), 5 min before testing	1st click: RO-20-1724 increased amplitude of P20 (at a dose of 0.25, 0.5, 1 mg/kg) and of N40 at a dose of (0.25, 0.5, 2.5 mg/kg) in CA3 area No effects on 2nd click	(Halene and Siegel 2008)
	Impaired by D-amphetamine, 0.5 mg/kg (mouse)	RO-20-1724 (0.25 mg/kg, sc), 5 min before testing	1st click: P20 no effect. N40 RO-20-1734 reversed deficit caused by D-amphetamine in CA3 area. No effects on 2nd click	(Halene and Siegel 2008)

This table is an adapted and updated version of the overview (Table 3) in Blokland et al. (2006)

PDE5

Prickaerts et al. (1997) were the first to describe memory enhancing effects of PDE5 inhibition, using the PDE5-I zaprinast. However, zaprinast is not selective for PDE5, as it also inhibits PDE1, 9, 10, and 11 (Bender and Beavo 2006). Recently, more highly selective PDE5 inhibitors have been developed mainly for the treatment of erection disorder, e.g sildenafil (Viagra), vardenafil (Levitra) and tadalafil (Cialis) (Setter et al. 2005). The next section will give a general summary of the available literature on PDE5-Is and cognition; a more detailed overview is provided in Table 5.

So far, several studies have shown positive effects of selective PDE5-Is on memory performance in the object recognition task in adult rats; zaprinast (Domek-Lopacinska and Strosznajder 2008; Prickaerts et al. 1997), sildenafil (Prickaerts et al. 2005; Prickaerts et al. 2002b) and vardenafil (Prickaerts et al. 2002b; Rutten et al. 2007b) improved memory consolidation. In addition, Rutten et al. (2005) showed that sildenafil also improved memory consolidation in mice in this task. Previous work from our group showed that zaprinast reversed the object memory deficits induced by the NOS inhibitor 7-nitroindazole in rats in

the object recognition task (Prickaerts et al. 1997). However, zaprinast was unable to reverse memory deficits in aged rats in this task (Domek-Lopacinska and Strosznajder 2008).

Several studies have shown spatial memory improvement in an adapted version of the elevated plus maze in mice (Patil et al. 2004a; Singh and Parle 2003) after treatment with a PDE5-I. Furthermore, sildenafil treatment ameliorated the deficits induced by diabetes or ECS in this task (Patil et al. 2004a; Patil et al. 2006). Previous studies showed no effects of PDE5-Is on spatial tasks in healthy rats, i.e. the water escape task or the Y maze (Prickaerts et al. 2004). However, since only one dose was tested in this study, further investigation will be needed. Finally, in hyperammonemia and portacaval shunt deficit models for liver failure, both sildenafil and zaprinast reversed spatial recognition deficits of rats in the Y maze (Erceg et al. 2006; Erceg et al. 2005a; Erceg et al. 2005b). Recent work adds to this since sildenafil reversed the effects the nitric oxide synthase (NOS) inhibitor L-NAME, in a complex maze learning paradigm (Devan et al. 2006; Devan et al. 2007).

Furthermore, various studies investigated the effects of PDE5-Is on active and passive avoidance learning in rats, mice and neonatal chicks. Although one study failed to show improvement in learning performance after sildenafil treatment in unimpaired and aged rats (Shafiei et al. 2006), others have shown improvements in unimpaired and aged mice and in neonatal chicks (Baratti and Boccia 1999; Campbell and Edwards 2006; Patil et al. 2004a). In contrast, Edwards et al. (2007) found that zaprinast could also have a negative effect on learning and memory when given at a high dose. Memory impairments in avoidance learning caused by scopolamine, diabetes or electro convulsive shocks in rats were reversed by sildenafil treatment (Devan et al. 2004; Patil et al. 2006). In addition, zaprinast as well as sildenafil reversed memory deficits caused by a model for diabetes in mice (Patil et al. 2004a).

Finally, a recent study showed that the PDE5-I sildenafil dose dependently improved performance in a prefrontal task, i.e. the object retrieval task (see above), in cynomolgus macaques (Rutten et al. 2008a).

Most research regarding the cognition enhancing effects of PDE5-Is so far has focused on animal preclinical models; there are only two papers in which the effects of the PDE5-I sildenafil on human cognition were investigated. Grass et al. (2001) have shown that 100 mg sildenafil enhanced performance in a simple reaction time test when given 1 h before testing. However, no effects were found on short-term memory, divided attention and other psychomotor tasks (Grass et al. 2001). In addition, Schultheiss et al (2001) studied the effects of sildenafil (100 mg, 1 h before testing) on auditory attention and word recognition. Again, no cognition enhancing effects were found with regard to the behavioral measures

In both studies, short-term memory tasks were performed that are thought to measure memory performance processes comparable to the object recognition task in rats. However, the object recognition task in animals usually measures more aspects of memory, such as that for object and for location, even though only the object memory itself might have been measured. The human tasks, on the other hand, only assess memory for words or pictures, or location, but never the combination of these aspects. Possibly, the fact that spatial information was lacking in the human studies has caused this discrepancy in findings.

Sildenafil changed certain components of event-related potentials (ERPs) in the study of Schultheiss and collegues (2001). The Nd component, although it only showed a

marginally significant effect, was increased after treatment with sildenafil. This indicates improved focused attention. The P3 component, which measures controlled processes of target selection, was significantly enhanced after administration of sildenafil (Schultheiss et al. 2001). Again, this is evidence for improvements after treatment with sildenafil. Finally, a reduced negativity between 150-250 ms was found in the word recognition experiment after sildenafil treatment; this may also indicate an effect on information processing although the exact role of this component remains uncertain (Schultheiss et al. 2001).

Several possible explanations for not finding any cognition enhancing effects after PDE5-I treatment in humans in contrast to the results in animal studies exist. First, only one dose of sildenafil on one specific time point was tested in both studies. Investigating different doses, both higher and lower, at different administration time points might reveal possible cognition enhancing effects in humans. In addition, a 'ceiling effect' might have occurred in the cognitive tasks; this means that healthy subjects in these studies already perform at their maximal level, so their performance can not be further improved. A final explanation might be that the number of participants was not sufficient, since only 6 participants were tested by Grass et al. (2001), whereas Schultheiss and co-workers (2001) examined 10 healthy participants.

Table 5 Overview of effects of PDE5-Is on cognition. icv: intracerebroventricular; ic: intracerebral; ip: intraperitoneal; LPS: lipopoly saccharine; NOS: nitric oxide synthase; ORT: object recognition task; po: per os; T1: trial 1; T2: trial 2; STZ: streptozotoon

Task (cognitive process, area involved)	Model (species)	Treatment	Results	Reference
Object recognition task (object memory, hippocampus and rhinal cortex)	Unimpaired (rat)	Sildenafil Citrate (1, 3 or 10 mg/kg, po). 30min before or immediately after first trial. (24h interval T1-T2)	Sildenafil (3mg/kg T0 or 10mg/kg T1-30min) improves memory consolidation	(Prickaerts et al. 2005)
	Unimpaired (rat)	Zaprinast (3 or 10mg/kg,ip) immediately after first trial. (4h interval T1-T2)	Zaprinast (10mg/kg) improved memory consolidation.	(Prickaerts et al. 1997)
	Unimpaired (rat)	Sildenafil (1, 3 or 10mg/kg,po) immediately after first trial. (24h interval T1-T2)	Sildenafil (3-10 mg/kg) improved memory consolidation in ORT.	(Prickaerts et al. 2002b)
	Unimpaired (rat)	Vardenafil (0.1, 0.3, 1 or 3 mg/kg,po) immediately after first trial. (24h interval T1-T2)	Vardenafil (0.3-3 mg/kg) improved memory consolidation in ORT.	(Prickaerts et al. 2002b)
	Unimpaired (rat)	Vardenafil (1mg/kg, po) immediately after, 1h, 3h or 6h after first trial. (24h interval T1-T2)	Vardenafil (1 mg/kg immediately after T1) improved memory consolidation in ORT	(Rutten et al. 2007b)

Task (cognitive process, area involved)	Model (species)	Treatment	Results	Reference
	Unimpaired (mouse)	Sildenafil (0.3, 1 or 3 mg/kg, po) immediately after first trial. (24h interval T1-T2)	Sildenafil (1 mg/kg) improved memory consolidation in ORT	(Rutten et al. 2005)
	Impaired by NOS inhibitor (rat)	7-nitroindazole (10 or 30mg/kg, ip) Zaprinast (3 or 10mg/kg, ip) immediately after first trial. (1h interval T1-T2)	Zaprinast (10 mg/kg) reversed the NOS-I (10mg/kg) deficit in ORT	(Prickaerts et al. 1997)
	Impaired by age, 3, 12 and 24 months old (rat)	Zaprinast (0.3 mg/kg, sc) 1h before first trial or immediately after first trial. (2h interval T1-T2)	Zaprinast 1h before T1 improved acquisition in 3 month-old animals. In addition, it improved consolidation in animals of 3 months when given immediately after T1.	(Domek-Lopacinska and Strosznajder 2008)
Adapted version of elevated plus-maze (spatial memory, hippocampus)	Unimpaired (mouse)	Sildenafil (2, 4 or 8 mg/kg, ip) 30min before or immediately after first trial	Sildenafil (8mg/kg) before T1 marginally increased spatial memory acquisition. Sildenafil (2, 4, 8mg/kg) imm. after T1 increased spatial memory retention.	(Singh and Parle 2003)
	Unimpaired (mouse) Age impaired (mouse)	Sildenafil (0.25, 0.5 or 1mg/kg, ip) immediately after first trial	Sildenafil improved spatial memory performance in young (0.5 and 1.0mg/kg) and aged (0.25-1 mg/kg) animals.	(Patil et al. 2004a)
	Unimpaired (mouse) Age impaired (mouse)	Zaprinast (0.5, 1 or 2mg/kg, ip) immediately after first trial	Zaprinast improved spatial memory performance in young (1.0 and 2.0mg/kg) and aged (0.5-2 mg/kg) animals.	(Patil et al. 2004a)
	Impaired by diabetes-STZ (rat)	Streptozotocin (STZ) (60mg/kg, ip) Sildenafil (0.25, 0.5 or 1 mg/kg, ip) immediately after training	Sildenafil (all doses) reversed STZ spatial memory deficits.	(Patil et al. 2006)

Task (cognitive process, area involved)	Model (species)	Treatment	Results	Reference
	Impaired by diabetes-LPS (mouse)	Lipopolysaccharine (LPS: 50ug, ip) and sildenafil (0.25, 0.5 or 1 mg/kg, ip) or zaprinast (0.5, 1 or 2mg/kg, ip) immediately after training	Sildenafil (0.5 and 1 mg/kg) and Zaprinast (1 and 2 mg/kg) reversed LPS spatial memory deficits.	(Patil et al. 2004a)
	Impaired by Electro convulsive shock (rat)	Shocks (0.2mA,0.2s/day for 15 days) Sildenafil (0.5, 1 or 2 mg/kg, ip) immediately after training	Sildenafil (all doses) reversed spatial memory deficits	(Patil et al. 2006)
Y-maze (spatial memory, hippocampus and cerebellum)	Unimpaired (rat)	Vardenfil (3mg/kg, po) daily after last trial.	No effects on spatial recognition	(Prickaerts et al. 2004)
	Impaired by Hyperammonemia (rat)	Sildenafil (50mg/L) in drinking water two days before training.	Sildenafil (in drink water) reversed spatial recognition deficits.	(Erceg et al. 2006)
	Impaired by Hyperammonemia (rat)	Ammonium acetate containing diet (28 days before testing) Zaprinast (50uM, 0.25 ul/h, 2 days before testing) in cerebral ventricle	Zaprinast (through minipump) reversed spatial recognition deficits.	(Erceg et al. 2005a)
	Impaired by Portacaval Shunts (rat)	Portacaval shunt operation 28 days before test. Sildenafil (50mg/L) in drinking water two days before training.	Sildenafil (in drink water) reversed spatial recognition deficits.	(Erceg et al. 2005b)
Water escape task (spatial memory, (hippocampus)	Unimpaired (rat)	Zaprinast (10mg/kg, ip) daily after last trial.	No effects on acquisition or retention of spatial memory	(Prickaerts et al. 2004)
Complex maze learning (learning, hippocampus)	Impaired by NOS inhibitor (rat)	L-NAME (60mg/kg,ip) 30min before training Sildenafil (1, 1.5, 3 or 4.5 mg/kg, ip) 15min before training	Sildenafil (1.5mg/kg) attenuated the L-NAME deficit in Maze learning.	(Devan et al. 2006)

Task (cognitive process, area involved)	Model (species)	Treatment	Results	Reference
	Impaired by NOS inhibitor (rat)	L-NAME (0, 45 µg/kg, i.c.v.) 30min before training Sildenafil (0, 1.5 or 3 mg/kg, ip) 15min before training	Sildenafil (3 mg/kg) attenuated the L-NAME deficit in Maze learning.	(Devan et al. 2007)
Active avoidance learning (learning, hippocampus)	Impaired by scopolamine, 0.75 mg/kg, ip, 30 min before training (rat)	Sildenafil (1.5, 3 or 4.5mg/kg,ip) 15min before training.	Sildenafil (3mg/kg) reversed the scopolamine deficit in active avoidance task.	(Devan et al. 2004)
	Unimpaired (mouse)	Sildenafil (1, 3, 10 or 30 mg/kg, i.p.) 30 min before training or immediately after training	Sildenafil (3mg/kg) improved performance (both 30min before and imm. after training) in active avoidance.	(Baratti and Boccia 1999)
Passive avoidance learning (learning, hippocampus)	Unimpaired (rat)	Sildenafil (1, 3, 10 or 20 mg/kg, ip) immediately after training in young and old rats.	Sildenafil has no effect on retention performance in passive avoidance.	(Shafiei et al. 2006)
	Unimpaired (neonate chick)	Zaprinast (0.1-750µM/side, ic) immediately after training	Zaprinast (>100uM) enhanced early consolidation.	(Campbell and Edwards 2006)
	Unimpaired (young chick)	Zaprinast (100 µM/side, ic) immediately after training. Retention times between 10-180 min.	Zaprinast impaired performance (at a retention of 40, 60, 90 and 120 min)	(Edwards and Lindley 2007)
	Unimpaired (mouse) Age impaired (mouse)	Sildenafil (0.25, 0.5 or 1mg/kg, ip) immediately after first trial	Sildenafil improved consolidation in young (0.5 and 1.0mg/kg) and aged (0.25-1 mg/kg) animals.	(Patil et al. 2004a)
	Unimpaired (mouse) Age impaired (mouse)	Zaprinast (0.5, 1 or 2mg/kg,ip) immediately after first trial	Zaprinast improved spatial memory performance in young (1.0 and 2.0mg/kg) and aged (0.5-2 mg/kg) animals.	(Patil et al. 2004a)
	Impaired by Diabetes (rat)	STZ (60mg/kg,ip) Sildenafil (0.25, 0.5 or 1 mg/kg, ip) immediately after training.	Sildenafil (all doses) reversed STZ memory deficit caused by diabetes.	(Patil et al. 2006)

Task (cognitive process, area involved)	Model (species)	Treatment	Results	Reference
	Impaired by Electro convulsive shock (rat)	Shocks (0.2mA,0.2s/day for 15 days) Sildenafil (0.5, 1, 2 mg/kg, ip) immediately after training.	Sildenafil (all doses) reversed memory deficit caused by ECS.	(Patil et al. 2006)
	Impaired by diabetes-LPS (mouse)	Lipopolysaccharine (LPS: 50ug, ip) and sildenafil (0.25, 0.5 or 1 mg/kg, ip) or zaprinast (0.5, 1, 2mg/kg,ip) immediately after training	Sildenafil (0.5 and 1 mg/kg) and Zaprinast (1 and 2 mg/kg) reversed LPS induced memory deficits.	(Patil et al. 2004b)
Object retrieval (executive functioning and response inhibition, prefrontal cortex)	Unimpaired (cynomolgus macaque)	Sildenafil (0.3, 1 or 3 mg/kg, im) 30 min before testing	Sildenafil (1, 3 mg/kg) improved object retrieval performance.	(Rutten et al. 2008a)
7 different psychophysical tests (psychophysical performance, various brain areas)	Unimpaired (humans)	Sildenafil (100 mg, po) 1h before testing	Sildenafil enhanced performance on the simple reaction time test; other tests no effect	(Grass et al. 2001)
Auditory selective attention & ERPs (attention, prefrontal cortex)	Unimpaired (humans)	Sildenafil (100 mg, po) 1h before testing	Sildenafil had no effect on the behavioral measurements of attention. However, an increase in the ERP components Nd and P3 indicates an improvement of attention.	(Schultheiss et al. 2001)
Verbal recognition memory & ERPs (memory & information processing, hippocampus & frontal cortex)	Unimpaired (humans)	Sildenafil (100 mg, po) 1h before testing	Sildenafil had no effect on the behavioral measurements of memory. However, a reduction in negativity between 150-250 ms might indicate an effect on information processing.	(Schultheiss et al. 2001)

PDE9

Table 6 Overview of effects of PDE9-I on cognition. po: per os; T1: trial 1; T2: trial 2; sc: subcutaneous

Task (cognitive process, area involved)	Model (species)	Treatment	Results	Reference
Object recognition task (object memory, hippocampus and rhinal cortex)	Unimpaired (rat)	BAY 73-6691 (0.1, 0.3, 1 or 3 mg/kg, po) 30 min before T1 (24h interval T1-T2)	BAY 73-6691 (0.1, 0.3 mg/kg) had an intermediate effect on memory consolidation	(van der Staay et al. 2008)
Passive avoidance learning (learning, hippocampus)	Impaired by scopolamine, 0.03 mg/kg, sc, 30 min before testing (rat)	BAY 73-6691 (0.3, 1 or 3 mg/kg, po) 60 minutes before testing	BAY 73-6691 (1, 3 mg/kg) attenuated the scopolamine induced retention deficit	(van der Staay et al. 2008)
Social recognition (social memory, hippocampus and amygdala)	Unimpaired (rat)	BAY 73-6691 (0, 0.03, 0.3 or 3 mg/kg, po) 60 min before the first trial (T1), immediately after T1 (24h interval T1-T2)	BAY 73-6691 (0.3, 3 mg/kg) 60 min before T1, or BAY 73-6691 (0.03, 0.3, 3 mg/kg) immediately after T1 and 60 min before T2 improved memory consolidation	(van der Staay et al. 2008)
	Unimpaired (rat)	BAY 73-6691 (0 or 1 mg/kg, po) 60 min before the first trial (T1), with a familiar juvenile or BAY 73-6691 (1 mg/kg, po) 60 min before the first trial (T1), with a novel juvenile (24h interval T1-T2)	BAY 73-6691 (1 mg/kg) improved memory consolidation with a familiar as well as a novel juvenile	(van der Staay et al. 2008)
	Unimpaired (mouse)	BAY 73-6691 (0, 0.03, 0.3 or 3 mg/kg, po) 30 min before the first trial (24h interval T1-T2)	BAY 73-6691 (0.3, 3 mg/kg) 30 min before T1 improved memory consolidation	(van der Staay et al. 2008)
T-maze (working memory, hippocampus)	Impaired by MK-801, 0.06 mg/kg, sc, 30 min before testing (mouse)	BAY 73-6691 (0, 1, 3 or 10 mg/kg, po) 60 min before testing	BAY 73-6691 (10 mg/kg) attenuated the MK-801 induced deficit in alternation rate	(van der Staay et al. 2008)

To our knowledge, only one paper has been published in which the effects of PDE9 inhibition on cognition are described (van der Staay et al. 2008). In this paper the potent and selective PDE9-I BAY-73-6691 was used (Wunder et al. 2005). It was shown that this PDE9-I improved memory consolidation in unimpaired rats and mice in the object recognition and social recognition task (van der Staay et al. 2008). Furthermore, this PDE9-I

reversed the MK-801 or scopolamine induced memory deficit in T-maze and the passive avoidance task, respectively (van der Staay et al. 2008). More detailed information can be found in Table 6.

PDE10

Only very recently, PDE10-Is have become a target for CNS research, especially concerning the cognitive deficits related to schizophrenia (Schmidt et al. 2008). In the next section, a summary of the available literature on PDE10-Is and cognition will be given; a more detailed overview can be found in Table 7.

Chronic treatment with the PDE10-I papaverine impaired spatial memory and reversal learning in unimpaired mice in the Morris water maze (Hebb et al. 2008). Administration of TP-10 did not have an effect on information processing in a prepulse inhibition task in unimpaired and MK-801 impaired mice (Schmidt et al. 2008). However, TP-10 reversed the auditory gating deficit caused by D-amphetamine in rats (Schmidt et al. 2008). Papaverine improved attention in the attention shifting task in rats that were impaired by subchronic phenylcyclohexylpiperidine (PCP) treatment, a model of schizophrenia, whereas no effect was found in unimpaired rats (Rodefer et al. 2005).

Several studies also used KO models to study the role of PDE10 in cognition. It was shown that PDE10A knock-out in a DBA1LacJ background had no effect on learning and memory in the passive avoidance and water escape task in mice (Siuciak et al. 2006; Siuciak et al. 2008b). In addition, these mice showed the same conditioned avoidance response as wild-type mice; however, these KO mice required more training to reach performance of wild-type animals (Siuciak et al. 2006; Siuciak et al. 2008b). On the other hand, PDE10A KO mice with a C57BL/6N background were unable to reach the performance of the wild-tape mice in this task (Siuciak et al. 2008b).

The data discussed in the previous paragraphs showed that PDE10-Is can improve cognition in impaired animals, but can also induce a cognitive impairment in healthy animals. There are several explanations that might account for these contradictory findings. First, the cognitive impairment in healthy animals caused by papaverine was the result of a subchronic treatment, which was not found after acute treatment in impaired animals. Secondly, different aspects of cognition were addressed in these studies. In the healthy animals, learning and memory were studied, whereas in the impaired animals information processing and attention were investigated. Thirdly, improving cognition of a healthy individual is not the same as restoring impaired cognition; the underlying processes, and thus the effect of a compound, may differ.

Table 7 Overview of effects of PDE10-Is on cognition. CAR: conditioned avoidance responding; ip: intraperitoneal; iv: intravenous; KO: knock-out; PCP: phenylcyclohexylpiperidine; PPI: prepulse inhibition; sc: subcutaneous

Task (cognitive process, area involved)	Model (species)	Treatment	Results	Reference
Passive avoidance learning (hippocampus)	Impaired PDE10A KO (mouse)	-	Apparent effect, but this could be explained by a locomotor effect	(Siuciak et al. 2006)
	Impaired PDE10A KO (mouse)	-	No effect	(Siuciak et al. 2008b)
Acquisition of conditioned avoidance responding (CAR) (learning, hippocampus)	Impaired PDE10A KO (mouse)	-	PDE10A-/- mice learned the task as well as PDE10A+/+ mice, but needed more training.	(Siuciak et al. 2006)
	Impaired by PDE10A KO; DBA1LacJ background (mouse)	-	KO mice learned the task as well as WT, but needed more training.	(Siuciak et al. 2008b)
	Impaired by PDE10A KO; C57BL/6N background (mouse)	-	KO mice learned needed more training and did not reach performance of WT.	(Siuciak et al. 2008b)
Morris water maze (spatial memory, hippocampus)	Impaired PDE10A KO (mouse)	-	Apparent effect, but this could be explained by a locomotor effect	(Siuciak et al. 2006)
	Unimpaired (mouse)	Chronic treatment of papaverine (0, 5, 10 or 20 mg/kg, sc) daily for 14 days. Then, same treatment either prior of 30 min after testing.	Papaverine (5 mg/kg, after testing) impaired latency and distance. In addition, papaverine (20 mg/kg, 30min before testing and 5 mg/kg 30 min after testing) increased the time spend in the old platform quadrant in reversal learning.	(Hebb et al. 2008)
Auditory gating (anesthetized) (information processing, frontal cortex)	Impaired by D-amphetamine, 1 mg/kg, iv, 5 min before testing (rat)	TP-10 (0, 3 mg/kg); 5 min before testing	TP-10 reversed auditory gating deficit	(Schmidt et al. 2008)
Prepulse inhibition (information processing, frontal cortex)	Unimpaired (mouse)	TP-10 (0, 0.32, 1, 3.2 or 10 mg/kg, sc) 30 min before testing	TP-10 had no effect on PPI or startle response	(Schmidt et al. 2008)

Task (cognitive process, area involved)	Model (species)	Treatment	Results	Reference
	Impaired by MK-801, 0.178 mg/kg, sc, 30 min before testing (mouse)	TP-10 (0, 1, 3.2 or 10 mg/kg, sc) 30 min before testing	TP-10 did not reverse PPI deficit	(Schmidt et al. 2008)
Attention set-shifting task (attention, prefrontal cortex)	Impaired by subchronic PCP treatment, 5 mg/kg, ip, twice a day for 7 days (rat)	Papaverine (0, 3, 10 or 30 mg/kg, ip)	Papaverine attenuated PCP induced deficits at all doses. No effect of papaverine on saline treated rats	(Rodefer et al. 2005)

MECHANISMS OF ACTION

There are several mechanisms of action which could account for the cognition enhancing effects of PDE-Is. First, it has been proposed that these effects could be the result of vasodilatory properties of PDE-Is. Secondly, cognition enhancement could be a consequence of emotional arousal. Finally, positive effects may be due to enhanced second messenger signaling (cAMP and/or cGMP) resulting in facilitated LTP processes. All three mechanisms will be discussed in the next sections.

Blood flow

An increase in blood flow and concomitantly an increase in glucose metabolism might be related to the observed cognitive enhancements after PDE inhibitor treatments as predominantly investigated and observed in rodents. This because PDE-Is increase levels of cAMP and cGMP, and vasodilatation properties can be attributed to both cyclic nucleotides (Dundore et al. 1993; Paterno et al. 1996).

Summarizing the rodent behavioural data with PDE5 inhibition (see Table 5) it appears that zaprinast and sildenafil are optimally effective at an oral dose of approximately 10 and 3 mg/kg, respectively. The effects of both zaprinast and sildenafil on blood pressure, which is negatively related to blood flow, have been sparsely investigated in conscious rodents. Administration of zaprinast does not decrease mean arterial blood pressure at a dose of 2 mg/kg (i.p.) in mice (Patil et al. 2004a) and 10 mg/kg (p.o.) in rats (Prickaerts et al. 1997). Yet, a decrease in blood pressure can be observed with zaprinast after systemic administration (i.v.) of doses higher than 10 mg/kg (Dundore et al. 1993).

One mg/kg sildenafil (i.p.) did not affect mean arterial blood pressure in mice up to six h after administration (Patil et al. 2004a). Yet sildenafil can decrease mean arterial blood pressure up to six h, but an oral dose of at least 10 mg/kg was needed in rats (Rehse et al. 1999). Sildenafil has also been tested directly on cerebral blood flow as measured with laser-Doppler flowmetry, although rats need to be anesthetized for this technique (Zhang et al. 2002). Surprisingly, localized cerebral blood flow was increased after oral administration of 2 mg/kg sildenafil.

Cerebral blood flow and glucose utilization have been investigated in mice with the [13N]ammonia uptake and [3H]2-deoxyglucose uptake technique (Ishikawa et al. 2002). It was found that within 5 min after 3 mg/kg rolipram (i.p.) administration, blood flow and glucose metabolism in the brain were both decreased by approximately 20 and 40%, respectively. At 30 min after administration glucose use was still decreased by 60%. 1 mg/kg rolipram was also tested on central glucose use, which was found to be decreased by 40% at 15 min after administration. Of note, these doses of rolipram are rather high and behaviourally effective doses are in general below 1 mg/kg (i.p.) (see Table 4). Increasing the dose of rolipram above 1 mg/kg will only result in sedation and locomotor depression.

Taken together, the PDE4-Is and PDE5-Is tested in rodents can have peripheral and central vascular and metabolic effects, but these effects occur after treatment with doses that are higher than required for cognition enhancement. Moreover, detailed inspection of the behavioral data already suggests that a uniform cerebrovascular effect is not sufficient to explain the differential effects on cognitive processes. For instance, administration of a cGMP analogue into the hippocampus improved early consolidation, whereas a comparable cAMP analogue had no effect (Bernabeu et al. 1996; Prickaerts et al. 2002a). Along similar lines, sildenafil improved early consolidation, whereas rolipram did not (Rutten et al. 2007b). On the other hand, late consolidation processes are improved by rolipram while sildenafil is ineffective. Once more, these findings indicate that it is not likely that cerebrovascular and metabolic effects explain the cognitive improvements as observed in rodents.

Sildenafil 100 mg has effects on the central nervous system of humans as evident from influenced evoked potential and reaction times (Grass et al. 2001; Schultheiss et al. 2001). The same dose of sildenafil has been shown to increase heart rate and decreased diastolic blood pressure in healthy subjects (Kruuse et al. 2002). However, sildenafil had no effect on bloodflow in the middle cerebral artery, just as there were no changes in radial and temporal artery diameters (Arnavaz et al. 2003; Kruuse et al. 2002). This indicates that effects on cognition after sildenafil administration are not likely to be related to cerebrovascular mechanisms in humans as well.

Emotional arousal

Anecdotic report and case studies describe emotional arousal (anxiety, aggression) in men taking sildenafil (Milman and Arnold 2002). In rats it has been demonstrated that sildenafil (1-3 mg/kg) has an anxiogenic effect (Kurt et al. 2004). Effects on emotion and arousal are likely, since animal studies have shown that central cGMP is involved in sympathetic activation (Krukoff 1998). Concomitantly, anxiolytics including benzodiazepines reduced the stress-induced increase in central cGMP levels (Tang et al. 1997). cAMP levels were reduced as well after benzodiazepines administration, as found in vitro (Niles and Wang 1999); although increases in cAMP have also been observed (Cherry et al. 2001). In line with the latter observation the PDE4-I rolipram (0.1 mg/kg) had an anxiolytic effect in rats (Silvestre et al. 1999). Yet it has to be noted again that the dose of rolipram is still relatively high and decreased locomotor activity might have interfered with the behavioural response. Nevertheless, it is evident that the cyclic nucleotides cAMP and cGMP play a role in arousal and emotional processes. Emotional arousal, to a certain maximum, is necessary for an optimal cognitive performance (Prickaerts and Steckler 2005). Thus, effects of PDE-Is

on cognition can be influenced by or attributed to effects on processes of emotions and arousal.

Long-term potentiation

Hippocampal LTP is the most established cellular model for the neuroplastic mechanisms that underlie learning and memory (Bliss and Collingridge 1993). LTP is described by the increase in the chemical strength of a synapse after tetanus stimulation that lasts for over an hour. Experimentally, a series of short, high frequency electric stimulations to a nerve cell synapse can strengthen, or potentiate, that synapse for several minutes to hours. Glutamate induces LTP via activation of the ionotropic NMDA receptor, after which calcium enters the cell triggering various pre- and postsynaptic changes. The mechanism of LTP and its relationship to learning and memory is quite complicated. It depends on the fine-tuning of various components of the glutamatergic system including ionotropic and metabotropic glutamate receptors, other neurochemical systems, second messengers and signal transduction pathways. Hippocampal LTP can, depending on the induction paradigm, last for less than 3 h or longer. The former is called early-phase LTP (E-LTP) and the later late-phase LTP (L-LTP). It has been suggested that E-LTP (or LTP1) can be transformed into L-LTP (LTP3), probably via an intermediate LTP2 form (Reymann and Frey 2007). Furthermore, it has been assumed that E-LTP is related to short-term memory and L-LTP to long-term memory, respectively (Izquierdo et al. 2002).

In general, both pre- and postsynaptic mechanisms are related to LTP and can involve the second messengers cAMP and cGMP. Figure 1 provides a schematic overview of the cellular processes related to LTP and second messenger signalling. More in detail, a postsynaptic cAMP/PKA/CREB pathway (Impey et al. 1996) and cGMP/PKG/CREB pathway (Lu et al. 1999) are involved in L-LTP. A postsynaptic calmodulin-dependent protein kinase II (CaMKII) pathway (Sweatt 1999) and presynaptic cGMP/PKG pathway (Arancio et al. 1995) have been implicated in E-LTP.

Since PDE-Is influence the levels of the second messengers cAMP and/or cGMP it can be argued that the procognitive effects of PDE-Is are related to the facilitation of LTP. Yet, only a limited number of studies have investigated the effects of PDE-Is on LTP. Most research has been aimed at the effects of PDE4 inhibition on LTP. The PDE4-I rolipram, when applied to hippocampal slices, has been shown to facilitate hippocampal LTP in rats and mice (Ahmed and Frey 2003; 2005; Gong et al. 2004; Navakkode et al. 2004; 2005). In addition, we recently demonstrated that the PDE9-I BAY 73-6691 amplified E-LTP elicited by weak tetanic stimulation in young Wistar rats (van der Staay et al. 2008). These findings are in line with observations of enhanced E-LTP after treatment with the PDE2-I BAY 60-7550 in rats (Boess et al. 2004). Finally, chronic administration (1 mg/kg/day, i.p.), for 15 days, of the PDE5-I sildenafil improved LTP in CA3-CA1 synapses of hippocampal slices in mice (Uthayathas et al. 2007). To our knowledge, no studies have investigated the effects of PDE10 inhibition on LTP. The few existing studies that investigated the effects of PDE-Is on LTP indicate that inhibition of PDEs may have a beneficial effect on synaptic plasticity. Since LTP is considered the underlying mechanism for learning and memory, it is relevant to evaluate the effects of PDE-Is on LTP in addition to and in parallel with behavioral studies.

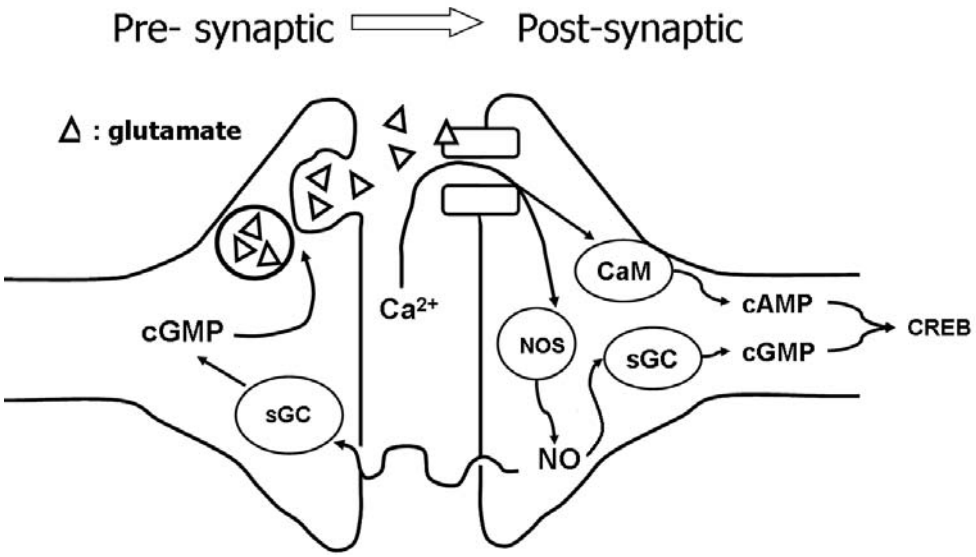

Figure 1 Ca2+ entry through the postsynaptic ionotropic NMDA receptor triggers LTP induction. Ca2+ results in the activation of CaMKII (a specific form of CaMK). Activated CaMKII stimulates the insertion into the membrane of the ionotropic AMPA receptor, which is involved in regular signal transduction through the generation of excitatory postsynaptic potentials (EPSPs). In addition CaMKII activates AC resulting in the production of the second messenger cAMP. The latter activates PKA, which has a positive effect on the transcription factor CREB (via MAP kinases possibly). CREB activation is known to result in an increased gene expression, including the genes for AMPA receptors and thus future signal transduction is enhanced. Ca2+ is also known to activate the enzyme NOS, which produces NO. The latter can activate GC, which produces the second messenger cGMP. There are indications that postsynaptically cGMP has similar effects as cAMP, but via activation of PKG. NO is also known to act as a retrograde messenger and can thus stimulate presynaptic GC. It has been found that cGMP stimulates the synthesis and release of glutamate via PKG

The process opposed to LTP is long-term depression (LTD), which decreases, or depresses, the strength of a synapse for a certain amount of time as a result of either strong or persistent weak stimulation. Several studies have shown that PDE4-Is and PDE5-Is can induce or reinforce LTD in the hippocampus and the striatum among others (Bailey et al. 2003; Calabresi et al. 1999; Navakkode et al. 2005). Recently it has been found that a deficit in LTD can result in memory impairment (Griffiths et al. 2008) which is in line with the theoretical neural-network models that depend on bidirectional synaptic plasticity (LTP and LTD) to mediate learning and memory (Malenka 1994). Accordingly, it is now evident that besides excitatory strengthening mechanisms in LTP, stabilization or suppression mechanisms, e.g. LTD, are also crucial for the regulation of synaptic plasticity (Abel et al. 1998). However, the exact underlying mechanisms remain elusive and the role of PDE-Is in these processes require further investigation.

TIME WINDOWS IN MEMORY PROCESSES

PDE2, PDE4 and PDE9 inhibition improved both short-term memory (STM) and long-term memory (LTM) (see Table 3, 4 and 6). PDE5 inhibition has only been investigated for LTM. Yet, based on the PDE9-I experiments (van der Staay et al. 2008), it might be

expected that PDE5 inhibition will result in STM improvements, though this needs to be confirmed in future studies. Taken together, treatment of rodents with different types of selective PDE-Is, which inhibit the degradation of the second messengers cAMP and/or cGMP, improved their STM as well as LTM. Furthermore, with respect to LTM it appears that for consolidation processes a distinction can be made between early consolidation (< 3 h) and late consolidation (> 3 h) with cGMP being involved in the former and cAMP in the latter (Bernabeu et al. 1996; Izquierdo et al. 2006; Prickaerts et al. 2002a; Rutten et al. 2007b). These findings suggest that different underlying mechanisms should explain consolidation processes. Or in more detail, are different forms of LTP involved in different phases of long-term memory consolidation?

Defining STM as not requiring protein synthesis may implicate that the time window of E-LTP corresponds with the duration of STM (1-3 h) , the definition often used in animal research (Izquierdo et al. 2002). Pre-synaptic cGMP is involved in E-LTP (LTP1) (Arancio et al. 1995), but cAMP is probably not (Nguyen and Woo 2003). Thus, it can be argued that rolipram should not improve STM. However, we found that rolipram can improve STM (Rutten et al. 2006). This effect might be explained by a general enhancement of synaptic transmission by increasing neurotransmitter availability, as rolipram has been found to activate the cognition-related cholinergic (Imanishi et al. 1997), but also noradrenergic and dopaminergic neurotransmitter systems (Schoffelmeer et al. 1985).

L-LTP (LTP2 and LTP3) is dependent on protein synthesis and last longer than 3 h (Reymann and Frey 2007). It can be assumed that L-LTP is related to LTM. Figure 2 illustrates the inter relationship between STM and LTM, with intermediate memory (IM) in between STM and LTM. It might be speculated that LTP2 is representing early consolidation/IM and LTP3 represents late consolidation/LTM. These questions clearly warrant further investigations.

E-LTP can be converted into L-LTP (Pang et al. 2004). This is in line with the idea that information in the STM can be transferred into LTM (Baddeley 2003). As pre-synaptic cGMP plays a role in E-LTP, theoretically, inhibition of cGMP degradation with for instance a PDE9-I should therefore be able to influence L-LTP/LTM via E-LTP/STM as well. But cGMP as well as cAMP are involved in post-synaptic L-LTP processes resulting in phosphorylation of the transcription factor CREB eventually. However, as described above, both cyclic nucleotides have different effects on consolidation processes. This implies that the signal transduction pathways are far more complex than known thus far. It seems likely that additional modulators are involved in regulating and mediating the timed effect of the second messengers cGMP and cAMP on memory processes.

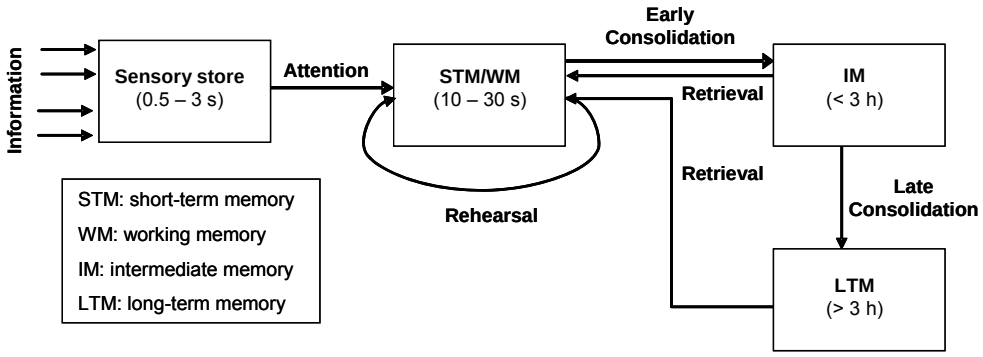

Figure 2 A schematic classification of memory identifying four distinct types of memory: sensory store, short-term memory (STM)/working memory (WM), intermediate memory (IM) and long-term memory (LTM). In the sensory store, all the incoming information from the sensory organs is accumulated and depending on attention processes certain items can be transferred to STM/WM (Baddeley 2003). These stores generally have limited capacity and duration. Information can be stored for a longer period of time ranging from hours to years. It is suggested that there are two stages involved, that is IM and LTM (Kesner and Hopkins 2006). The time frames of the three stages of STM, IM and LTM are not clearly defined and depend on the definitions used by the researcher. Especially between animal and human research the definitions of the time windows tend to vary a lot (from seconds to hours). In addition, the exact role of different brain areas in this respect is not fully clear yet. But it is evident that the hippocampus plays a key role and is particularly involved in intermediate memory processes (Kesner and Hopkins 2006). We propose that information is processed from STM to IM via early consolidation and subsequently from IM to LTM via late consolidation. In addition, we assume that STM is supported by transient changes in neuronal transmission, not requiring gene expression and protein synthesis whereas IM and LTM are maintained by more stable and permanent neuronal changes that are dependent on protein synthesis (Izquierdo et al. 2002). cGMP-specific PDE-Is might be able to influence STM via enhanced LTP1. In addition, cAMP-specific PDE-Is influence STM probably via an increased neurotransmitter release directly. Furthermore, LTP2 might represent IM and should be specifically influenced by cGMP-specific PDE inhibition. Finally, LTM is likely represented by LTP3 which should be influenced by cAMP-specific PDE inhibition

Targetting cognitive functioning

The application of PDE-Is in studies of animal cognition enhancement has been fruitful and these studies have extended our fundamental knowledge about the possible underlying cellular and molecular mechanisms of learning and memory and other cognitive functions. However, to predict which classes of PDE-Is are possibly the most effective cognition enhancers, in either preclinical or clinical studies, depends on various factors.

First, it is important to know the exact localization of specific PDE enzymes in the normal brain (see also Table 1). The localization of the enzymes might predict that certain cognitive functions that are primarily located in specific brain structures may be enhanced by some PDE-Is, but not by others. For example PDE10 is predominantly expressed in striatal areas (Schmidt et al. 2008) and is therefore a target for schizophrenia. In contrast, PDE4 is highly expressed in the hippocampus and cortex (Perez-Torres et al. 2000) and is therefore considered a better target for cognition enhancement. Of note, the development of a specific antibody against a selective PDE, preferentially of the level of isoform type, will more specifically target a PDE for a certain cognitive function (Fujita et al. 2007).

Secondly, it must be taken into account that the constitution of the brain changes with age and the distribution of PDEs can be modified by the aging process As a consequence, a PDE-I can improve cognition in young subjects, but impair cognition in old subjects. Likewise, Ramos et al. (2003) demonstrated that rolipram had a positive effect on prefrontal cortex-dependent working memory in young rhesus monkeys, but negative effect on working memory in aged rhesus monkeys. However, rolipram improved performance in the passive avoidance task, a test of hippocampus-dependent memory, in both young and aged mice (Barad et al. 1998). With advancing age, opposite profiles between the function of PKA in the hippocampus and prefrontal cortex were suggested to explain the results of Ramos et al. (2003); i.e. the prefrontal cortex showed indices of increased PKA activity, while the hippocampus exhibited evidence of decreased PKA activity (Ramos et al. 2003). In addition, is has been shown that expression of PDE5 is strongly reduced in brains of Alzheimer's disease patients (Reyes-Irisarri et al. 2007). However, PDE2 and PDE9 do not show this Alzheimer related reduction in expression patterns, but show the same distribution as in healthy age-matched controls (Reyes-Irisarri et al. 2007). Along similar lines, PDE5 inhibition did not improve object memory in aged rats (Domek-Lopacinska and Strosznajder 2008). Consequently, when developing a PDE-I for treatment of the cognitive decline resulting from Alzheimer's disease, PDE2-Is and PDE9-Is may be a better target in this population than PDE5-Is.

Thirdly, since most PDEs are transcribed by several genes, which give rise to multiple PDE splice variants and isoforms, further investigation into possible isoform-specific effects of PDE-Is are a field of great interest. For example, four isoforms of PDE4 mRNA have been found; PDE4A, PDE4B, PDE4C and PDE4D. Indirect evidence suggests that PDE4A and PDE4B are involved in signaling pathways related to affective (Ye et al. 2000) and memory (Ahmed and Frey 2003) processes, respectively. Recently, the antidepressant potential of PDE4A in the hippocampus has been found to be related to specific splice variants of this PDE4 isoform (D'Sa et al. 2005). The same probably holds for PDE4B and memory (Ahmed and Frey 2005) or schizophrenia (Siuciak et al. 2008a). PDE4D KO mice have already been generated and these animals display both an antidepressant and pro-cognitive profile (Zhang et al. 2002). Furthermore, it has been observed, that the expression of the majority of PDE4D isoforms (1-9) was reduced in the hippocampus of patients with Alzheimer's disease compared to healthy adults. Interestingly, PDE4D1 and PDE4D2 were increased in the brains Alzheimer's patients (McLachlan et al. 2007). These findings underscore the relevance of further investigations into the role of isoform specific PDEs in cognition enhancement.

Furthermore, the most widely used PDE4-I in behavioral studies, rolipram, produces severe dose-limiting emetic side effects including headache, gastric hyper secretion and severe emesis (e.g. nausea) in humans (Zhu et al. 2001). Novel PDE4-Is are thought to produce less emetic side effects, but thus far no human cognition studies have been reported using these second generation PDE4-Is. Thus far, only PDE5-Is can be prescribed to humans. However, particularly cardiovascular effects limit their usefulness as a general treatment for cognitive disorders, since patients with cardiovascular indications cannot be included. In addition, central effects including visual disturbances and headache, limits the use of PDE5-I such as sildenafil (Kruuse et al. 2002). Especially chronic treatment with

these drugs could be disadvantageous. Again, an isoform specific PDE-I could circumvent the above mentioned side effects.

FUTURE DIRECTIONS

In this review we summarized all recent available literature of the cognition enhancing effects of PDE-Is in preclinical studies. It has been shown that inhibitors of PDE2, PDE4, PDE5, PDE9 and PDE10 improve a wide range of cognitive processes, including information processing, attention, learning, executive functioning and response inhibition, in various behavioral models within different species. We argue that it is unlikely that blood flow is the mechanism underlying these procognitive effects. We feel that LTP appears to be a better substrate for the cognition enhancing properties of PDE-Is.

Despite accumulating evidence for the procognitive effects of PDE-Is, further investigation is still required. First, more localization studies are required to obtain more knowledge about the localization of the specific PDE isoforms in different brain areas. In addition, the exact underlying working mechanisms of selective PDE-I have to be investigated by using central administration paradigms, blood flow measurements and parallel LTP experiments. Clearly, it is crucial to translate the procognitive findings in animals to human subjects. Since PDE5-I are already clinically accepted for the treatment of erectile dysfunction, these drugs can be readily tested in human subjects. Besides neuropsychological tasks to address cognitive functioning, imaging studies (EEG and fMRI) are necessary elucidate the central mechanisms underlying the cognition enhancing effects of PDE inhibition.

Taken together, PDE-Is offer a promising target for cognitive enhancement. Yet, the future for cognition enhancing PDE-Is lies in the development of isoform specific PDE-Is, that are present in the aged or Alzheimer diseased brain, and that have limited aversive side effect profiles within the effective dose range for cognition enhancement. Suitable candidates appear to be PDE2-Is or PDE9-Is, although little is known about their side effect profiles and isoform specificity.

ACKNOWLEGDEMENTS

J.P is supported by the EU Framework 6 Integrated Project NEWMOOD (LSHM-CT-2004-503474). In addition, the authors would like to thank Anke Sambeth for her useful comments.

REFERENCES

Abel T, Martin KC, Bartsch D, Kandel ER (1998) Memory suppressor genes: inhibitory constraints on the storage of long-term memory. *Science* 279: 338-41

Ahmed T, Frey JU (2003) Expression of the specific type IV phosphodiesterase gene PDE4B3 during different phases of long-term potentiation in single hippocampal slices of rats in vitro. *Neuroscience* 117: 627-38

Ahmed T, Frey JU (2005) Phosphodiesterase 4B (PDE4B) and cAMP-level regulation within different tissue fractions of rat hippocampal slices during long-term potentiation in vitro. *Brain Res* 1041: 212-22

Andreeva SG, Dikkes P, Epstein PM, Rosenberg PA (2001) Expression of cGMP-specific phosphodiesterase 9A mRNA in the rat brain. *J Neurosci* 21: 9068-76

Arancio O, Kandel ER, Hawkins RD (1995) Activity-dependent long-term enhancement of transmitter release by presynaptic 3',5'-cyclic GMP in cultured hippocampal neurons. *Nature* 376: 74-80

Arnavaz A, Aurich A, Weissenborn K, Hartmann U, Emrich HM, Schneider U (2003) Effect of sildenafil (Viagra) on cerebral blood flow velocity: a pilot study. *Psychiatry Res* 122: 207-9

Bach ME, Barad M, Son H, Zhuo M, Lu YF, Shih R, Mansuy I, Hawkins RD, Kandel ER (1999) Age-related defects in spatial memory are correlated with defects in the late phase of hippocampal long-term potentiation in vitro and are attenuated by drugs that enhance the cAMP signaling pathway. *Proc Natl Acad Sci USA* 96: 5280-5

Baddeley A (2003) Working memory: looking back and looking forward. *Nat Rev Neurosci* 4: 829-39

Bailey CP, Trejos JA, Schanne FA, Stanton PK (2003) Pairing elevation of [cyclic GMP] with inhibition of PKA produces long-term depression of glutamate release from isolated rat hippocampal presynaptic terminals. *Eur J Neurosci* 17: 903-8

Barad M, Bourtchouladze R, Winder DG, Golan H, Kandel E (1998) Rolipram, a type IV-specific phosphodiesterase inhibitor, facilitates the establishment of long-lasting long-term potentiation and improves memory. *Proc Natl Acad Sci USA* 95: 15020-5

Baratti CM, Boccia MM (1999) Effects of sildenafil on long-term retention of an inhibitory avoidance response in mice. *Behav Pharmacol* 10: 731-7

Bender AT, Beavo JA (2006) Cyclic nucleotide phosphodiesterases: molecular regulation to clinical use. *Pharmacol Rev* 58: 488-520

Bernabeu R, Schmitz P, Faillace MP, Izquierdo I, Medina JH (1996) Hippocampal cGMP and cAMP are differentially involved in memory processing of inhibitory avoidance learning. *Neuroreport* 7: 585-8

Best JD, Berghmans S, Hunt JJ, Clarke SC, Fleming A, Goldsmith P, Roach AG (2008) Non-associative learning in larval zebrafish. *Neuropsychopharmacology* 33: 1206-15

Billingsley ML, Polli JW, Balaban CD, Kincaid RL (1990) Developmental expression of calmodulin-dependent cyclic nucleotide phosphodiesterase in rat brain. *Brain Res Dev Brain Res* 53: 253-63

Blaney PH (1986) Affect and memory: a review. *Psychol Bull* 99: 229-46

Bliss TV, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 361: 31-9

Blokland A, Schreiber R, Prickaerts J (2006) Improving memory: a role for phosphodiesterases. *Curr Pharm Des* 12: 2511-23

Boess FG, Hendrix M, van der Staay FJ, Erb C, Schreiber R, van Staveren W, de Vente J, Prickaerts J, Blokland A, Koenig G (2004) Inhibition of phosphodiesterase 2 increases neuronal cGMP, synaptic plasticity and memory performance. *Neuropharmacology* 47: 1081-92

Bolger GB, Rodgers L, Riggs M (1994) Differential CNS expression of alternative mRNA isoforms of the mammalian genes encoding cAMP-specific phosphodiesterases. *Gene* 149: 237-44

Bourtchouladze R, Lidge R, Catapano R, Stanley J, Gossweiler S, Romashko D, Scott R, Tully T (2003) A mouse model of Rubinstein-Taybi syndrome: defective long-term memory is ameliorated by inhibitors of phosphodiesterase 4. *Proc Natl Acad Sci USA* 100: 10518-22

Braun NN, Reutiman TJ, Lee S, Folsom TD, Fatemi SH (2007) Expression of phosphodiesterase 4 is altered in the brains of subjects with autism. *Neuroreport* 18: 1841-4

Cahill L, Prins B, Weber M, McGaugh JL (1994) Beta-adrenergic activation and memory for emotional events. *Nature* 371: 702-4

Calabresi P, Gubellini P, Centonze D, Sancesario G, Morello M, Giorgi M, Pisani A, Bernardi G (1999) A critical role of the nitric oxide/cGMP pathway in corticostriatal long-term depression. *J Neurosci* 19: 2489-99

Campbell E, Edwards T (2006) Zaprinast consolidates long-term memory when administered to neonate chicks trained using a weakly reinforced single trial passive avoidance task. *Behav Brain Res* 169: 181-5

Cherry JA, Davis RL (1999) Cyclic AMP phosphodiesterases are localized in regions of the mouse brain associated with reinforcement, movement, and affect. *J Comp Neurol* 407: 287-301

Cherry JA, Thompson BE, Pho V (2001) Diazepam and rolipram differentially inhibit cyclic AMP-specific phosphodiesterases PDE4A1 and PDE4B3 in the mouse. *Biochim Biophys Acta* 1518: 27-35

Cho CH, Cho DH, Seo MR, Juhnn YS (2000) Differential changes in the expression of cyclic nucleotide phosphodiesterase isoforms in rat brains by chronic treatment with electroconvulsive shock. *Exp Mol Med* 32: 110-4

Comery TA, Martone RL, Aschmies S, Atchison KP, Diamantidis G, Gong X, Zhou H, Kreft AF, Pangalos MN, Sonnenberg-Reines J, Jacobsen JS, Marquis KL (2005) Acute gamma-secretase inhibition improves contextual fear conditioning in the Tg2576 mouse model of Alzheimer's disease. *J Neurosci* 25: 8898-902

Costa DA, Cracchiolo JR, Bachstetter AD, Hughes TF, Bales KR, Paul SM, Mervis RF, Arendash GW, Potter H (2007) Enrichment improves cognition in AD mice by amyloid-related and unrelated mechanisms. *Neurobiol Aging* 28: 831-44

D'Sa C, Eisch AJ, Bolger GB, Duman RS (2005) Differential expression and regulation of the cAMP-selective phosphodiesterase type 4A splice variants in rat brain by chronic antidepressant administration. *Eur J Neurosci* 22: 1463-75

Davis HP, Squire LR (1984) Protein synthesis and memory: a review. *Psychol Bull* 96: 518-59

Dent G, White SR, Tenor H, Bodtke K, Schudt C, Leff AR, Magnussen H, Rabe KF (1998) Cyclic nucleotide phosphodiesterase in human bronchial epithelial cells: characterization of isoenzymes and functional effects of PDE inhibitors. *Pulm Pharmacol Ther* 11: 47-56

Devan BD, Bowker JL, Duffy KB, Bharati IS, Jimenez M, Sierra-Mercado D, Jr., Nelson CM, Spangler EL, Ingram DK (2006) Phosphodiesterase inhibition by sildenafil citrate attenuates a maze learning impairment in rats induced by nitric oxide synthase inhibition. *Psychopharmacology* (Berl) 183: 439-45

Devan BD, Pistell PJ, Daffin LW, Jr., Nelson CM, Duffy KB, Bowker JL, Bharati IS, Sierra-Mercado D, Spangler EL, Ingram DK (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: 134-40

Devan BD, Sierra-Mercado D, Jr., Jimenez M, Bowker JL, Duffy KB, Spangler EL, Ingram DK (2004) Phosphodiesterase inhibition by sildenafil citrate attenuates the learning impairment induced by blockade of cholinergic muscarinic receptors in rats. *Pharmacol Biochem Behav* 79: 691-9

DeZazzo J, Tully T (1995) Dissection of memory formation: from behavioral pharmacology to molecular genetics. *Trends Neurosci* 18: 212-8

Domek-Lopacinska K, 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

Dundore RL, Clas DM, Wheeler LT, Habeeb PG, Bode DC, Buchholz RA, Silver PJ, Pagani ED (1993) Zaprinast increases cyclic GMP levels in plasma and in aortic tissue of rats. *Eur J Pharmacol* 249: 293-7

Edwards TM, Lindley N (2007) Phosphodiesterase type 5 inhibition coupled to strong reinforcement results in two periods of transient retention loss in the young chick. *Behav Brain Res* 183: 231-5

Egawa T, Mishima K, Matsumoto Y, Iwasaki K, Fujiwara M (1997) Rolipram and its optical isomers, phosphodiesterase 4 inhibitors, attenuated the scopolamine-induced impairments of learning and memory in rats. *Jpn J Pharmacol* 75: 275-81

Erceg S, Monfort P, Cauli O, Montoliu C, Llansola M, Piedrafita B, Felipo V (2006) Role of extracellular cGMP and of hyperammonemia in the impairment of learning in rats with chronic hepatic failure. Therapeutic implications. *Neurochem Int* 48: 441-6

Erceg S, Monfort P, Hernandez-Viadel M, Llansola M, Montoliu C, Felipo V (2005a) Restoration of learning ability in hyperammonemic rats by increasing extracellular cGMP in brain. *Brain Res* 1036: 115-21

Erceg S, Monfort P, Hernandez-Viadel M, Rodrigo R, Montoliu C, Felipo V (2005b) Oral administration of sildenafil restores learning ability in rats with hyperammonemia and with portacaval shunts. *Hepatology* 41: 299-306

Fawcett L, Baxendale R, Stacey P, McGrouther C, Harrow I, Soderling S, Hetman J, Beavo JA, Phillips SC (2000) Molecular cloning and characterization of a distinct human phosphodiesterase gene family: PDE11A. *Proc Natl Acad Sci USA* 97: 3702-7

Fisher DA, Smith JF, Pillar JS, St Denis SH, Cheng JB (1998a) Isolation and characterization of PDE8A, a novel human cAMP-specific phosphodiesterase. *Biochem Biophys Res Commun* 246: 570-7

Fisher DA, Smith JF, Pillar JS, St Denis SH, Cheng JB (1998b) Isolation and characterization of PDE9A, a novel human cGMP-specific phosphodiesterase. *J Biol Chem* 273: 15559-64

Frith C (1996) Neuropsychology of schizophrenia, what are the implications of intellectual and experiential abnormalities for the neurobiology of schizophrenia? *Br Med Bull* 52: 618-26

Fujishige K, Kotera J, Michibata H, Yuasa K, Takebayashi S, Okumura K, Omori K (1999) Cloning and characterization of a novel human phosphodiesterase that hydrolyzes both cAMP and cGMP (PDE10A). *J Biol Chem* 274: 18438-45

Fujita M, Imaizumi M, D'Sa C, Zoghbi SS, Crescenzo MS, Hong J, Musachio JL, Gee AD, Seidel J, Green MV, Pike VW, Duman RS, Innis RB (2007) In vivo and in vitro measurement of brain phosphodiesterase 4 in rats after antidepressant administration. *Synapse* 61: 78-86

Gamanuma M, Yuasa K, Sasaki T, Sakurai N, Kotera J, Omori K (2003) Comparison of enzymatic characterization and gene organization of cyclic nucleotide phosphodiesterase 8 family in humans. *Cell Signal* 15: 565-74

Ghelardini C, Galeotti N, Gualtieri F, Romanelli MN, Bucherelli C, Baldi E, Bartolini A (2002) DM235 (sunifiram): a novel nootropic with potential as a cognitive enhancer. *Naunyn Schmiedebergs Arch Pharmacol* 365: 419-26

Giordano D, De Stefano ME, Citro G, Modica A, Giorgi M (2001) Expression of cGMP-binding cGMP-specific phosphodiesterase (PDE5) in mouse tissues and cell lines using an antibody against the enzyme amino-terminal domain. *Biochim Biophys Acta* 1539: 16-27

Gong B, Vitolo OV, Trinchese F, Liu S, Shelanski M, Arancio O (2004) Persistent improvement in synaptic and cognitive functions in an Alzheimer mouse model after rolipram treatment. *J Clin Invest* 114: 1624-34

Grass H, Klotz T, Fathian-Sabet B, Berghaus G, Engelmann U, Kaferstein H (2001) Sildenafil (Viagra): is there an influence on psychological performance? *Int Urol Nephrol* 32: 409-12

Griffiths S, Scott H, Glover C, Bienemann A, Ghorbel MT, Uney J, Brown MW, Warburton EC, Bashir ZI (2008) Expression of Long-Term Depression Underlies Visual Recognition Memory. *Neuron* 58: 186-94

Halene TB, Siegel SJ (2008) Antipsychotic-like properties of PDE4 inhibitors - Evaluation of RO-20-1724 with auditory event related potentials and prepulse inhibition of startle. *J Pharmacol Exp Ther* 326: 230-9

Hayashi M, Matsushima K, Ohashi H, Tsunoda H, Murase S, Kawarada Y, Tanaka T (1998) Molecular cloning and characterization of human PDE8B, a novel thyroid-specific isozyme of 3',5'-cyclic nucleotide phosphodiesterase. *Biochem Biophys Res Commun* 250: 751-6

Hayashi M, Shimada Y, Nishimura Y, Hama T, Tanaka T (2002) Genomic organization, chromosomal localization, and alternative splicing of the human phosphodiesterase 8B gene. *Biochem Biophys Res Commun* 297: 1253-8

Hebb AL, Robertson HA, Denovan-Wright EM (2008) Phosphodiesterase 10A inhibition is associated with locomotor and cognitive deficits and increased anxiety in mice. *Eur Neuropsychopharmacol* 18: 339-63

Hetman JM, Soderling SH, Glavas NA, Beavo JA (2000) Cloning and characterization of PDE7B, a cAMP-specific phosphodiesterase. *Proc Natl Acad Sci USA* 97: 472-6

Holthues H, Vollrath L (2004) The phototransduction cascade in the isolated chick pineal gland revisited. *Brain Res* 999: 175-80

Hotston M, Jeremy JY, Bloor J, Greaves NS, Persad R, Angelini G, Shukla N (2007) Homocysteine and copper interact to promote type 5 phosphodiesterase expression in rabbit cavernosal smooth muscle cells: mediation by superoxide and inhibition with sildenafil. *Asian J Androl* 10: 905-13

Huang Z, Dias R, Jones T, Liu S, Styhler A, Claveau D, Otu F, Ng K, Laliberte F, Zhang L, Goetghebeur P, Abraham WM, Macdonald D, Dube D, Gallant M, Lacombe P, Girard Y, Young RN, Turner MJ, Nicholson DW, Mancini JA (2007) L-454,560, a potent and selective PDE4 inhibitor with in vivo efficacy in animal models of asthma and cognition. *Biochem Pharmacol* 73: 1971-81

Imanishi T, Sawa A, Ichimaru Y, Miyashiro M, Kato S, Yamamoto T, Ueki S (1997) Ameliorating effects of rolipram on experimentally induced impairments of learning and memory in rodents. *Eur J Pharmacol* 321: 273-8

Impey S, Mark M, Villacres EC, Poser S, Chavkin C, Storm DR (1996) Induction of CRE-mediated gene expression by stimuli that generate long-lasting LTP in area CA1 of the hippocampus. *Neuron* 16: 973-82

Ishikawa M, Hosoi R, Kobayashi K, Nishimura T, Inoue O (2002) Rolipram depresses [(3)H]2-deoxyglucose uptake in mouse brain and heart in vivo. *Eur J Nucl Med Mol Imaging* 29: 1212-5

Ito M, Nishikawa M, Fujioka M, Miyahara M, Isaka N, Shiku H, Nakano T (1996) Characterization of the isoenzymes of cyclic nucleotide phosphodiesterase in human platelets and the effects of E4021. *Cell Signal* 8: 575-81

Izquierdo I, Barros DM, Mello e Souza T, de Souza MM, Izquierdo LA, Medina JH (1998) Mechanisms for memory types differ. *Nature* 393: 635-6

Izquierdo I, Bevilaqua LR, Rossato JI, Bonini JS, Medina JH, Cammarota M (2006) Different molecular cascades in different sites of the brain control memory consolidation. *Trends Neurosci* 29: 496-505

Izquierdo LA, Barros DM, Vianna MR, Coitinho A, deDavid e Silva T, Choi H, Moletta B, Medina JH, Izquierdo I (2002) Molecular pharmacological dissection of short- and long-term memory. *Cell Mol Neurobiol* 22: 269-87

Kanes SJ, Tokarczyk J, Siegel SJ, Bilker W, Abel T, Kelly MP (2007) Rolipram: a specific phosphodiesterase 4 inhibitor with potential antipsychotic activity. *Neuroscience* 144: 239-46

Kesner RP, Hopkins RO (2006) Mnemonic functions of the hippocampus: a comparison between animals and humans. *Biol Psychol* 73: 3-18

Kobayashi T, Gamanuma M, Sasaki T, Yamashita Y, Yuasa K, Kotera J, Omori K (2003) Molecular comparison of rat cyclic nucleotide phosphodiesterase 8 family: unique expression of PDE8B in rat brain. *Gene* 319: 21-31

Kotera J, Fujishige K, Omori K (2000) Immunohistochemical localization of cGMP-binding cGMP-specific phosphodiesterase (PDE5) in rat tissues. *J Histochem Cytochem* 48: 685-93

Krukoff TL (1998) Central regulation of autonomic function: no brakes? *Clin Exp Pharmacol Physiol* 25: 474-8

Kruuse C, Thomsen LL, Jacobsen TB, Olesen J (2002) The phosphodiesterase 5 inhibitor sildenafil has no effect on cerebral blood flow or blood velocity, but nevertheless induces headache in healthy subjects. *J Cereb Blood Flow Metab* 22: 1124-31

Kurt M, Bilge SS, Aksoz E, Kukula O, Celik S, Kesim Y (2004) Effect of sildenafil on anxiety in the plus-maze test in mice. *Pol J Pharmacol* 56: 353-7

Lal S, Sharma RK, McGregor C, Macaulay RJ (1999) Immunohistochemical localization of calmodulin-dependent cyclic phosphodiesterase in the human brain. *Neurochem Res* 24: 43-9

Loughney K, Snyder PB, Uher L, Rosman GJ, Ferguson K, Florio VA (1999) Isolation and characterization of PDE10A, a novel human 3', 5'-cyclic nucleotide phosphodiesterase. *Gene* 234: 109-17

Lu YF, Kandel ER, Hawkins RD (1999) Nitric oxide signaling contributes to late-phase LTP and CREB phosphorylation in the hippocampus. *J Neurosci* 19: 10250-61

Malenka RC (1994) Synaptic plasticity in the hippocampus: LTP and LTD. *Cell* 78: 535-8

Martins TJ, Mumby MC, Beavo JA (1982) Purification and characterization of a cyclic GMP-stimulated cyclic nucleotide phosphodiesterase from bovine tissues. *J Biol Chem* 257: 1973-9

Mattson MP, Chan SL, Duan W (2002) Modification of brain aging and neurodegenerative disorders by genes, diet, and behavior. *Physiol Rev* 82: 637-72

McGaugh JL (1989) Dissociating learning and performance: drug and hormone enhancement of memory storage. *Brain Res Bull* 23: 339-45

McLachlan CS, Chen ML, Lynex CN, Goh DL, Brenner S, Tay SK (2007) Changes in PDE4D isoforms in the hippocampus of a patient with advanced Alzheimer disease. *Arch Neurol* 64: 456-7

Merck & Co. I (2006) Randomized, double blind, placebo controlled treatment study. MK-0952 is a phosphodiesterase type IV (PDE4) inhibitor in development for improvement of cognitive impairment in mild-tomoderate. Clinicaltrials.gov

Alzheimer's disease. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US): Available from: http://clinicaltrials.gov/show/NCT00362024 NLM Identifier: NCT

Messier C (2004) Glucose improvement of memory: a review. *Eur J Pharmacol* 490: 33-57

Milman HA, Arnold SB (2002) Neurologic, psychological, and aggressive disturbances with sildenafil. *Ann Pharmacother* 36: 1129-34

Miro X, Perez-Torres S, Palacios JM, Puigdomenech P, Mengod G (2001) Differential distribution of cAMP-specific phosphodiesterase 7A mRNA in rat brain and peripheral organs. *Synapse* 40: 201-14

Monti B, Berteotti C, Contestabile A (2006) Subchronic Rolipram Delivery Activates Hippocampal CREB and Arc, Enhances Retention and Slows Down Extinction of Conditioned Fear. *Neuropsychopharmacology* 31: 278-86

Morin F, Lugnier C, Kameni J, Voisin P (2001) Expression and role of phosphodiesterase 6 in the chicken pineal gland. *J Neurochem* 78: 88-99

Murad F, Mittal CK, Arnold WP, Katsuki S, Kimura H (1978) Guanylate cyclase: activation by azide, nitro compounds, nitric oxide, and hydroxyl radical and inhibition by hemoglobin and myoglobin. *Adv Cyclic Nucleotide Res* 9: 145-58

Nagakura A, Niimura M, Takeo S (2002) Effects of a phosphodiesterase IV inhibitor rolipram on microsphere embolism-induced defects in memory function and cerebral cyclic AMP signal transduction system in rats. *Br J Pharmacol* 135: 1783-93

Navakkode S, Sajikumar S, Frey JU (2004) The type IV-specific phosphodiesterase inhibitor rolipram and its effect on hippocampal long-term potentiation and synaptic tagging. *J Neurosci* 24: 7740-4

Navakkode S, Sajikumar S, Frey JU (2005) Mitogen-activated protein kinase-mediated reinforcement of hippocampal early long-term depression by the type IV-specific phosphodiesterase inhibitor rolipram and its effect on synaptic tagging. *J Neurosci* 25: 10664-70

Nguyen PV, Woo NH (2003) Regulation of hippocampal synaptic plasticity by cyclic AMP-dependent protein kinases. *Prog Neurobiol* 71: 401-37

Niles LP, Wang J (1999) Diazepam inhibits forskolin-stimulated adenylyl cyclase activity in human tumour cells. *Pharmacol Toxicol* 85: 153-6

Pang PT, Teng HK, Zaitsev E, Woo NT, Sakata K, Zhen S, Teng KK, Yung WH, Hempstead BL, Lu B (2004) Cleavage of proBDNF by tPA/plasmin is essential for long-term hippocampal plasticity. *Science* 306: 487-91

Parrott A, Morinan A, Moss M, Scholey A (2004) Understanding drugs and behaviour. *Wiley*, Wiley

Paterno R, Faraci FM, Heistad DD (1996) Role of Ca(2+)-dependent K+ channels in cerebral vasodilatation induced by increases in cyclic GMP and cyclic AMP in the rat. *Stroke* 27: 1603-8

Patil CS, Jain NK, Singh VP, Kulkarni SK (2004a) Differential Effect of the PDE5 Inhibitors, Sildenafil and Zaprinast, in Aging- and Lippolysaccharide-Induced Cognitive Dysfunction in Mice. *Drug Dev Res* 63: 66-75

Patil CS, Singh VP, Kulkarni SK (2006) Modulatory effect of sildenafil in diabetes and electroconvulsive shock-induced cognitive dysfunction in rats. *Pharmacol Rep* 58: 373-80

Patil CS, Singh VP, Singh S, Kulkarni SK (2004b) Modulatory Effect of the PDE-5 Inhibitor Sildenafil in Diabetic Neuropathy. *Pharmacology* 72: 192-5

Perez-Torres S, Cortes R, Tolnay M, Probst A, Palacios JM, Mengod G (2003) Alterations on phosphodiesterase type 7 and 8 isozyme mRNA expression in Alzheimer's disease brains examined by in situ hybridization. *Exp Neurol* 182: 322-34

Perez-Torres S, Miro X, Palacios JM, Cortes R, Puigdomenech P, Mengod G (2000) Phosphodiesterase type 4 isozymes expression in human brain examined by in situ hybridization histochemistry and[3H]rolipram binding autoradiography. Comparison with monkey and rat brain. *J Chem Neuroanat* 20: 349-74

Polli JW, Kincaid RL (1994) Expression of a calmodulin-dependent phosphodiesterase isoform (PDE1B1) correlates with brain regions having extensive dopaminergic innervation. *J Neurosci* 14: 1251-61

Prickaerts J, de Vente J, Honig W, Steinbusch HW, Blokland A (2002a) cGMP, but not cAMP, in rat hippocampus is involved in early stages of object memory consolidation. *Eur J Pharmacol* 436: 83-7

Prickaerts J, Sik A, van der Staay FJ, de Vente J, Blokland A (2005) Dissociable effects of acetylcholinesterase inhibitors and phosphodiesterase type 5 inhibitors on object recognition memory: acquisition versus consolidation. *Psychopharmacology (Berl)* 177: 381-90

Prickaerts J, Sik A, van Staveren WC, Koopmans G, Steinbusch HW, van der Staay FJ, de Vente J, Blokland A (2004) Phosphodiesterase type 5 inhibition improves early memory consolidation of object information. *Neurochem Int* 45: 915-28

Prickaerts J, Steckler T (2005) Effects of glucocorticoids on emotion and memory processes in animals. In: Steckler T, Kalin N, Reul JMHM (eds) Handbook of Stress and the Brain, Part 1: The Neurobiology of Stress. Elsevier, Amsterdam, pp 359-86

Prickaerts J, Steinbusch HW, Smits JF, de Vente J (1997) Possible role of nitric oxide-cyclic GMP pathway in object recognition memory: effects of 7-nitroindazole and zaprinast. *Eur J Pharmacol* 337: 125-36

Prickaerts J, van Staveren WC, Sik A, Markerink-van Ittersum M, Niewohner U, van der Staay FJ, Blokland A, de Vente J (2002b) 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: 351-61

Ramos BP, Birnbaum SG, Lindenmayer I, Newton SS, Duman RS, Arnsten AF (2003) Dysregulation of protein kinase a signaling in the aged prefrontal cortex: new strategy for treating age-related cognitive decline. *Neuron* 40: 835-45

Ramos BP, Stark D, Verduzco L, van Dyck CH, Arnsten AF (2006) Alpha2A-adrenoceptor stimulation improves prefrontal cortical regulation of behavior through inhibition of cAMP signaling in aging animals. *Learn Mem* 13: 770-6

Randt CT, Judge ME, Bonnet KA, Quartermain D (1982) Brain cyclic AMP and memory in mice. *Pharmacol Biochem Behav* 17: 677-80

Reed TM, Browning JE, Blough RI, Vorhees CV, Repaske DR (1998) Genomic structure and chromosome location of the murine PDE1B phosphodiesterase gene. *Mamm Genome* 9: 571-6

Rehse K, Scheffler H, Reitner N (1999) Interaction of Viagra with the NO donors molsidomine and RE 2047 with regard to antithrombotic and blood pressure lowering activities. *Arch Pharm (Weinheim)* 332: 182-4

Reinhardt RR, Chin E, Zhou J, Taira M, Murata T, Manganiello VC, Bondy CA (1995) Distinctive anatomical patterns of gene expression for cGMP-inhibited cyclic nucleotide phosphodiesterases. *J Clin Invest* 95: 1528-38

Rentero C, Monfort A, Puigdomenech P (2003) Identification and distribution of different mRNA variants produced by differential splicing in the human phosphodiesterase 9A gene. *Biochem Biophys Res Commun* 301: 686-92

Repaske DR, Corbin JG, Conti M, Goy MF (1993) A cyclic GMP-stimulated cyclic nucleotide phosphodiesterase gene is highly expressed in the limbic system of the rat brain. *Neuroscience* 56: 673-86

Reyes-Irisarri E, Markerink-Van Ittersum M, Mengod G, de Vente J (2007) Expression of the cGMP-specific phosphodiesterases 2 and 9 in normal and Alzheimer's disease human brains. *Eur J Neurosci* 25: 3332-8

Reyes-Irisarri E, Perez-Torres S, Miro X, Martinez E, Puigdomenech P, Palacios JM, Mengod G (2008) Differential distribution of PDE4B splice variant mRNAs in rat brain and the effects of systemic administration of LPS in their expression. *Synapse* 62: 74-9

Reymann KG, Frey JU (2007) The late maintenance of hippocampal LTP: requirements, phases, 'synaptic tagging', 'late-associativity' and implications. *Neuropharmacology* 52: 24-40

Richter W, Jin SL, Conti M (2005) Splice variants of the cyclic nucleotide phosphodiesterase PDE4D are differentially expressed and regulated in rat tissue. *Biochem J* 388: 803-11

Rodefer JS, Murphy ER, Baxter MG (2005) PDE10A inhibition reverses subchronic PCP-induced deficits in attentional set-shifting in rats. *Eur J Neurosci* 21: 1070-6

Rutten K, Basile JL, Prickaerts J, Blokland A, Vivian JA (2008a) Selective PDE inhibitors rolipram and sildenafil improve object retrieval performance in adult cynomolgus macaques. *Psychopharmacology (Berl)* 196: 643-8

Rutten K, Lieben C, Smits L, Blokland A (2007a) The PDE4 inhibitor rolipram reverses object memory impairment induced by acute tryptophan depletion in the rat. *Psychopharmacology (Berl)* 192: 275-82

Rutten K, Misner DL, Works M, Blokland A, Novak TJ, Santarelli L, Wallace-Boone T (2008b) Enhanced long-term potentiation and impaired learning in phosphodiesterase 4D knockout (PDE4D-/-) mice. *Eur J Neurosci* 28: 625-32

Rutten K, Prickaerts J, Blokland A (2006) Rolipram reverses scopolamine-induced and time-dependent memory deficits in object recognition by different mechanisms of action. *Neurobiol Learn Mem* 85: 132-8

Rutten K, Prickaerts J, Hendrix M, van der Staay FJ, Sik A, Blokland A (2007b) 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: 107-12

Rutten K, Prickaerts J, Schaenzle G, Rosenbrock H, Blokland A (2008c) Sub-chronic rolipram treatment leads to a persistent improvement in long-term object memory in rats. *Neurobiol Learn Mem* 90: 569-75

Rutten K, Vente JD, Sik A, Ittersum MM, Prickaerts J, Blokland A (2005) The selective PDE5 inhibitor, sildenafil, improves object memory in Swiss mice and increases cGMP levels in hippocampal slices. *Behav Brain Res* 164: 11-6

Salanova M, Chun SY, Iona S, Puri C, Stefanini M, Conti M (1999) Type 4 cyclic adenosine monophosphate-specific phosphodiesterases are expressed in discrete subcellular compartments during rat spermiogenesis. *Endocrinology* 140: 2297-306

Sasaki T, Kotera J, Omori K (2002) Novel alternative splice variants of rat phosphodiesterase 7B showing unique tissue-specific expression and phosphorylation. *Biochem J* 361: 211-20

Schmidt CJ, Chapin DS, Cianfrogna J, Corman ML, Hajos M, Harms JF, Hoffman WE, Lebel LA, McCarthy SA, Nelson FR, Proulx-Lafrance C, Majchrzak MJ, Ramirez AD, Schmidt K, Seymour PA, Siuciak JA, Tingley Iii FD, Williams RD, Verhoest PR, Menniti FS (2008) Preclinical characterization of selective PDE10A inhibitors: A new therapeutic approach to the treatment of schizophrenia. *J Pharmacol Exp Ther* 325: 681-90

Schmidt HH, Lohmann SM, Walter U (1993) The nitric oxide and cGMP signal transduction system: regulation and mechanism of action. *Biochim Biophys Acta* 1178: 153-75

Schoffelmeer AN, Wardeh G, Mulder AH (1985) Cyclic AMP facilitates the electrically evoked release of radiolabelled noradrenaline, dopamine and 5-hydroxytryptamine from rat brain slices. *Naunyn Schmiedebergs Arch Pharmacol* 330: 74-6

Schultheiss D, Muller SV, Nager W, Stief CG, Schlote N, Jonas U, Asvestis C, Johannes S, Munte TF (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

Seeger TF, Bartlett B, Coskran TM, Culp JS, James LC, Krull DL, Lanfear J, Ryan AM, Schmidt CJ, Strick CA, Varghese AH, Williams RD, Wylie PG, Menniti FS (2003) Immunohistochemical localization of PDE10A in the rat brain. *Brain Res* 985: 113-26

Setter SM, Iltz JL, Fincham JE, Campbell RK, Baker DE (2005) Phosphodiesterase 5 inhibitors for erectile dysfunction. *Ann Pharmacother* 39: 1286-95

Shafiei M, Mahmoudian M, Rostami P, Nemati F (2006) Effect of sildenafil (Viagra) on memory retention of a passive avoidance response in rats. *Acta Physiol Hung* 93: 53-9

Shah BH, Catt KJ (2004) GPCR-mediated transactivation of RTKs in the CNS: mechanisms and consequences. *Trends Neurosci* 27: 48-53

Shakur Y, Holst LS, Landstrom TR, Movsesian M, Degerman E, Manganiello V (2001) Regulation and function of the cyclic nucleotide phosphodiesterase (PDE3) gene family. *Prog Nucleic Acid Res Mol Biol* 66: 241-77

Silvestre JS, Fernandez AG, Palacios JM (1999) Effects of rolipram on the elevated plus-maze test in rats: a preliminary study. *J Psychopharmacol* 13: 274-7

Singh N, Parle M (2003) Sildenafil improves acquisition and retention of memory in mice. *Indian J Physiol Pharmacol* 47: 318-24

Siuciak JA, Chapin DS, McCarthy SA, Martin AN (2007) Antipsychotic profile of rolipram: efficacy in rats and reduced sensitivity in mice deficient in the phosphodiesterase-4B (PDE4B) enzyme. *Psychopharmacology (Berl)* 192: 415-24

Siuciak JA, McCarthy SA, Chapin DS, Fujiwara RA, James LC, Williams RD, Stock JL, McNeish JD, Strick CA, Menniti FS, Schmidt CJ (2006) Genetic deletion of the striatum-enriched phosphodiesterase PDE10A: evidence for altered striatal function. *Neuropharmacology* 51: 374-85

Siuciak JA, McCarthy SA, Chapin DS, Martin AN (2008a) Behavioral and neurochemical characterization of mice deficient in the phosphodiesterase-4B (PDE4B) enzyme. *Psychopharmacology (Berl)* 197: 115-26

Siuciak JA, McCarthy SA, Chapin DS, Martin AN, Harms JF, Schmidt CJ (2008b) Behavioral characterization of mice deficient in the phosphodiesterase-10A (PDE10A) enzyme on a C57/Bl6N congenic background. *Neuropharmacology* 54: 417-27

Soderling SH, Bayuga SJ, Beavo JA (1998) Identification and characterization of a novel family of cyclic nucleotide phosphodiesterases. *J Biol Chem* 273: 15553-8

Soderling SH, Bayuga SJ, Beavo JA (1999) Isolation and characterization of a dual-substrate phosphodiesterase gene family: PDE10A. *Proc Natl Acad Sci USA* 96: 7071-6

Sonnenburg WK, Rybalkin SD, Bornfeldt KE, Kwak KS, Rybalkina IG, Beavo JA (1998) Identification, quantitation, and cellular localization of PDE1 calmodulin-stimulated cyclic nucleotide phosphodiesterases. *Methods* 14: 3-19

Stearns G, Evangelista M, Fadool JM, Brockerhoff SE (2007) A mutation in the cone-specific pde6 gene causes rapid cone photoreceptor degeneration in zebrafish. *J Neurosci* 27: 13866-74

Sweatt JD (1999) Toward a molecular explanation for long-term potentiation. *Learn Mem* 6: 399-416

Tang AH, Franklin SR, Carter DB, Sethy VH, Needham LM, Jacobsen EJ, Von Voigtlander PF (1997) Anxiolytic-like effects of PNU-101017, a partial agonist at the benzodiazepine receptor. *Psychopharmacology (Berl)* 131: 255-63

Uthayathas S, Parameshwaran K, Karuppagounder S, Ilbasmis Tamer I, Degim T, Suppiramaniam V, Dhanasekaran M (2007) Phosphodiesterase 5 inhibition enhances hippocampal long term potentiation in mice. Society for Neuroscience. Neuroscience Meeting Planner, San Diego, CA

van der Staay FJ, Rutten K, Barfacker L, Devry J, Erb C, Heckroth H, Karthaus D, Tersteegen A, van Kampen M, Blokland A, Prickaerts J, Reymann KG, Schroder UH, Hendrix M (2008) The novel selective PDE9 inhibitor BAY 73-6691 improves learning and memory in rodents. *Neuropharmacology* 55: 908-18

van Staveren WC, Markerink-van Ittersum M (2005) Localization of cyclic guanosine 3',5'-monophosphate-hydrolyzing phosphodiesterase type 9 in rat brain by nonradioactive in situ hydridization. *Methods Mol Biol* 307: 75-84

van Staveren WC, Steinbusch HW, Markerink-van Ittersum M, Behrends S, de Vente J (2004) Species differences in the localization of cGMP-producing and NO-responsive elements in the mouse and rat hippocampus using cGMP immunocytochemistry. *Eur J Neurosci* 19: 2155-68

Van Staveren WC, Steinbusch HW, Markerink-Van Ittersum M, Repaske DR, Goy MF, Kotera J, Omori K, Beavo JA, De Vente J (2003) mRNA expression patterns of the cGMP-hydrolyzing phosphodiesterases types 2, 5, and 9 during development of the rat brain. *J Comp Neurol* 467: 566-80

Wang P, Wu P, Egan RW, Billah MM (2001) Human phosphodiesterase 8A splice variants: cloning, gene organization, and tissue distribution. *Gene* 280: 183-94

Wang P, Wu P, Egan RW, Billah MM (2003) Identification and characterization of a new human type 9 cGMP-specific phosphodiesterase splice variant (PDE9A5). Differential tissue distribution and subcellular localization of PDE9A variants. *Gene* 314: 15-27

Wunder F, Tersteegen A, Rebmann A, Erb C, Fahrig T, Hendrix M (2005) Characterization of the first potent and selective PDE9 inhibitor using a cGMP reporter cell line. *Mol Pharmacol* 68: 1775-181

Yan C, Bentley JK, Sonnenburg WK, Beavo JA (1994) Differential expression of the 61 kDa and 63 kDa calmodulin-dependent phosphodiesterases in the mouse brain. *J Neurosci* 14: 973-84

Yan C, Zhao AZ, Bentley JK, Beavo JA (1996) The calmodulin-dependent phosphodiesterase gene PDE1C encodes several functionally different splice variants in a tissue-specific manner. *J Biol Chem* 271: 25699-706

Yanaka N, Kotera J, Ohtsuka A, Akatsuka H, Imai Y, Michibata H, Fujishige K, Kawai E, Takebayashi S, Okumura K, Omori K (1998) Expression, structure and chromosomal localization of the human cGMP-binding cGMP-specific phosphodiesterase PDE5A gene. *Eur J Biochem* 255: 391-9

Ye Y, Jackson K, O'Donnell JM (2000) Effects of repeated antidepressant treatment of type 4A phosphodiesterase (PDE4A) in rat brain. *J Neurochem* 74: 1257-62

Zhang HT, Crissman AM, Dorairaj NR, Chandler LJ, O'Donnell JM (2000) Inhibition of cyclic AMP phosphodiesterase (PDE4) reverses memory deficits associated with NMDA receptor antagonism. *Neuropsychopharmacology* 23: 198-204

Zhang HT, Huang Y, Jin SL, Frith SA, Suvarna N, Conti M, O'Donnell JM (2002) Antidepressant-like profile and reduced sensitivity to rolipram in mice deficient in the PDE4D phosphodiesterase enzyme. *Neuropsychopharmacology* 27: 587-95

Zhang HT, Huang Y, Suvarna NU, Deng C, Crissman AM, Hopper AT, De Vivo M, Rose GM, O'Donnell JM (2005) Effects of the novel PDE4 inhibitors MEM1018 and MEM1091 on memory in the radial-arm maze and inhibitory avoidance tests in rats. *Psychopharmacology (Berl)* 179: 613-9

Zhang HT, O'Donnell JM (2000) Effects of rolipram on scopolamine-induced impairment of working and reference memory in the radial-arm maze tests in rats. *Psychopharmacology (Berl)* 150: 311-6

Zhang HT, Zhao Y, Huang Y, Dorairaj NR, Chandler LJ, O'Donnell JM (2004) Inhibition of the phosphodiesterase 4 (PDE4) enzyme reverses memory deficits produced by infusion of the MEK inhibitor U0126 into the CA1 subregion of the rat hippocampus. *Neuropsychopharmacology* 29: 1432-9

Zhu J, Mix E, Winblad B (2001) The antidepressant and antiinflammatory effects of rolipram in the central nervous system. *CNS Drug Rev* 7: 387-98

CHAPTER 3

Phosphodiesterase type 5 (PDE5) inhibition improves object recognition memory: Indications for central and peripheral mechanisms

Olga A. H. Reneerkens, Kris Rutten, Sven Akkerman, Arjan Blokland, Christopher L. Shaffer, Frank S. Menniti, Harry W. M. Steinbusch & Jos Prickaerts

Published in Neurobiology of Learning and Memory (2012), 97(4), 370-379

ABSTRACT

A promising target for memory improvement is phosphodiesterase type 5 (PDE5), which selectively hydrolyzes cyclic guanosine monophosphate (cGMP). In rodents, PDE5 inhibitors (PDE5-Is) have been shown to improve memory performance in many behavioural paradigms. However, it is questioned whether the positive effects in animal studies result from PDE5 inhibition in the central nervous system or the periphery. Therefore, we studied the effects of PDE5 inhibition on memory and determined whether compound penetration of the blood-brain barrier (BBB) is required for this activity. Two selective PDE5-Is, vardenafil and UK-343,664, were tested in the object recognition task (ORT) in both a MK-801- and scopolamine-induced memory deficit model, and a time-delay model without pharmacological intervention. Compounds were dosed 30 min before the learning trial of the task. To determine if the PDE5-Is crossed the BBB, their concentrations were determined in plasma and brain tissue collected 30 min after oral administration. Vardenafil improved object recognition memory in all three variants of the ORT. UK-343,664 was ineffective at either preventing MK-801-induced memory disruption or time-dependent memory decay. However, UK-343,664 attenuated the memory impairment of scopolamine. Vardenafil crossed the BBB whereas UK-343,664 did not. Further, co-administration of UK-343,664 and scopolamine did not alter the brain partitioning of either molecule. This suggests that the positive effect of UK-343,664 on scopolamine-induced memory decay might arise from peripheral PDE5 inhibition. The results herein suggest that there may be multiple mechanisms that mediate the efficacy of PDE5 inhibition to improve memory performance in tasks such as the ORT and that these involve PDE5 located both within and outside of the brain. To further elucidate the underlying mechanisms, the cellular and subcellular localization of PDE5 needs to be determined.

INTRODUCTION

With the seminal findings of Barad et al. (1998) on the role of PDE4 in the regulation of long term potentiation (LTP) and learning and memory , there has grown a broad interest in the phosphodiesterases (PDE) as molecular targets to treat neuropsychiatric dysfunction (e.g. Halene and Siegel 2007; Menniti et al. 2006). PDEs are enzymes that inactivate the second messenger molecules cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). There are eleven families of PDEs, distinguished by molecular properties, substrate specificity, and regulation (Bender and Beavo 2006). These enzymes are expressed in unique and overlapping patterns throughout the body, and in particular in the CNS (Lakics et al. 2010). Both cAMP and cGMP play important roles in regulating processes of neuroplasticity, including LTP, that form the neurophysiological bases of learning and memory (Bliss and Collingridge 1993; Frey et al. 1993; Son et al. 1998). Pharmacological inhibitors of different PDE families offer means to modulate discreet cyclic nucleotide signalling pathways involved in different aspects of learning and memory, possibly for therapeutic benefit.

A PDE family of particular interest with regard to pharmacological targeting is PDE type 5 (PDE5). PDE5 inhibitors (PDE5-Is), including sildenafil, tadalafil, and vardenafil, are the first PDE-Is to achieve widespread clinical use, especially for the treatment of conditions caused by vascular dysfunction. PDE5 is specific for the metabolic inactivation of cGMP. The enzyme is expressed at high levels in smooth muscle of discreet vascular beds, where PDE5 inhibition causes increases in smooth muscle cGMP which results in vascular relaxation. Initially, PDE5-Is were of interest to reduce systemic blood pressure but were not sufficiently efficacious for commercial development. However, PDE5 is expressed at high levels in the vascular bed of the penis and these compounds found therapeutic utility and commercial success in the treatment of male erectile dysfunction. PDE5 is also expressed at high levels in the lung, and PDE5-Is are now being used successfully to treat pulmonary hypertension. It has also been found that PDE5 inhibition regulates vascular tone in the prostate and there is growing interest in the use of PDE5-Is for treatment of benign prostatic hyperplasia. Thus, the success of PDE5-Is derives from the fundamental role of the enzyme in the regulation of vascular tone, with disease utility tied to enzyme localization in specific vascular beds (for a review see also Ghofrani et al. 2006; Puzzo et al. 2008).

cGMP signaling cascades are prominently represented in the control of neuronal function including the pre- and post-synaptic mediation of different forms of neuroplasticity (Kleppisch and Feil 2009; Son et al. 1998). Thus, it is of interest whether PDE5 is involved in the regulation of one or more such neuronal signalling cascades and whether PDE5-Is may impact cognitive function. In fact, there are now a number of reports of PDE5-Is improving learning and memory performance in animals. This includes improved memory for novel objects in the object recognition task (ORT) (Domek-Lopacinska and Strosznajder 2008; Prickaerts et al. 2005; Rutten et al. 2007b; Rutten et al. 2009; van Donkelaar et al. 2008), avoidance learning (Baek et al. 2011; Baratti and Boccia 1999; Boccia et al. 2011; Devan et al. 2004) and complex maze learning in rodents (Devan et al. 2004) (for an overview see Chapter 2). Particularly interesting is the finding that chronic treatment with a PDE5-I caused a long lasting improvement in memory function and reduced plaque load in a mouse model of the amyloid deposition of Alzheimer's disease (AD) (Puzzo et al. 2009), although the

decrease in amyloid burden has not been replicated in a more recent study (Cuadrado-Tejedor et al. 2011). However, PDE5 mRNA and protein is expressed at only low levels or not observed in forebrain regions thought to mediate learning and memory function in these types of tasks (see Discussion). This raises the question of localization of the PDE5 target and mechanism of action that accounts for the pro-cognitive effects of PDE5-Is. Previously, we reported that cognitive enhancing doses of PDE5-Is do not affect cerebral blood flow and glucose utilization, indicating that such vascular effects do not account for pro-cognitive efficacy (Rutten et al. 2009). In the present study, we take another approach to localize the PDE5 target involved in the pro-cognitive action of PDE5 inhibitors by investigating whether PDE5-Is must cross the blood-brain barrier (BBB) to improve memory for novel objects.

We examined the efficacy of UK-343,664, a PDE5-I that is assumed to only poorly cross the BBB (Abel et al. 2001; Walker et al. 2001), to improve memory in three variants of the ORT: a 24 h delay interval where memory degrades over time or a 1 h interval where memory is disrupted by administration of scopolamine or MK-801. The scopolamine-induced memory deficit model is a widely used cognitive impairment model (Klinkenberg and Blokland 2010). This anti-cholinergic agent has shown to impair memory in several behavioral tests including the ORT (e.g. Rutten et al. 2006; Schreiber et al. 2007). After scopolamine, the MK-801-induced memory deficit model is the second most commonly used deficit model for preclinical cognition research (van der Staay et al. 2011). MK-801 is an N-methyl-D-aspartate (NMDA) receptor antagonist that disrupts in particular short-term memory (STM) and attention processes (e.g. Boess et al. 2004; van der Staay et al. 2008; Zhang et al. 2000), thereby causing cognitive deficits affiliated to schizophrenia (Kiss et al. 2010; Moghaddam and Jackson 2003; Vardigan et al. 2010). Therefore one has to be aware that the effects of MK-801 on a memory performance cannot be seen separately from STM and attention processes. The dose of MK-801 used in the present study is known to impair memory in rodents, but without causing sensorimotor impairments, motivational effects and/or signs of intoxication (van der Staay et al. 2011). We compare the efficacy of UK-343,664 in these assays to that of vardenafil, which is assumed to more readily cross the BBB (Prickaerts et al. 2004b) and which has previously been reported to be efficacious in the ORT (Prickaerts et al. 2004b; Rutten et al. 2007b; Rutten et al. 2009; van Donkelaar et al. 2008). These results are discussed with regard to the localization of the PDE5 target(s) for these compounds and the role of central and peripheral mechanisms in modulating object recognition memory.

METHODS AND MATERIALS

Animals

All behavioural and related treatment were approved by the local ethical committee for animal experiments of Maastricht University and met governmental guidelines. Five batches of twenty-four 4-month-old male Wistar rats (Harlan, The Netherlands) were used with average body weights of 351 g (± 2.67, batch 1 vardenafil 1 h interval MK-801), 498 g (± 4.80, batch 2 vardenafil 1h interval scopolamine), 364 g (± 3.93, batch 3 UK-343,664 1 h interval MK-801), 409 g (± 4.23, batch 4 UK-343,664 interval scopolamine) and 406 g (± 5.86, batch 5 UK-343,664 24 h interval with vardenafil as a positive control). The animals

were housed individually in standard cages on sawdust bedding in an air-conditioned room (about 20°C). They were kept on a 12/12-hour reversed light/dark cycle (lights on from 19.00 to 7.00 h) and had free access to food and water. The rats were housed in the same room as where they were tested. A radio, which was playing softly, provided background noise in the room. All testing was done between 9.00 and 18.00 h.

Neuropharmacokinetics studies were conducted at BioDuro, Pharmaceutical Product Development Inc. (Beijing, PRC) in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996). Male Wistar Han rats (250–300 g, 2.5-months old; Vital River Laboratories, Beijing, PRC) were maintained on a 12 h light-dark cycle in a temperature- and humidity-controlled environment with free access to food and water throughout the studies.

Treatment

MK-801 and scopolamine were prepared daily and dissolved in saline. Based on previous results (data not shown), a dose of 0.125 mg/kg was selected for MK-801 and a dose of 0.1 mg/kg for scopolamine, since these doses impaired recognition memory without affecting exploratory activity. Administration (1 ml/kg) was intraperitoneal (i.p.) (30 min before T1).

Vardenafil and UK-343,664 were first dissolved in 1.5 ml ethanol with 2% Tween 80. After extraction of ethanol via vaporization under N2 gas, the end volume of 0.5% methylcellulose was added. Both compounds (2 ml/kg) were given by oral gavage (p.o.). For vardenafil, the doses tested are 0.3 - 3 mg/kg in the 1 h interval and 3 mg/kg in the 24 h interval. For the latter, vardenafil was used as a positive control and the dose of 3 mg/kg was based on previous findings (Prickaerts et al. 2005; Prickaerts et al. 2002). For UK-343,664 the doses tested were 1-30 mg/kg in the MK-801 deficit model and 3-30 mg/kg in the scopolamine deficit model and 24 h interval. All compounds were administered 30 min before T1. Vardenafil was kindly donated by BAYER (Wuppertal, Germany) and UK-343,664 was a gift from Pfizer Inc (Groton, CT, USA). The experimenter was blind to the compounds and doses tested. Sixteen animals were used for each condition in the vardenafil 1 h interval studies; twelve for the UK-343,664 1 h interval studies and the 24 h interval study. Of note, control conditions (vehicle, saline and MK-801) were always tested in 24 animals, as part of the training protocol (see below).

For neuropharmacokinetics studies, all drugs were tested alone or in behaviourally tested combinations of MK-801 (0.125 mg/kg, i.p.), scopolamine (0.1 mg/kg, i.p.), vardenafil (3 mg/kg, p.o.) and/or UK-343,664 (30 mg/kg, p.o.). Three animals were dosed per treatment regimen.

Object recognition memory

The ORT was performed as described elsewhere (Prickaerts et al. 1997). The apparatus consisted of a circular arena, 83 cm in diameter. Half of the 40 cm high wall was made of gray polyvinyl chloride, the other half of transparent polyvinyl chloride. Two objects were placed in a symmetrical position about 10 cm away from the gray wall. Each object was available in triplicate. We used four different objects: 1) a cone consisting of a gray

polyvinyl chloride base (maximal diameter 18 cm) with a collar on top made of brass (total height 16 cm), 2) a standard 1 l transparent glass bottle (diameter 10 cm, height 22 cm) filled with water, 3) a massive metal cube (10.0 x 5.0 x 7.5 cm) with two holes (diameter 1.9 cm), and 4) a massive aluminium cube with a tapering top (13.0 x 8.0 x 8.0 cm). These objects are presented in a semi-random manner to prevent the same objects being used in two consecutive test sessions. As a result, the time between presentations of similar objects to a certain animals is at least one week. Rats could not displace the objects. Fluorescent red tubes and a light bulb provided a constant illumination of about 8 lux on the floor of the apparatus and light intensity was equal throughout the apparatus.

A testing session comprised two trials. The duration of each trial was 3 min. During the first trial (T1) the apparatus contained two identical objects (samples). A rat was always placed in the apparatus facing the wall at the middle of the front (transparent) segment. After the first exploration period the rat was placed back in its home cage. Subsequently, after a predetermined delay interval, the rat was returned to the apparatus for the second trial (T2), but now with two dissimilar objects, a familiar one (the sample) and a new one. The times spent in exploring each object during T1 and T2 were recorded manually with a personal computer.

Exploration was defined as follows: directing the nose to the object at a distance of no more than 2 cm and/or touching the object with the nose. Sitting on the object was not considered exploratory behaviour. In order to avoid the presence of olfactory trails the objects were always thoroughly cleaned with 70% ethanol. All combinations and locations of objects were used in a balanced manner to reduce potential biases due to preferences for particular locations or objects.

In previous studies we have found that rodents show a good object memory performance with a 1 h delay interposed between T1 and T2 (Rutten et al. 2007a; van Donkelaar et al. 2008). However, when a 24 h delay interval is used, the animals do not discriminate between the novel and familiar object in T2, indicating that they do not remember the object that was presented in T1 (Prickaerts et al. 2004b; Rutten et al. 2007b). Using a 4 h delay, the discrimination performance is intermediate between the performance of 1 h and 24 h delay, suggesting a delay-dependent forgetting in this task (Sik et al. 2003). Based on this experience, in the present study we used a 1 h interval to test the memory enhancing effects of PDE-Is in the MK-801 as well as the scopolamine deficit model and a 24 h interval to test the effects of UK-343,664 without an additional pharmacological intervention.

In the first week, the animals were handled daily and adapted to the procedure in two days, i.e. they were allowed to explore the apparatus (without any objects) twice for 3 min each day. Next, the rats were adapted to the testing and p.o. (2 ml/kg) and i.p. (1 ml/kg) administration procedures by a saline injection 30 min before T1 until they showed a stable discrimination performance, i.e. a good discrimination at 1 h interval and no discrimination at 24 h interval (after about 1 week). After this, testing of the control conditions began. The animals used for the 1 h delay interval experiments were all treated with saline or MK-801/scopolamine together with the vehicle of the PDE5-Is, 30 min before T1. More specifically, batch 1 and 3 were treated with MK-801 (0.125 mg/kg, i.p.), batch 2 and 4 with scopolamine (0.1 mg/kg, i.p.). The rats used in the 24 h delay interval, batch 5, were only treated with the vehicle (p.o.) 30 min before T1. Subsequently, the PDE5-Is were tested:

vardenafil (0.3-3 mg/kg, p.o.) in combination with MK-801 in batch 1 and with scopolamine in batch 2, UK-343,664 (1-30 mg/kg, p.o. in MK-801 deficit model; 3-30 mg/kg in scopolamine deficit model) in combination with MK-801 in batch 3 and in combination with scopolamine in batch 4, and UK-343,664 (3-30 mg/kg, p.o.) in a 24 h interval with vardenafil (3 mg/kg, p.o.) as a positive control in batch 5. In total, each animal in each batch was tested 6-7 times and did not show any decline in performance or exploratory behaviour. Doses/vehicle per compound were tested randomly with a wash-out period of at least one day in between test sessions.

Determination of vardenafil, UK-343,664, MK-801 and scopolamine concentrations in plasma and brain samples

Following the sequential administration (2 ml/kg for p.o. doses, 1 ml/kg for i.p. doses) of compounds (i.e. PDE5-I/vehicle (p.o.) followed immediately by scopolamine/MK-801/saline (i.p.)), each rat was placed under isoflurane anesthesia at 0.5 h post-dose. Blood samples were obtained by cardiac puncture and collected into EDTA-containing tubes, which were stored on wet ice until plasma isolation. Subsequently, the whole brain was extracted, rinsed of excess blood with ice-cold saline, placed into a tared vial, weighed and frozen on dry ice. All plasma and brain tissue were stored at -20 °C until processing for bioanalysis.

Compound quantification within collected plasma and brain was performed at BioDuro, Pharmaceutical Product Development Inc. For bioanalytical sample preparation, plasma was used as is, while brain samples were first homogenized in a 4-fold volume (w/v) of saline. Both matrices were processed for the quantification of dosed compound using liquid-liquid extraction methodology followed by a characterized liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay. Individual standard curves were prepared in respective control matrices, an appropriate dynamic range was achieved for each compound, and instrument settings and potentials were adjusted to optimize the MS signal for each analyte. Raw data were processed using Analyst Software version 1.4.2 (AB Sciex Inc., Ontario, Canada). The lower limit of quantification (LLOQ) for all compounds was 1 nanogram per millilitre (ng/ml) for plasma and 2.5 nanogram per gram (ng/g) for brain.

Statistical analysis

The basic measures in the ORT were the times spent exploring an object during T1 and T2. Table 1 depicts how these measures of the ORT (*e*1, *e*2 and *d*2) were calculated. *e*1 and *e*2 are measures of the total exploration time of both objects during T1 and T2 respectively. *d*2 is considered as index measure of discrimination between the new and the familiar objects. In fact, *d*2 is a relative measure of discrimination that corrects for exploration activity (*e*2). Thus, there should be no differences in *d*2 indices between experiments with similar treatments at similar intervals.

Table 1 Measures involved in the object recognition test. *e*1 is the measure of the time spent in exploring both identical objects (*a*1 and *a*2) in the T1, and *e*2 is the measure of the time spent in exploring both the familiar (*a*) and new object (*b*) in the T2; *d*2 is the measure of discrimination between the new and familiar objects

Exploration	Discrimination
$e1 = a1 + a2$	
$e2 = a + b$	$d2 = (b - a) / e2$

One-sample t-statistics were performed in order to assess whether *d*2 differed from zero per treatment condition (within comparison). Effects between the different conditions were assessed by a one-way ANOVA (between comparisons). In case of a statistically reliable dose effect, comparisons between means of the different doses were analyzed in more detail using post hoc Bonferroni t-tests ($P < 0.05$).

If the plasma or brain sample of a compound was below the quantification limit (BQL), but one or more of the other samples in the same (compound) group had measurable values, the BQL was treated as zero. The levels of vardenafil, UK-343,664, MK-801 and scopolamine in plasma and brain samples as well as the brain/plasma ratio were analysed with a non-parametric Kruskal Wallis test because of the low number of subjects per group, i.e. 3. In case of a statistically reliable condition effect, comparisons between means of the different treatments were analyzed in further detail using pairwise non-paramatric Mann-Whitneys tests with adjusted p-values for the number of tests.

RESULTS

Effects of vardenafil on MK-801-induced memory deficits

The results of vardenafil treatment, 30 min before T1 in combination with MK-801, are summarized in Table 2. There were no differences between treatment conditions in the level of exploration in T1 (*e*1: $F(4,95) = 1.07$, n.s.) and in T2 (*e*2: $F(4,95) = 2.47$, n.s.).

Table 2 Results of treatment with vardenafil on exploration time; drug administration (p.o.) was 30 min before T1. The delay interval between T1 and T2 was 1 h. Mean values (± SEM) of total exploration time (s) during the T1 (*e*1) and T2 (*e*2). n = 24 per vehicle condition; n = 16 per experimental condition. MK = MK-801, var = vardenafil

PO	vehicle	vehicle	0.3 mg/kg var	1 mg/kg var	3 mg/kg var
IP	saline	0.125 mg/kg MK	0.125 mg/kg MK	0.125 mg/kg MK	0.125 mg/kg MK
e1	15.35 (1.17)	17.18 (1.27)	18.61 (1.48)	19.84 (1.39)	18.88 (1.67)
*e*2	17.94 (0.92)	19.08 (1.14)	20.38 (1.55)	24.13 (2.02)	20.88 (2.06)

The effects of vardenafil on the relative discrimination index *d*2 are presented in Figure 1. One sample t-tests showed that the *d*2 value of the vehicle/saline and 1 mg/kg vardenafil conditions differed from zero, in contrast to the vehicle/MK-801 and vardenafil 0.3 mg/kg and 3 mg/kg conditions. When comparing between groups, differences were found for the *d*2 index ($F(4,95) = 8.42$, $P < 0.001$). Post hoc Bonferroni comparisons revealed that the *d*2 values were higher in the vehicle/saline and 1 mg/kg vardenafil conditions than in the vehicle/MK-801 condition (see Figure 1). In addition, the *d*2 was higher in vehicle/saline than in the vardenafil 0.3 mg/kg condition.

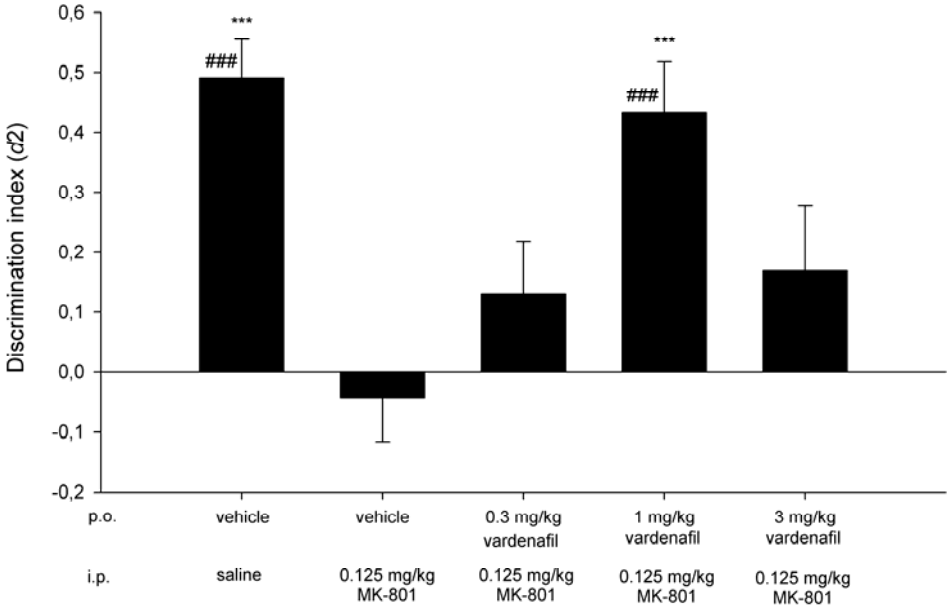

Figure 1 Effects of vardenafil on discrimination performance (*d*2) in the ORT (mean ± SEM). All drugs were given 30 min before T1. Interval was 1 h. 1 mg/kg vardenafil completely reversed the MK-801-induced memory deficit. A difference from the MK-801 condition is depicted with asterisks (Bonferroni t-tests, ***: P < 0.001). A difference from zero is depicted with # (One sample t-tests, ###: P < 0.001)

Effects of vardenafil on scopolamine-induced memory deficits

The results of the vardenafil treatment, 30 min before T1, are summarized in Table 3. There were no differences found between treatment conditions in the level of exploration in T1 (*e*1: F (4,95) = 0.95, n.s.). In T2, there were no differences between treatment conditions in the level of exploration either (*e*2: F(4,95) = 1.53, n.s.).

Table 3 Results of treatment with vardenafil on exploration time; drug administration (p.o.) was 30 min before T1. The delay interval between T1 and T2 was 1 h. Mean values (± SEM) of total exploration time (s) during the T1 (*e*1) and T2 (*e*2). n=24 per vehicle condition; n = 16 per experimental condition. Scop = scopolamine, var = vardenafil

PO	vehicle	vehicle	0.3 mg/kg var	1 mg/kg var	3 mg/kg var
IP	saline	0.1 mg/kg scop	0.1 mg/kg scop	0.1 mg/kg scop	0.1 mg/kg scop
*e*1	18.94 (1.25)	16.76 (1.32)	18.39 (1.51)	17.48 (1.99)	20.83 (2.03)
*e*2	22.36 (1.59)	20.34 (2.57)	18.13 (1.03)	19.15 (1.98)	25.54 (3.03)

The effects of vardenafil treatment on the relative discrimination index *d*2 are graphically presented in Figure 2. One sample t-tests showed that *d*2 values of vardenafil 1 mg/kg, 3 mg/kg and vehicle/saline condition differed from zero (see Figure 2). In contrast, the vehicle/scopolamine and 0.3 mg/kg vardenafil conditions showed no differences from zero. When comparing between groups, differences were found for the *d*2 index (F(4,95) = 9.47, P < 0.001). The *d*2 values were higher for the vardenafil 1 mg/kg, 3 mg/kg and

vehicle/saline conditions than for the vehicle/scopolamine and vardenafil 0.3 mg/kg conditions (Bonferroni t-tests; see Figure 2).

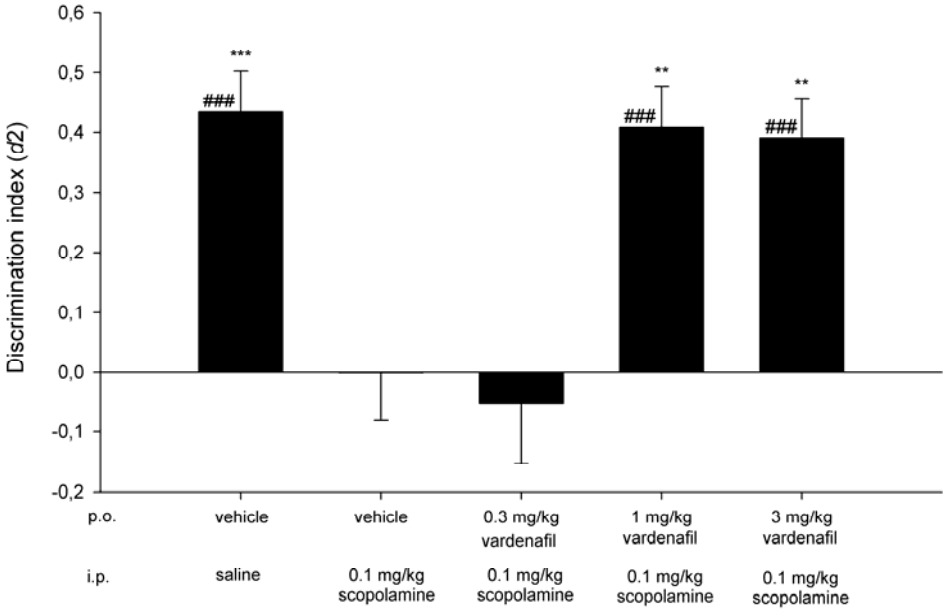

Figure 2 Effects of vardenafil on the discrimination performance (*d*2) in the ORT (mean ± SEM). All drugs were given 30 min before T1. Interval was 1 h. 1 and 3 mg/kg vardenafil completely reversed the scopolamine-induced memory deficit. A difference from the scopolamine condition is depicted with asterisks (Bonferroni t-tests, **: P < 0.01; ***: P < 0.001). A difference from zero is depicted with # (One sample t-tests, ###: P < 0.001)

Effects of UK-343,664 on MK-801-induced memory deficits

The results of UK-343,664 treatment, 30 min before T1, are summarized in Table 4. No differences were found between treatment conditions in the level of exploration in T1 (*e*1: F (5,94) = 0.41, n.s.). In T2, there were also no differences between treatment conditions in the level of exploration either (*e*2: F(5,94) = 1.69, n.s.).

Table 4 Results of treatment with UK-343,664 on exploration time; drug administration (p.o.) was 30 min before T1. The delay interval between T1 and T2 was 1 h. Mean values (± SEM) of total exploration time (s) during the T1 (*e*1) and T2 (*e*2). n = 24 per vehicle condition; n = 12 per experimental condition. MK = MK-801, UK = UK-343,664

PO	vehicle	vehicle	1 mg/kg UK	3 mg/kg UK	10 mg/kg UK	30 mg/kg UK
IP	saline	0.125 mg/kg MK	0.125 mg/kg MK	0.125 mg/kg MK	0.125 mg/kg MK	0.125 mg/kg MK
*e*1	19.70 (1.35)	20.04 (0.98)	20.48 (1.83)	19.36 (1.57)	19.86 (1.68)	22.17 (1.15)
*e*2	20.86 (1.11)	20.07 (1.47)	18.59 (1.63)	23.13 (1.69)	22.76 (1.64)	17.56 (1.28)

One sample t-tests showed that *d*2 values of the vehicle/saline condition differed from zero. In contrast, the other conditions showed no differences from zero. The effects of UK-

343,664 treatment on the *d*2 are presented in Figure 3. When comparing between groups, differences were found for the *d*2 index (F(5,94) = 9.28, P < 0.001). The *d*2 values were higher for the vehicle/saline condition than for the other conditions (see Figure 3) (Bonferroni t-tests).

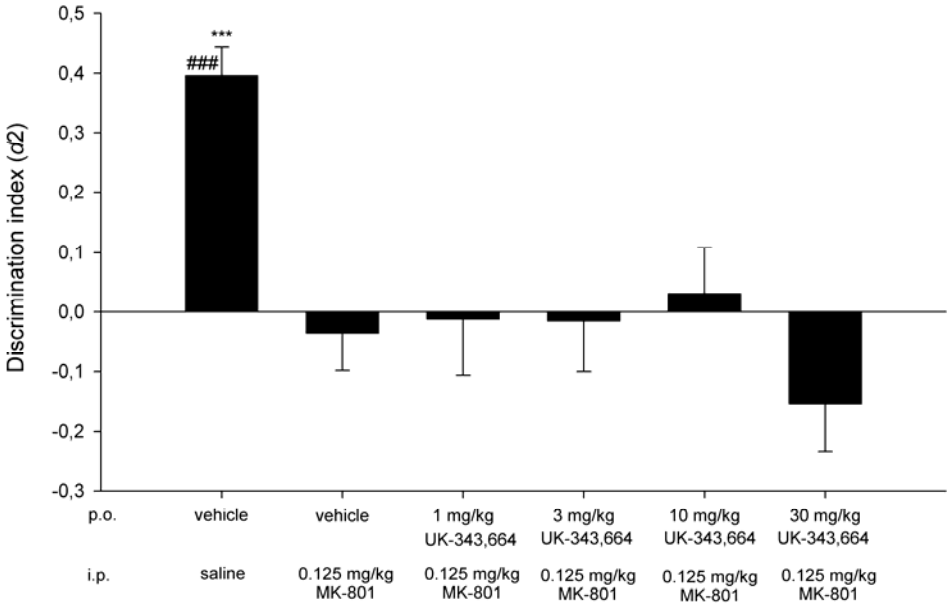

Figure 3 Effects of UK-343,664 on the discrimination performance (*d*2) in the ORT (mean ± SEM). All drugs were given 30 min before T1. Interval was 1 h. UK-343,664 did not reverse the MK-801-induced memory deficit. A difference from the MK-801 condition is depicted with asterisks (Bonferroni t-tests, ***: P < 0.001). A difference from zero is depicted with # (One sample t-tests, ###: P < 0.001)

Effects of UK-343,664 on scopolamine-induced memory deficits

The results of the UK-343,664 treatment, 30 min before T1, are summarized in Table 5. There were no differences found between treatment conditions in the level of exploration in T1 (*e*1: F (4,83) = 0.90, n.s.). In T2, there were no differences between treatment conditions in the level of exploration either (*e*2: F(4,83) = 0.69, n.s.).

Table 5 Results of treatment with UK-343,664 on exploration time; drug administration (p.o.) was 30 min before T1. The delay interval between T1 and T2 was 1 h. Mean values (± SEM) of total exploration time (s) during the T1 (*e*1) and T2 (*e*2). n=24 per vehicle condition; n = 12 per experimental condition. Scop = scopolamine, UK = UK-343,664

PO	vehicle	vehicle	3 mg/kg UK	10 mg/kg UK	30 mg/kg UK
IP	saline	0.1 mg/kg scop	0.1 mg/kg scop	0.1 mg/kg scop	0.1 mg/kg scop
*e*1	18.73 (0.94)	18.28 (1.10)	16.07 (1.34)	17.46 (1.78)	20.08 (2.16)
*e*2	22.74 (1.09)	21.14 (1.23)	23.26 (2.73)	19.31 (1.48)	21.38 (2.76)

The effects of UK-343,664 treatment on the relative discrimination index *d*2 are graphically presented in Figure 4. One sample t-tests showed that *d*2 values of UK-343,664 10 mg/kg, 30 mg/kg and vehicle/saline condition differed from zero (see Figure 4). In contrast, the vehicle/scopolamine and 3 mg/kg UK-343,664 conditions showed no differences from zero. When comparing between groups, differences were found for the *d*2 index ($F(4,83) = 6.48$, $P < 0.001$). The *d*2 values were higher for the UK-343,664 30 mg/kg and vehicle/saline conditions than for the vehicle/scopolamine and UK-343,664 3 mg/kg conditions (Bonferroni t-tests; see Figure 4).

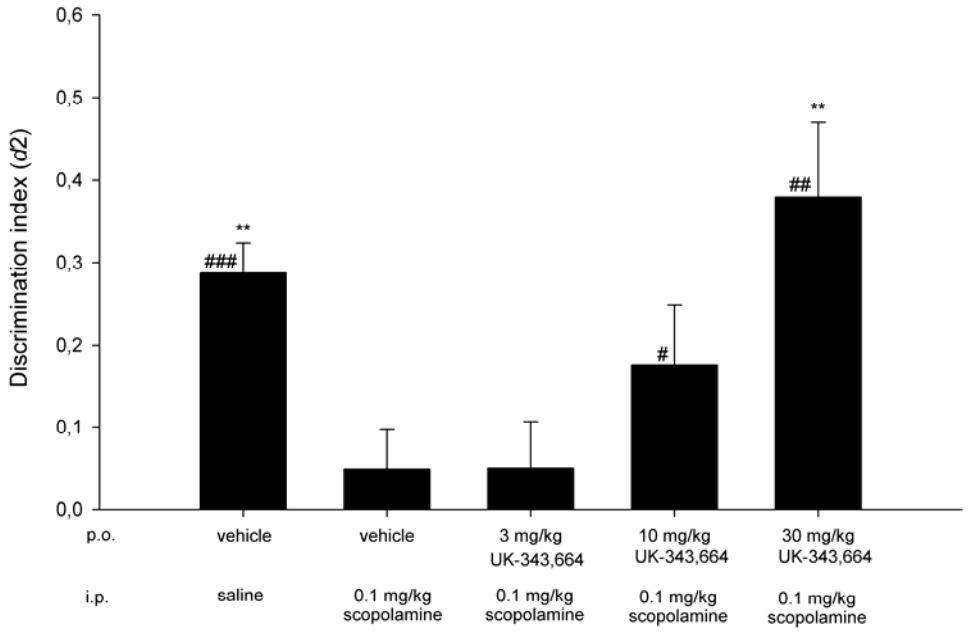

Figure 4 Effects of UK-343,664 on the discrimination performance (*d*2) in the ORT (mean ± SEM). All drugs were given 30 min before T1. Interval was 1 h. 30 mg/kg UK-343,664 completely reversed the scopolamine-induced memory deficit, whereas 10 mg/kg had an intermediate effect. A difference from the scopolamine condition is depicted with asterisks (Bonferroni t-tests, **: $P < 0.01$). A difference from zero is depicted with # (One sample t-tests, #: $P < 0.05$; ##: $P < 0.01$; ###: $P < 0.001$)

Effects of UK-343,664 or vardenafil on memory performance in a 24 h interval

The results of PDE5-I treatment, 30 min before T1, are summarized in Table 6. There were no differences between treatment conditions in the level of exploration in T1 (*e*1: $F(4,71) = 1.56$, n.s.) nor in T2 (*e*2: $F(4,71) = 0.27$, n.s.).

The effects of PDE5 inhibition on the relative discrimination index *d*2 in a 24 h interval are presented in Figure 5. One sample t-tests showed that the *d*2 value of the vardenafil (3 mg/kg) condition differed from zero, in contrast to the vehicle and UK-343,664 conditions. When comparing between groups, differences were found for the *d*2 index ($F(4,71) = 3.26$, $P < 0.05$). Bonferroni post hoc t-tests ($P < 0.05$) comparisons revealed that the *d*2 values only differed between the vardenafil and the 3 mg/kg UK-343,664 condition (see Figure 5).

Table 6 Results of treatment with UK-343,663 or vardenafill on exploration time; drug administration (p.o.) was 30 min before T1. The delay interval between T1 and T2 was 24 h. Mean values (± SEM) of total exploration time (s) during the T1 (*e*1) and T2 (*e*2). n = 24 per vehicle condition; n = 12 per experimental condition. UK = UK-343,664, var = vardenafil

PO	vehicle	3 mg/kg UK	10 mg/kg UK	30 mg/kg UK	3 mg/kg var
*e*1	19.37 (1.64)	19.01 (2.10)	20.67 (1.46)	17.11 (2.23)	14.43 (1.16)
*e*2	20.88 (1.19)	22.07 (2.55)	20.90 (1.03)	19.84 (2.07)	22.50 (2.78)

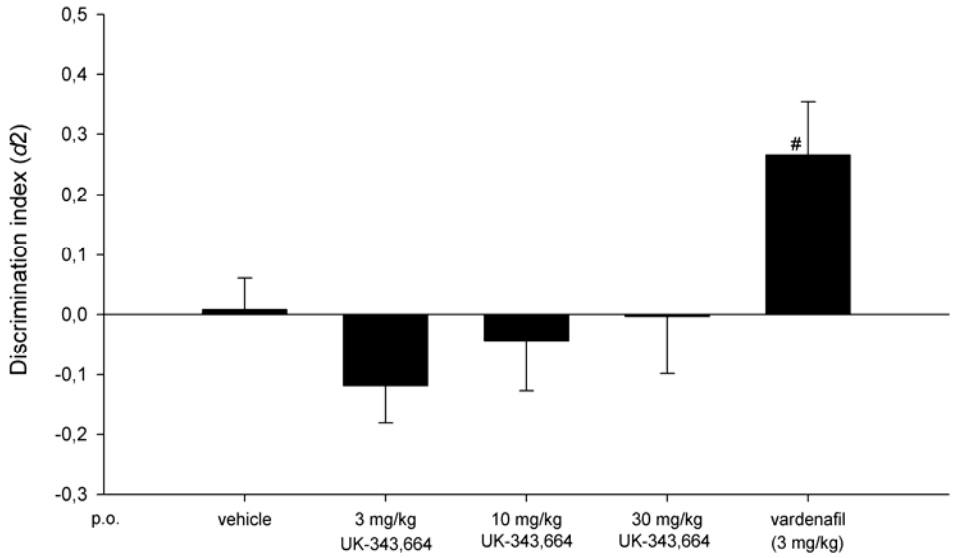

Figure 5 Effects of PDE5 inhibition on the discrimination performance (*d*2) in the ORT (mean ± SEM). All drugs were given 30 min before T1. Interval was 24 h. Vardenafil had an intermediate effect on memory. A difference from zero is depicted with # (One sample t-tests, #: $P < 0.05$)

Plasma and brain concentrations of vardenafil, UK-343,664, MK-801 and scopolamine

Total plasma (C_p) and total brain (C_b) compound concentrations, as well as brain-to-plasma ratios (C_b:C_p), of vardenafil and UK-343,664 30 min after administration are summarized in Table 7. Paralleling the behavioural experiments, both molecules' C_p and C_b were determined in combination with saline, MK-801 (0.125 mg/kg, i.p.) or scopolamine (0.1 mg/kg, i.p.). For vardenafil, mean C_p and C_b were > LLOQ in all conditions; C_p, C_b and C_b:C_p did not differ between groups (respectively $\chi 2 = 5.96$, n.s; $\chi 2 = 3.31$, n.s; $\chi 2 = 1.56$, n.s.). For UK-343,664, mean C_p was > LLOQ in all groups and no difference between these groups was found ($\chi 2 = 4.39$, n.s.). However, UK-343,664 C_b was > LLOQ in only one of three animals in the MK-801 condition.

For both MK-801 and scopolamine, C_p, C_b and C_b:C_p were determined 30 min after their administration (Table 8). Paralleling the behavioural experiments, both molecules' C_p and C_b were determined in combination with vehicle, vardenafil (3 mg/kg, p.o.) or UK-343,664 (30 mg/kg, p.o.); both were evaluated twice in combination with vehicle (i.e. in both the vardenafil and the UK-343,664 paradigm). For MK-801, mean C_p and C_b were > LLOQ in all

conditions. No differences in C_p ($\chi 2$ = 4.54, n.s.) or C_b:C_p ($\chi 2$ = 1.70) were observed between groups, whereas one was detected for C_b ($\chi 2$ = 6.27, P < 0.05). However, non-parametric adjusted pairwise comparisons revealed no detailed effects on C_b between separate MK-801-dosed groups. Additionally, scopolamine mean C_p and C_b were > LLOQ in all conditions, but no differences between groups were found (plasma $\chi 2$ = 2.68, n.s.; brain $\chi 2$ = 3.46, n.s.; ratio $\chi 2$ = 0.55, n.s.).

Table 7 Mean (±SEM) neuropharmacokinetics (N=3/condition) of vardenafil and UK-343,664 30 min after PO administration (3 mg/kg and 30 mg/kg, respectively) concomitant with saline, MK-801 (0.125 mg/kg, i.p.) or scopolamine (0.1 mg/kg, i.p.). For both compounds, the LLOQ for plasma and brain were 1 ng/ml and 2.5 ng/g, respectively. n.a.: not applicable; BQL: below quantification limit

	Saline	MK-801 (0.125 mg/kg)	Scopolamine (0.1 mg/kg)
Vardenafil (3 mg/kg)			
Cp (ng/ml)	38.3 (2.1)	22.1 (5.3)	10.2 (8.0)
Cb (ng/g)	4.3 (0.2)	2.7 (1.5)	1.2 (1.2)
Cb:Cp	0.11 (0.01)	0.15 (0.04)	0.14 (n.a.)
UK-343,664 (30 mg/kg)			
Cp (ng/ml)	47.3 (7.1)	50.1 (17.1)	5.8 (5.8)
Cb (ng/g)	BQL	1.1 (1.1)	BQL
Cb:Cp	n.a.	0.05 (n.a.)	n.a.

Table 8 Mean (±SEM) neuropharmacokinetics (N=3/condition except for vehicle (N=6/condition)) of MK-801 and scopolamine 30 min after i.p. administration (0.125 mg/kg and 0.1 mg/kg, respectively) concomitant with vehicle, vardenafil (3 mg/kg, p.o.) or UK-343,664 (30 mg/kg, p.o.). For both compounds, the LLOQ for plasma and brain were 1 ng/mL and 2.5 ng/g, respectively. n.a.: not applicable

	Vehicle	Vardenafil (3 mg/kg)	UK-343,664 (30 mg/kg)
MK-801 (0.125 mg/kg)			
Cp (ng/ml)	6.0 (1.3)	6.4 (3.6)	15.1 (2.3)
Cb (ng/g)	48.2 (5.2)	50.6 (8.2)	85.4 (5.3)
Cb:Cp	9.4 (1.5)	12.6 (4.7)	5.8 (0.5)
Scopolamine (0.1 mg/kg)			
Cp (ng/ml)	4.5 (0.8)	3.9 (0.8)	2.7 (0.4)
Cb (ng/g)	16.8 (1.3)	16.3 (3.9)	12.0 (0.5)
Cb:Cp	4.2 (0.7)	4.1 (0.2)	4.7 (0.8)

DISCUSSION

The ORT is a one trial learning and memory task tapping an intrinsic drive to explore and remember aspects of novelty in the environment (Ennaceur 2010; Ennaceur and Delacour 1988). The tasks require discrimination between novel and familiar objects. A number of lines of evidence indicate that the core circuitry that computes this discrimination resides within the perirhinal cortex and is involved in ORT performance (Brown and

Aggleton 2001; Winters and Bussey 2005). Hippocampal processing is additionally involved in object recollection (Mumby 2001). Studies with pharmacological agents reveal a large number of diverse molecular targets that impact object recognition and memory (e.g. de Bruin et al. 2011; Ennaceur et al. 1989; Prickaerts et al. 2005; Prickaerts et al. 2012). Mapping the effects of these pharmacological agents to circuitry and signaling mechanisms increase our understanding of the molecular underpinnings of object recognition and memory. Furthermore, the ability of these compounds to improve ORT performance suggests that such agents may be of therapeutic value to treat cognitive dysfunction in humans. The key to realizing this potential therapeutic value is mapping these molecular mechanisms to relevant disease mechanisms. There are a number of reports of PDE5-Is improving performance in the ORT (Domek-Lopacinska and Strosznajder 2008; Prickaerts et al. 2005; Rutten et al. 2007b; Rutten et al. 2009; van Donkelaar et al. 2008). However, PDE5 expression is limited in the CNS, particularly in forebrain regions at the core of processing object recognition and memory. Therefore, we asked the provocative question: does the PDE5 target for the action of the inhibitors in the ORT lie solely within the brain?

In the present study, we compared the effects of two PDE5-Is in several ORT variants. One compound, vardenafil, was assumed to cross the BBB (Prickaerts et al. 2004a). In the present study we confirmed this assumption, finding that vardenafil had a brain-to-plasma ratio (C_b:C_p) of 0.11 30 min after its oral administration. The other PDE5-I, UK-343,664, was predicted to poorly permeate the BBB because of its physiochemical properties and P-glycoprotein-mediated efflux liability (Abel et al. 2001; Walker et al. 2001). In fact, UK-343,664 (30 mg/kg, p.o.) resulted in easily quantifiable total plasma concentrations, whereas total brain concentrations were below the LLOQ resulting in a C_b:C_p < 0.05. The approximate cerebral blood volume relative to total unperfused brain volume is 0.04 (Hitchcock and Pennington 2006), thus a C_b:C_p >0.04 determined in this study if a PDE5-I was indeed brain penetrant. Collectively, these data confirm the brain penetration of vardenafil and strongly imply the lack of brain partitioning by UK-343,664.

In the behavioural studies, vardenafil improved object recognition memory in three ORT variants, namely, where memory degrades as a function of time or where memory is disrupted by the muscarinic antagonist scopolamine or the NMDA antagonist MK-801. UK-343,664, was not effective in preventing delay-dependent memory decay or memory disruption by MK-801. These results suggest that there is PDE5 within the brain that mediates the effects of such inhibitors on object recognition and memory in these paradigms. Along similar lines, a recent study of Puzzo et al. (2009) indicated that penetration of the BBB is crucial for the beneficial effects of PDE5-Is in a transgenic APP/PS1 mouse model of Alzheimer's disease. It was shown that chronic sildenafil treatment improved spatial working memory in a radial arm maze and contextual fear conditioning, whereas treatment with tadalafil, which is assumed to not cross the BBB, did not improve memory in these tasks (Puzzo et al. 2009).

However, we found that both vardenafil and UK-343,664 ameliorated the disruptive effect of scopolamine. Co-administration of scopolamine with vardenafil or UK-343,664 did not alter the C_b:C_p of the PDE5-Is, consistent with no evidence in the literature suggesting scopolamine compromises BBB integrity. Co-administration of either vardenafil or UK-343,664 with scopolamine also had no effect on scopolamine neuropharmacokinetics. Thus, the parsimonious conclusion is that the effect of UK-343,664 in the scopolamine model is

mediated by systemic PDE5. Therefore, the question remains why the effective dose of UK-343,664 (30 mg/kg, p.o.) in the scopolamine test is not effective in a delay-dependent memory decay test (i.e. natural forgetting), while vardenafil (3 mg/kg, p.o.) is effective in both tests. The memory enhancing effect of UK-343,664 is therefore likely related to its combination with central and/or possible peripheral effects of scopolamine. Interactions of UK-343,664 or vardenafil with MK-801 can be ruled out based on the behavioral and neuropharmacokinetic data.

The behavioural results with vardenafil can be considered in terms of data on the localization of PDE5 in the rat brain. In the first analysis using in situ hybridization in rat, Kotera et al. (1997) reported robust expression of PDE5A mRNA in Purkinje cells of the cerebellum, but in no other brain region. Van Staveren et al. (2003) subsequently confirmed PDE5 mRNA expression in Purkinje cells. In addition, PDE5 mRNA was observed in scattered cells in the hippocampus, dentate gyrus, and cortex. However, PDE5 mRNA was not detected in these brain regions of Alzheimer's disease patients and healthy age-matched controls (Reyes-Irisarri et al. 2007). Immunohistochemical analysis by several groups using different PDE5 antibodies observed robust expression of PDE5 protein in rodent Purkinje cells (Giordano et al. 2001; Kotera et al. 2000; Menniti et al. 2009; Shimizu-Albergine et al. 2003). PDE5 protein expression has also been detected in spinal cord motor neurons (Menniti et al. 2009; Nakamizo et al. 2003) and in the cell bodies of midbrain mesencephalic 5 neurons (Kruse et al. 2006; Menniti et al. 2009). Rare PDE5-expressing cells in cortical areas in rats have also been reported (Menniti et al. 2009). Thus, PDE5 expression in Purkinje neurons is amply confirmed and PDE5 protein is also consistently observed in identified brain stem and spinal cord neurons. In contrast, PDE5 expression in forebrain, particularly in those regions most directly implicated in object recognition and memory, is uncertain; PDE5 mRNA and protein expression in forebrain is not detected in some studies and, when detected, is restricted to scattered cells of unknown phenotype (Menniti et al. 2009). There is, however, biochemical evidence of PDE5 activity in the hippocampus. In rat hippocampal slices, incubation with a PDE5-I and a nitric oxide donor elevated cGMP detected by immunocytochemistry. The increase in cGMP was localized to astrocytes in the CA1, varicose neuronal fibers in the CA2/CA3 region and in some fibers in the CA1 and dentate gyrus (Prickaerts et al. 2002). In slices from mouse hippocampus, cGMP increases were observed in astrocytes and CA3 varicosities (Rutten et al. 2005).

The biochemical/functional effects of PDE5-Is in the hippocampus links PDE5 to circuitry implicated in object discrimination. However, further study is needed to reconcile these functional effects of PDE5 inhibition with the apparently very limited forebrain PDE5 expression. The sparse cellular expression pattern suggests the possibility that the enzyme is localized to one or more neuronal interneuron population. PDE5-expressing interneurons, even if numerically small, could exert powerful, differential effects on network-level signal processing that could contribute to the efficacy of PDE5-Is in the ORT. Further confirmation of expression and identification of the sparse cell types that putatively express the enzyme in forebrain, including object memory-related rhinal and hippocampal areas, is needed as a step towards evaluating this possibility.

The unexpected finding that UK-343,664 ameliorated the effect of scopolamine on ORT performance suggests that for the scopolamine paradigm, inhibition of a PDE5 target outside the BBB also impacts object recognition and memory. PDE5 is highly expressed in

discreet vascular beds throughout the body, including some cerebrovascular beds (Kruuse et al. 2003; Kruuse et al. 2002; Menniti et al. 2009). Thus, it is reasonable to speculate that a locus for the peripheral effect of PDE5 inhibition in the ORT resides in the cerebrovasculature. Our previous studies indicate no effect of vardenafil on local cerebral glucose utilization and cerebral blood flow in the hippocampus and perirhinal areas that might account for an effect in ORT (Rutten et al. 2009). Kruuse and colleagues (2009) also reported lack of effects of PDE5-Is on cerebrovascular responsiveness in humans. Thus, if cerebrovascular PDE5 is the target of the inhibitors in the ORT, the mechanism does not appear to be hemodynamic.

In summary, the results of the present study suggest that there may be multiple mechanisms that mediate the efficacy of PDE5 inhibition to improve performance in cognitive tasks such as the ORT and that these involve enzyme located both within and outside of the brain. In many respects, these results are surprising. That one locus of action is in inside the brain is surprising, given the sparse (or absent) expression of the enzyme in forebrain regions most significantly involved in object recognition and memory. That the other locus could be outside the brain is simply astonishing. PDE5-Is are widely used clinically to treat vascular disorders, where these compounds have been safe and well-tolerated. However, initial studies of such compounds in humans have not enhanced cognitive function (Goff et al. 2009; Schultheiss et al. 2001), although a recent study investigating the effects of PDE5 inhibition in patients with erectile dysfunction revealed a positive effect on several cognitive tasks (Shim et al. 2011). Further preclinical research is clearly needed to better identify the enzyme targets and mechanisms that account for activity in nootropic tasks such as the ORT and this may guide future clinical studies into cognitive function, ideally as a step towards realizing a new therapeutic use of PDE5-Is.

REFERENCES

Abel S, Beaumont KC, Crespi CL, Eve MD, Fox L, Hyland R, Jones BC, Muirhead GJ, Smith DA, Venn RF, Walker DK (2001) Potential role for P-glycoprotein in the non-proportional pharmacokinetics of UK-343,664 in man. *Xenobiotica* 31: 665-76

Baek SB, Bahn G, Moon SJ, Lee J, Kim KH, Ko IG, Kim SE, Sung YH, Kim BK, Kim TS, Kim CJ, Shin MS (2011) The phosphodiesterase type-5 inhibitor, tadalafil, improves depressive symptoms, ameliorates memory impairment, as well as suppresses apoptosis and enhances cell proliferation in the hippocampus of maternal-separated rat pups. *Neurosci Lett* 488: 26-30

Barad M, Bourtchouladze R, Winder DG, Golan H, Kandel E (1998) Rolipram, a type IV-specific phosphodiesterase inhibitor, facilitates the establishment of long-lasting long-term potentiation and improves memory. *Proc Natl Acad Sci USA* 95: 15020-5

Baratti CM, Boccia MM (1999) Effects of sildenafil on long-term retention of an inhibitory avoidance response in mice. *Behav Pharmacol* 10: 731-7

Bender AT, Beavo JA (2006) Cyclic nucleotide phosphodiesterases: molecular regulation to clinical use. *Pharmacol Rev* 58: 488-520

Bliss TV, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 361: 31-9

Boccia MM, Blake MG, Krawczyk MC, Baratti CM (2011) Sildenafil, a selective phosphodiesterase type 5 inhibitor, enhances memory reconsolidation of an inhibitory avoidance task in mice. *Behav Brain Res* 220: 319-24

Boess FG, Hendrix M, van der Staay FJ, Erb C, Schreiber R, van Staveren W, de Vente J, Prickaerts J, Blokland A, Koenig G (2004) Inhibition of phosphodiesterase 2 increases neuronal cGMP, synaptic plasticity and memory performance. *Neuropharmacology* 47: 1081-92

Brown MW, Aggleton JP (2001) Recognition memory: what are the roles of the perirhinal cortex and hippocampus? *Nat Rev Neurosci* 2: 51-61

Cuadrado-Tejedor M, Hervias I, Ricobaraza A, Puerta E, Perez-Roldan JM, Garcia-Barroso C, Franco R, Aguirre N, Garcia-Osta A (2011) Sildenafil restores cognitive function without affecting beta-amyloid burden in a mouse model of Alzheimer's disease. *Br J Pharmacol* 164: 2029-41

de Bruin NM, Prickaerts J, van Loevezijn A, Venhorst J, de Groote L, Houba P, Reneerkens O, Akkerman S, Kruse CG (2011) Two novel 5-HT6 receptor antagonists ameliorate scopolamine-induced memory deficits in the object recognition and object location tasks in Wistar rats. *Neurobiol Learn Mem* 96: 392-402

Devan BD, Sierra-Mercado D, Jr., Jimenez M, Bowker JL, Duffy KB, Spangler EL, Ingram DK (2004) Phosphodiesterase inhibition by sildenafil citrate attenuates the learning impairment induced by blockade of cholinergic muscarinic receptors in rats. *Pharmacol Biochem Behav* 79: 691-9

Domek-Lopacinska K, 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

Ennaceur A (2010) One-trial object recognition in rats and mice: methodological and theoretical issues. *Behav Brain Res* 215: 244-54

Ennaceur A, Cavoy A, Costa JC, Delacour J (1989) A new one-trial test for neurobiological studies of memory in rats. II: Effects of piracetam and pramiracetam. *Behav Brain Res* 33: 197-207

Ennaceur A, Delacour J (1988) A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behav Brain Res* 31: 47-59

Frey U, Huang YY, Kandel ER (1993) Effects of cAMP simulate a late stage of LTP in hippocampal CA1 neurons. *Science* 260: 1661-4

Ghofrani HA, Osterloh IH, Grimminger F (2006) Sildenafil: from angina to erectile dysfunction to pulmonary hypertension and beyond. *Nat Rev Drug Discov* 5: 689-702

Giordano D, De Stefano ME, Citro G, Modica A, Giorgi M (2001) Expression of cGMP-binding cGMP-specific phosphodiesterase (PDE5) in mouse tissues and cell lines using an antibody against the enzyme amino-terminal domain. *Biochim Biophys Acta* 1539: 16-27

Goff DC, Cather C, Freudenreich O, Henderson DC, Evins AE, Culhane MA, Walsh JP (2009) A placebo-controlled study of sildenafil effects on cognition in schizophrenia. *Psychopharmacology (Berl)* 202: 411-7

Halene TB, Siegel SJ (2007) PDE inhibitors in psychiatry--future options for dementia, depression and schizophrenia? *Drug Discov Today* 12: 870-8

Hitchcock SA, Pennington LD (2006) Structure-brain exposure relationships. *J Med Chem* 49: 7559-83

Kiss T, Hoffmann WE, Hajos M (2010) Delta oscillation and short-term plasticity in the rat medial prefrontal cortex: modelling NMDA hypofunction of schizophrenia. *Int J Neuropsychopharmacol*: 1-14

Kleppisch T, Feil R (2009) cGMP signalling in the mammalian brain: role in synaptic plasticity and behaviour. *Handb Exp Pharmacol*: 549-79

Klinkenberg I, Blokland A (2010) The validity of scopolamine as a pharmacological model for cognitive impairment: a review of animal behavioral studies. *Neurosci Biobehav Rev* 34: 1307-50

Kotera J, Fujishige K, Omori K (2000) Immunohistochemical localization of cGMP-binding cGMP-specific phosphodiesterase (PDE5) in rat tissues. *J Histochem Cytochem* 48: 685-93

Kotera J, Yanaka N, Fujishige K, Imai Y, Akatsuka H, Ishizuka T, Kawashima K, Omori K (1997) Expression of rat cGMP-binding cGMP-specific phosphodiesterase mRNA in Purkinje cell layers during postnatal neuronal development. *Eur J Biochem* 249: 434-42

Kruse LS, Sandholdt NT, Gammeltoft S, Olesen J, Kruuse C (2006) Phosphodiesterase 3 and 5 and cyclic nucleotide-gated ion channel expression in rat trigeminovascular system. *Neurosci Lett* 404: 202-7

Kruuse C, Hansen AE, Larsson HB, Lauritzen M, Rostrup E (2009) Cerebral haemodynamic response or excitability is not affected by sildenafil. *J Cereb Blood Flow Metab* 29: 830-9

Kruuse C, Thomsen LL, Birk S, Olesen J (2003) Migraine can be induced by sildenafil without changes in middle cerebral artery diameter. *Brain* 126: 241-7

Kruuse C, Thomsen LL, Jacobsen TB, Olesen J (2002) The phosphodiesterase 5 inhibitor sildenafil has no effect on cerebral blood flow or blood velocity, but nevertheless induces headache in healthy subjects. *J Cereb Blood Flow Metab* 22: 1124-31

Lakics V, Karran EH, Boess FG (2010) Quantitative comparison of phosphodiesterase mRNA distribution in human brain and peripheral tissues. *Neuropharmacology* 59: 367-74

Menniti FS, Faraci WS, Schmidt CJ (2006) Phosphodiesterases in the CNS: targets for drug development. *Nat Rev Drug Discov* 5: 660-70

Menniti FS, Ren J, Coskran TM, Liu J, Morton D, Sietsma DK, Som A, Stephenson DT, Tate BA, Finklestein SP (2009) Phosphodiesterase 5A inhibitors improve functional recovery after stroke in rats: optimized dosing regimen with implications for mechanism. *J Pharmacol Exp Ther* 331: 842-50

Moghaddam B, Jackson ME (2003) Glutamatergic animal models of schizophrenia. *Ann N Y Acad Sci* 1003: 131-7

Mumby DG (2001) Perspectives on object-recognition memory following hippocampal damage: lessons from studies in rats. *Behav Brain Res* 127: 159-81

Nakamizo T, Kawamata J, Yoshida K, Kawai Y, Kanki R, Sawada H, Kihara T, Yamashita H, Shibasaki H, Akaike A, Shimohama S (2003) Phosphodiesterase inhibitors are neuroprotective to cultured spinal motor neurons. *J Neurosci Res* 71: 485-95

Prickaerts J, Koopmans G, Blokland A, Scheepens A (2004a) Learning and adult neurogenesis: survival with or without proliferation? *Neurobiol Learn Mem* 81: 1-11

Prickaerts J, Sik A, van der Staay FJ, de Vente J, Blokland A (2005) Dissociable effects of acetylcholinesterase inhibitors and phosphodiesterase type 5 inhibitors on object recognition memory: acquisition versus consolidation. *Psychopharmacology (Berl)* 177: 381-90

Prickaerts J, Sik A, van Staveren WC, Koopmans G, Steinbusch HW, van der Staay FJ, de Vente J, Blokland A (2004b) Phosphodiesterase type 5 inhibition improves early memory consolidation of object information. *Neurochem Int* 45: 915-28

Prickaerts J, Steinbusch HW, Smits JF, de Vente J (1997) Possible role of nitric oxide-cyclic GMP pathway in object recognition memory: effects of 7-nitroindazole and zaprinast. *Eur J Pharmacol* 337: 125-36

Prickaerts J, van Goethem NP, Chesworth R, Shapiro G, Boess FG, Methfessel C, Reneerkens OA, Flood DG, Hilt D, Gawryl M, Bertrand S, Bertrand D, Konig G (2012) EVP-6124, a novel and selective alpha7 nicotinic acetylcholine receptor partial agonist, improves memory performance by potentiating the acetylcholine response of alpha7 nicotinic acetylcholine receptors. *Neuropharmacology* 62: 1099-110

Prickaerts J, van Staveren WC, Sik A, Markerink-van Ittersum M, Niewohner U, van der Staay FJ, Blokland A, de Vente J (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: 351-61

Puzzo D, Sapienza S, Arancio O, Palmeri A (2008) Role of phosphodiesterase 5 in synaptic plasticity and memory. *Neuropsychiatr Dis Treat* 4: 371-87

Puzzo D, Staniszewski A, Deng SX, Privitera L, Leznik E, Liu S, Zhang H, Feng Y, Palmeri A, Landry DW, Arancio O (2009) Phosphodiesterase 5 inhibition improves synaptic function, memory, and amyloid-beta load in an Alzheimer's disease mouse model. *J Neurosci* 29: 8075-86

Reyes-Irisarri E, Markerink-Van Ittersum M, Mengod G, de Vente J (2007) Expression of the cGMP-specific phosphodiesterases 2 and 9 in normal and Alzheimer's disease human brains. *Eur J Neurosci* 25: 3332-8

Rutten K, Lieben C, Smits L, Blokland A (2007a) The PDE4 inhibitor rolipram reverses object memory impairment induced by acute tryptophan depletion in the rat. *Psychopharmacology (Berl)* 192: 275-82

Rutten K, Prickaerts J, Blokland A (2006) Rolipram reverses scopolamine-induced and time-dependent memory deficits in object recognition by different mechanisms of action. *Neurobiol Learn Mem* 85: 132-8

Rutten K, Prickaerts J, Hendrix M, van der Staay FJ, Sik A, Blokland A (2007b) 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: 107-12

Rutten K, Van Donkelaar EL, Ferrington L, Blokland A, Bollen E, Steinbusch HW, Kelly PA, Prickaerts JH (2009) Phosphodiesterase inhibitors enhance object memory independent of cerebral blood flow and glucose utilization in rats. *Neuropsychopharmacology* 34: 1914-25

Rutten K, Vente JD, Sik A, Ittersum MM, Prickaerts J, Blokland A (2005) The selective PDE5 inhibitor, sildenafil, improves object memory in Swiss mice and increases cGMP levels in hippocampal slices. *Behav Brain Res* 164: 11-6

Schreiber R, Vivian J, Hedley L, Szczepanski K, Secchi RL, Zuzow M, van Laarhoven S, Moreau JL, Martin JR, Sik A, Blokland A (2007) Effects of the novel 5-HT(6) receptor antagonist RO4368554 in rat models for cognition and sensorimotor gating. *Eur Neuropsychopharmacol* 17: 277-88

Schultheiss D, Muller SV, Nager W, Stief CG, Schlote N, Jonas U, Asvestis C, Johannes S, Munte TF (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

Shim YS, Pae CU, Kim SW, Kim HW, Kim JC, Koh JS (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: 109-14

Shimizu-Albergine M, Rybalkin SD, Rybalkina IG, Feil R, Wolfsgruber W, Hofmann F, Beavo JA (2003) Individual cerebellar Purkinje cells express different cGMP phosphodiesterases (PDEs): in vivo phosphorylation of cGMP-specific PDE (PDE5) as an indicator of cGMP-dependent protein kinase (PKG) activation. *J Neurosci* 23: 6452-9

Sik A, van Nieuwehuyzen P, Prickaerts J, Blokland A (2003) Performance of different mouse strains in an object recognition task. *Behav Brain Res* 147: 49-54

Son H, Lu YF, Zhuo M, Arancio O, Kandel ER, Hawkins RD (1998) The specific role of cGMP in hippocampal LTP. *Learn Mem* 5: 231-45

van der Staay FJ, Rutten K, Barfacker L, Devry J, Erb C, Heckroth H, Karthaus D, Tersteegen A, van Kampen M, Blokland A, Prickaerts J, Reymann KG, Schroder UH, Hendrix M (2008) The novel selective PDE9 inhibitor BAY 73-6691 improves learning and memory in rodents. *Neuropharmacology* 55: 908-18

van der Staay FJ, Rutten K, Erb C, Blokland A (2011) Effects of the cognition impairer MK-801 on learning and memory in mice and rats. *Behav Brain Res* 220: 215-29

van Donkelaar EL, Rutten K, Blokland A, Akkerman S, Steinbusch HW, Prickaerts J (2008) Phosphodiesterase 2 and 5 inhibition attenuates the object memory deficit induced by acute tryptophan depletion. *Eur J Pharmacol* 600: 98-104

Van Staveren WC, Steinbusch HW, Markerink-Van Ittersum M, Repaske DR, Goy MF, Kotera J, Omori K, Beavo JA, De Vente J (2003) mRNA expression patterns of the cGMP-hydrolyzing phosphodiesterases types 2, 5, and 9 during development of the rat brain. *J Comp Neurol* 467: 566-80

Vardigan JD, Huszar SL, McNaughton CH, Hutson PH, Uslaner JM (2010) MK-801 produces a deficit in sucrose preference that is reversed by clozapine, D-serine, and the metabotropic glutamate 5 receptor positive allosteric modulator CDPPB: relevance to negative symptoms associated with schizophrenia? *Pharmacol Biochem Behav* 95: 223-9

Walker DK, Beaumont KC, Comby P, Evans KM, Gedge JI, Halliday RC, Roffey SJ, Wright PA (2001) Pharmacokinetics and metabolism of a selective PDE5 inhibitor (UK-343,664) in rat and dog. *Xenobiotica* 31: 651-64

Winters BD, Bussey TJ (2005) Transient inactivation of perirhinal cortex disrupts encoding, retrieval, and consolidation of object recognition memory. *J Neurosci* 25: 52-61

Zhang HT, Crissman AM, Dorairaj NR, Chandler LJ, O'Donnell JM (2000) Inhibition of cyclic AMP phosphodiesterase (PDE4) reverses memory deficits associated with NMDA receptor antagonism. *Neuropsychopharmacology* 23: 198-204

CHAPTER 4

Inhibition of phoshodiesterase type 2 or type 10 reverses object memory deficits induced by scopolamine or MK-801

Olga A. H. Reneerkens, Kris Rutten, Eva Bollen, Thorsten Hage, Arjan Blokland, Harry W. M. Steinbusch & Jos Prickaerts

Published in Behavioural Brain Research (2013), 236(1), 16-22

ABSTRACT

The objective of this study was to assess the effects of phosphodiesterase type 2 (PDE2) and type 10 (PDE10) inhibition on memory function in the object recognition task using the scopolamine- and MK-801-induced memory deficit model. The effects of the PDE2 inhibitor BAY 60-7550 and the PDE10 inhibitor PQ-10 on object recognition performance were investigated in the scopolamine (0.1 mg/kg, i.p.) or MK-801 (0.125 mg/kg, i.p.) model. BAY 60-7550 was tested at a dose of 0.3 - 3 mg/kg (p.o.) in both models; PQ-10 was tested at doses of 0.1 – 1 mg/kg (p.o.) in the scopolamine model and 0.3 – 3 mg/kg in the MK-801 model. All compounds were injected 30 min before the learning trial. Both BAY 60-7550 (1 mg/kg) and PQ-10 (0.3 mg/kg) attenuated the scopolamine-induced memory deficit. The MK-801-induced memory deficit was reversed after treatment with each PDE inhibitor at a dose of 1 mg/kg or higher. PQ-10 was highly brain penetrant, whereas 60-7550 levels in the brain were very low after oral treatment. We concluded that since BAY 60-7550 and PQ-10 reversed both scopolamine- and MK-801-induced memory deficits, this supports the notion that dual substrate PDE inhibitors might be suitable candidates for cognition enhancement.

INTRODUCTION

Recently, phosphodiesterase inhibitors (PDE-Is) have gained increased attention as a possible cognition enhancer (for review see Blokland et al. 2006 and Chapter 2). So far, eleven subclasses of phosphodiesterases (PDEs) are identified and depending on the subclass they belong to, these enzymes hydrolyze cyclic adenosine monophosphate (cAMP) and/or cyclic guanosine monophosphate (cGMP). cAMP and cGMP are second messengers which play an important role in glutamatergic signal transduction, including processes underlying long term potentiation (LTP) (Bailey et al. 1996; Chien et al. 2003; Frey et al. 1993; Son et al. 1998), which is the proposed neurophysiological correlate of memory (Bliss and Collingridge 1993). Indeed, several PDE-Is have been found to increase e.g. neuronal excitability (Threlfell et al. 2009) and facilitate LTP (Boess et al. 2004). Furthermore, cAMP and cGMP are assumed to directly affect neurotransmitter release, including acetylcholine (Imanishi et al. 1997). It has indeed been shown that central application of analogues of these second messengers can have memory enhancing effects in rodents (e.g. Bernabeu et al. 1996; Matsumoto et al. 2006; Prickaerts et al. 2002). Therefore, PDE-Is may modulate learning and memory processes, as they are supposed to enhance second messenger signaling in the brain.

The pro-cognitive effects of novel compounds on memory processes are often assessed by means of pharmacological deficit models, which temporarily impair memory performance. A widely used model to study the effects of drug administration before the learning trial is the scopolamine model (for review see Klinkenberg and Blokland 2010). This anti-cholinergic agent has shown to impair memory in several behavioural tests including the object recognition task (ORT, e.g. Rutten et al. 2006; Schreiber et al. 2007). The ORT, also known as the novel object recognition (NOR) task, is a one trial learning test (Akkerman et al. 2012). In a previous study we have shown that the cAMP-selective PDE4-I rolipram reversed a scopolamine-induced memory deficit in the ORT in rats (Rutten et al. 2006). In addition, rolipram was shown to reverse the effects of scopolamine in several other behavioural tests (Egawa et al. 1997; Ghelardini et al. 2002; Imanishi et al. 1997; Zhang et al. 2000). In comparison to PDE4 inhibition, the literature on effects of dual substrate, i.e. both cAMP and cGMP, PDE-Is including those for PDE2 and PDE10 on memory processes in deficit models in the ORT are very limited. To our knowledge, only Van Donkelaar et al. (van Donkelaar et al. 2008) reported that the PDE2-I BAY 60-7550 given 30 min before T1 can reverse a memory deficit caused by acute tryptophan depletion (ATD), which causes a temporal lowering of serotonin in the brain (e.g. van Donkelaar et al. 2008).

After scopolamine, the MK-801 induced memory deficit model is the second most commonly used deficit model used in preclinical cognition research (van der Staay et al. 2011). MK-801 is an N-methyl-D-aspartic acid (NMDA) antagonist which impairs among others memory function and attention processes (e.g. Boess et al. 2004; van der Staay et al. 2008; Zhang et al. 2000) and is therefore assumed to be more affiliated to cognitive deficits related to schizophrenia. Therefore, the MK-801 model (for review see van der Staay et al. 2011) would be a useful addition to the scopolamine model, since recent studies (e.g. Grauer et al. 2009; Schmidt et al. 2008) have focused on PDE10 inhibition as a treatment for positive, negative and cognitive symptoms of schizophrenia. In addition, previous studies have demonstrated that PDE2-I, PDE4-Is and PDE9-I reverse MK-801 induced working

memory, social odor recognition or avoidance learning deficits in a variety of behavioural tasks in rodents (Boess et al. 2004; Grauer et al. 2009; van der Staay et al. 2008; Zhang et al. 2000; Zhang et al. 2005).

The aim of the present study was to further characterize the effects of PDE2-I and PDE10-I on memory function. Therefore, we investigated the effects of the PDE2-I BAY 60-7550 and the PDE10-I PQ-10 on object recognition memory in scopolamine- and MK-801-induced deficit models. PQ-10 belongs to the same class as the PDE10 inhibitor papaverine, but IC50 literature suggests that is about 5 times more potent then papaverine, yet still about 10 times less potent the known PDE10 inhibitors TP-10 and MP-10 (Alderton et al. 2009; Siuciak 2008). To our knowledge the PQ-10 inhibitor has not been tested before on cognitive behaviour. We also determined the amount of BAY 60-7550 and PQ-10 in the blood plasma and brain tissue after treatment to gain insight into their brain penetration properties. We hypothesized that both PDE-Is could reverse the effects of scopolamine and MK-801 in the ORT. To our knowledge, this is the first time effects of these two different putative cognition enhancing drugs are assessed in deficit models related to dementia and schizophrenia.

METHODS AND MATERIALS

Animals

All experimental procedures were approved by the local ethical committees for animal experiments of the Maastricht University or biocrea and met governmental guidelines. For the behavioural experiments, 24 four-month-old male Wistar rats (Harlan, Horst, The Netherlands) were used with average body weights of 498 g (± 5 (standard error of mean, SEM), batch 1) and 351 g (± 3 (SEM), batch 2) for respectively the scopolamine and the MK-801 experiments. The animals were housed individually in standard Makrolon cages on sawdust bedding in an air-conditioned room (about 20°C). They were kept on a 12/12-h reversed light/dark cycle (lights on from 19.00 to 7.00 h) and had free access to food and water. The rats were housed in the same room as where they were tested. A radio, which was playing softly, provided background noise in the room. All testing was done between 9.00 and 18.00 h. Six animals were chosen randomly for the determination of BAY 60-7550 and PQ-10 levels in blood plasma and brain. For the additional BAY 60-7550 experiment performed at biocrea, 9 two-month-old female Wistar rats (Charles River, Sulzfeld, Germany) were used with an average body weight of 200-300 g. The animals had free access to food and water.

Treatment

Scopolamine hydrobromide and MK-801 hydrogen maleate (Sigma Aldrich, Zwijndrecht, The Netherlands) were prepared daily and dissolved in saline. Based on previous results (data not shown), a dose of 0.125 mg/kg was selected for MK-801 and a dose of 0.1 mg/kg for scopolamine, since these doses impaired recognition memory without affecting exploratory activity. Furthermore, these specific doses of scopolamine and MK-801 are generally accepted as memory impairer in rodents, without causing sensorimotor

impairments or motivational effect (Klinkenberg and Blokland 2010; 2011; van der Staay et al. 2011), although possible additional effects on attention cannot be ruled out completely. Administration (1 ml/kg) was intraperitoneal (i.p.) (30 min before T1).

BAY 60-7550 and PQ-10 were first dissolved in 1.5 ml 100% ethanol with 2% Tween 80. After extraction of ethanol via vaporization under N2 gas, the compounds were dissolved in 0.5% methylcellulose. BAY 60-7550 was tested at the doses of 0.3 - 3 mg/kg, PQ-10 was tested at the doses of 0.1 - 1 mg/kg in the scopolamine and 0.3 - 3 mg/kg in the MK-801 model, respectively. Both compounds were given by oral gavage (p.o.) 30 min before T1 in an injection volume of 2 ml/kg. BAY 60-7550 was a kind gift from BAYER (Wuppertal, Germany) and PQ-10 was kindly donated by Johnson & Johnson (Beerse, Belgium). The experimenter was blind to the compounds and doses tested. Twenty-four animals (batch 1) were used for each condition in the scopolamine study; sixteen (batch 2) for the MK-801 study. Of note, control conditions (saline, MK-801 and scopolamine) were always tested in 24 animals as part of the training protocol (see below). Visual inspection of the rats following drug administration indicated no gross side effects (e.g. sensorimotor effects or sedation) following drug administration.

Object recognition memory

The ORT was performed as described elsewhere (Ennaceur et al. 1997; Prickaerts et al. 1997). The apparatus consisted of a circular arena, 83 cm in diameter. Half of the 40 cm high wall was made of gray polyvinyl chloride, the other half of transparent polyvinyl chloride. Two objects were placed in a symmetrical position about 10 cm away from the gray wall. We used four different sets of objects: 1) a cone consisting of a gray polyvinyl chloride base (maximal diameter 18 cm) with a collar on top made of brass (total height 16 cm), 2) a standard 1 l transparent glass bottle (diameter 10 cm, height 22 cm) filled with water, 3) a solid metal cube (10.0 x 5.0 x 7.5 cm) with two holes (diameter 1.9 cm), and 4) a solid aluminium cube with a tapering top (13.0 x 8.0 x 8.0 cm). A rat could not displace the objects. Fluorescent red tubes and a light bulb provided a constant illumination of about 8 lux on the floor of the apparatus and the light intensity was equal in the different parts of the apparatus.

A testing session comprised two trials. The duration of each trial was 3 min. During T1 the apparatus contained two identical objects (samples). A rat was always placed in the apparatus facing the wall at the middle of the front (transparent) segment. After the first exploration period the rat was put back in its home cage. Subsequently, after a predetermined delay interval, the rat was put back in the apparatus for T2, but now with two dissimilar objects, a familiar one (the sample) and a new one. The times spent in exploring each object during T1 and T2 were recorded manually with a personal computer.

Exploration was defined as follows: directing the nose to the object at a distance of no more than 2 cm and/or touching the object with the nose. Sitting on the object was not considered as exploratory behaviour. In order to avoid the presence of olfactory trails the objects were always thoroughly cleaned with 70% ethanol. All combinations and locations of objects were used in a balanced manner to reduce potential biases due to preferences for particular locations or objects.

In several studies we have demonstrated that rodents show good object memory performance when a 1 h delay is interposed between T1 and T2 (Rutten et al. 2007a; van Donkelaar et al. 2008). However, when a 24 h delay interval is used, animals did not discriminate between the novel and familiar object in T2, indicating that they did not remember the object that was presented in T1 (Prickaerts et al. 2004; Rutten et al. 2007b). Using a 4 h delay, the discrimination performance was between the performance of the 1 h and 24 h delay, suggesting a delay-dependent forgetting in this task (Sik et al. 2003). In the present study a 1 h interval was used to test the memory enhancing effects of PDE-Is in the scopolamine and MK-801 deficit model.

In the first week, the animals were handled daily and adapted to the procedure in two days, i.e. they were allowed to explore the apparatus (without any objects) twice for 3 minutes each day. Next, they were adapted to the testing and p.o. and i.p. administration procedures by a saline injection (2 ml/kg p.o., 1 ml/kg i.p.) 30 min before T1 until a stable discrimination performance was observed, i.e. a good discrimination at 1 h interval and no discrimination at 24 h interval (all animals performed as expected). After this, testing of the control conditions began, i.e. animals were treated with saline, scopolamine (0.1 mg/kg, i.p.) or MK-801 (0.125 mg/kg, i.p.) together with the vehicle of the PDE-Is (p.o.), 30 min before T1. Subsequently, BAY 60-7550 (0.3-3 mg/kg, p.o.) and PQ-10 (0.1-1 mg/kg p.o. in the scopolamine, 0.3-3 mg/kg p.o. in the MK-801 model) were tested in both deficit models. A wash-out period of at least one day was applied between testing session. The control conditions (saline, scopolamine and MK-801) were tested in 24 animals. In addition, each PDE-I was tested in 24 (scopolamine study, batch 1) or 16 (MK-801 study, batch 2) animals.

Determination of BAY-60-7550 and PQ-10 levels in plasma and brain samples

BAY 60-7550 (1 mg/kg) and PQ-10 (1 mg/kg) were administered p.o. 30 min before blood collection (300 µl) from the saphenous vein into a heparinized tube (microcuvette CB300, Sarstedt, Germany). Sampled blood was centrifuged at 3000g for 10 min at 4 °C. After blood sampling, rats were killed by decapitation and the heads were immerged in liquid nitrogen for 5 s. Subsequently, the brain was removed and rinsed with distilled water. Plasma and brains were stored at -80 °C until compound determination took place.

Plasma and brain levels of BAY 60-7550 and PQ-10 were determined using a qualified research UPLC-MS/MS method. After solubilisation (with methanol) and protein precipitation (with acetonitrile), plasma and brain samples were quantified on a reversed phase UPLC-column (Acquity UPLC C18 1.7 µ BEH, 50x2.1mm; Waters, Milford, US). Mobile phases consisted of 0.1 % FA (solvent A) and acetonitrile (solvent B). Chromatographic separation was obtained by gradient elution (90 % solvent A; 10 % solvent B starting conditions to 10 % solvent A; 90 % solvent B in 1 min) at a flow rate of 0.8 ml/min. Total run time was 1.7 min.

UPLC-MS/MS analysis was carried out on a API-4000 MS/MS (Applied Biosystems, Toronto, Canada), which was coupled to an UPLC-system (Waters, Milford, US). The MS/MS, operated in the positive ion mode using the TurboIonSpray□-interface (electrospray ionization), was optimized for the quantification of the compounds (MRM transition for BAY 60-7550 was 477.2 > 459. MRM transition for PQ-10 was 404.2 > 258). The limit of quantification was determined for each compound separately; for BAY 60-7550 the limits were 0.2 ng/ml for plasma samples and 1 ng/g for brain samples; for PQ-10 the limits were 1

ng/ml and 10 ng/g respectively. The intra batch accuracy from independent QC samples was about 20% for plasma and brain samples. Brain samples were homogenized in milliQ-water (1/10 w/w).

For the additional determination of the levels of the PDE2-I BAY 60-7550 in blood plasma and brain tissue, BAY 60-7550 (10 mg/kg) was dissolved in vehicle solution (5% ethanol 10% solutol in water) and was administered p.o. 30, 60 and 240 min before blood (200 µl) collection by puncture of the ophthalmic venous plexus into Brand-micro-haematocrit-tubes applying a slight isoflurane anesthesia. Collected blood samples were centrifuged at 2000g for 10 min at 4 °C. After blood sampling, rats were killed by decapitation. The brain was removed, washed with cooled PBS buffer at 4 °C, dried with a paper towel and immerged into liquid nitrogen for 5-10 s. Plasma and brains stored at -80 °C until compound determination took place.

Plasma and brain levels of BAY 60-7550 were determined using a LC-ESI-MS/MS method. After solubilisation (with methanol) and protein precipitation (with acetonitrile), plasma and brain samples were quantified on a reversed phase HPLC-column (Luna C18, 50x2 mm, 3 µm; Phenomenex, Germany). The mobile phases consisted of 1 mM ammonium acetate with 5% acetonitril (solvent A) and acetonitrile (solvent B). Chromatographic separation was obtained by gradient elution (90 % solvent A; 10 % solvent B starting conditions to 0 % solvent A; 100 % solvent B in 3 min) at a flow rate of 0.6 ml/min.

The LC-ESI-MS/MS analysis for BAY 60-7550 was carried out on a API-3000 triple quad mass spectrometer (Applied Biosystems, Toronto, Canada) with Turbo ion spray interface working in positive ion mode. The MS/MS system was coupled to a binary pump LC-system HP Agilent 1100 (Agilent Technologies, Santa Clara, US) and optimized for the quantification of the test compound BAY 60-7550 (MRM transition was 477.3 > 459.3). MRM transition for PQ-10 was 404.2 > 258). The determined limit of quantification for BAY 60-7550 was 0.64 ng/ml and 0.64 ng/g for plasma and brain samples, respectively.

Statistical analysis

The basic measures in the ORT were the times spent exploring an object during T1 and T2. Table 1 depicts how these measures of the ORT ($e1$, $e2$ and $d2$) were calculated. $e1$ and $e2$ are measures of the total exploration time of both objects during T1 and T2 respectively. $d2$ is considered as index measure of discrimination between the new and the familiar objects. In fact, $d2$ is a relative measure of discrimination that corrects for exploration activity ($e2$). Thus, there should be no differences in $d2$ indices between experiments with similar treatments at similar intervals.

Table 1 Measures involved in the object recognition test. $e1$ is the measure of the time spent in exploring both identical objects ($a1$ and $a2$) in the first trial, and $e2$ is the measure of the time spent in exploring both the familiar (a) and new object (b) in the second trial; $d2$ is the measure of discrimination between the new and familiar objects

Exploration	Discrimination
$e1 = a1 + a2$	
$e2 = a + b$	$d2 = (b - a) / e2$

One-sample t-statistics were performed in order to assess whether *d*2 differed from zero per treatment condition (within comparison). Effects between the different conditions were assessed by one-way ANOVA (between comparisons). In case of a statistically reliable dose effect, comparisons between means of the different doses were analyzed in more detail using post hoc Bonferroni t-tests.

RESULTS

Effects of BAY 60-7550 and PQ-10 on scopolamine-induced memory deficits

The results of the BAY 60-7550 and PQ-10 treatment, 30 min before T1, are summarized in Table 2. No differences were observed between treatment conditions in the level of exploration in T1 (*e*1: F (7,191) = 1.41, n.s.) or T2 (*e*2: F(7,191) = 0.15, n.s.).

Table 2 Effects of treatment with BAY 60-7550 and PQ-10 in the scopolamine-induced memory deficit model on exploration time. Drug administration (p.o.) was 30 min before T1. The delay interval between T1 and T2 was 1 h. Mean values (± SEM) of total exploration time (s) during T1 (*e*1) and T2 (*e*2). n = 24 per condition. BAY = BAY 60-7550, PQ = PQ-10, scop = scopolamine; all in mg/kg. No differences from the scopolamine condition were found

PO	vehicle	vehicle	BAY 0.3	BAY 1	BAY 3	PQ 0.1	PQ 0.3	PQ 1
IP	saline	scop	scop 0.1	scop 0.1	scop 0.1	scop 0.1	scop 0.1	scop 0.1
*e*1	17.15	16.76	18.98	18.62	19.22	17.83	19.60	21.15
	(1.26)	(1.32)	(1.32)	(1.27)	(1.27)	(0.91)	(1.22)	(1.16)
*e*2	20.15	20.34	21.06	19.16	19.83	19.88	19.50	19.70
	(9.00)	(2.57)	(1.53)	(1.03)	(1.44)	(1.18)	(1.41)	(1.11)

The effects of BAY 60-7550 and PQ-10 treatment on the relative discrimination index *d*2 are presented in Figure 1. One sample t-tests showed that *d*2 values of the saline, 1 - 3 mg/kg BAY 60-7550 and 0.3 - 1 mg/kg PQ-10 conditions differed from zero. In contrast, the scopolamine, 0.3 mg/kg BAY 60-7550 and 0.1 mg/kg PQ-10 conditions showed no differences from zero. When comparing between groups, differences were found for the *d*2 index ($F(7,191) = 18.87$, $P < 0.001$). Accordingly, the *d*2 values for the saline, 1 – 3 mg/kg BAY 60-7550 and 0.3 – 1 mg/kg PQ-10 conditions were higher than for the scopolamine, 0.3 mg/kg BAY-60-7550 and 0.1 mg/kg PQ-10 conditions (Bonferroni t-tests, see Figure 1).

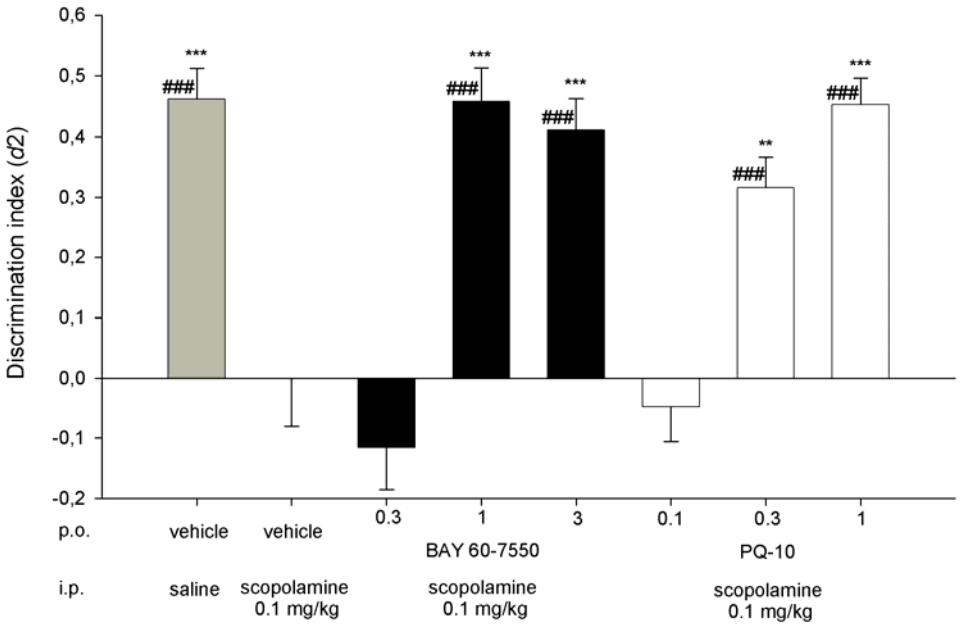

Figure 1 Effects of BAY 60-7550 and PQ-10 on discrimination performance (*d*2) the ORT (means ± SEM). All drugs were given 30 min before T1. Interval was 1h. BAY 60-7550 as well as PQ-10 completely reversed the scopolamine-induced memory deficit. A difference from the scopolamine condition is depicted with asterisks (Bonferroni t-tests, **: P < 0.01; ***: P < 0.001). A difference from zero is depicted with # (one sample t-tests, ###: P < 0.001). n=24 per condition

Effects of BAY 60-7550 and PQ-10 on MK-801-induced memory deficits

The results of the BAY 60-7550 or PQ-10 treatment, 30 min before T1 in combination with MK-801, are summarized in Table 3. Differences between treatment were observed in the level of exploration in T1 (*e*1: $F(7,143) = 5.49$, $P < 0.001$.). When comparing between groups with a post hoc analysis (Bonferroni t-tests, $P < 0.05$), the level of exploration in T1 in the 0.3 and 1 mg/kg PQ-10 condition was higher than in the saline condition. Also, the level of exploration in T1 was higher in the 3 mg/kg PQ-10 than in the other conditions, except for 0.3 and 1 mg/kg PQ-10. In T2, no differences were observed between treatment conditions in the level of exploration (*e*2: $F(7,143) = 1.90$, n.s.).

The effects of BAY 60-7550 and PQ-10 (injected 30 min before T1) on the relative discrimination index *d*2 are presented in Figure 2. One sample t-tests showed that the *d*2 value of the saline, BAY 60-7550 1-3mg/kg and PQ-10 1-3 mg/kg condition differed from zero, in contrast to the MK-801, BAY 60-7550 0.3 mg/kg and PQ-10 0.3 mg/kg conditions. Accordingly, when comparing between groups, differences were found for the *d*2 index ($F(7,143) = 9.05$, $P < 0.001$). Bonferroni post hoc t-tests ($P < 0.05$) comparisons revealed that the *d*2 values were higher in the saline, BAY 60-7550 1 - 3 mg/kg and PQ-10 1-3 mg/kg conditions than in the MK-801 and PQ-10 0.3 mg/kg conditions. In addition, the *d*2 was higher in saline than in the BAY 60-7550 0.3 mg/kg condition.

Table 3 Effects of treatment with BAY 60-7550 and PQ-10 in the MK-801-induced memory deficit model on exploration time. Drug administration (p.o.) was 30 min before T1. The delay interval between T1 and T2 was 1 h. n = 24 per vehicle condition, n = 16 per experimental condition. Mean values (± SEM) of total exploration time (s) during T1 (*e*1) and T2 (*e*2). BAY = BAY 60-7550, PQ = PQ-10, MK = MK-801; all in mg/kg. A difference from the MK-801 condition is depicted with asterisks (Bonferroni t-tests, **: P < 0.01)

PO IP	vehicle saline	vehicle MK	BAY 0.3 MK 0.125	BAY 1 MK 0.125	BAY 3 MK 0.125	PQ 0.3 MK 0.125	PQ 1 MK 0.125	PQ 3 MK 0.125
*e*1	15.35 (1.17)	17.18 (1.27)	18.06 (1.60)	18.64 (1.66)	18.42 (1.38)	23.18 (1.80)	23.03 (1.78)	26.38 (2.38)**
*e*2	17.94 (0.92)	19.08 (1.14)	19.24 (1.28)	22.44 (2.13)	20.99 (2.20)	22.00 (1.88)	22.28 (1.79)	24.25 (1.63)

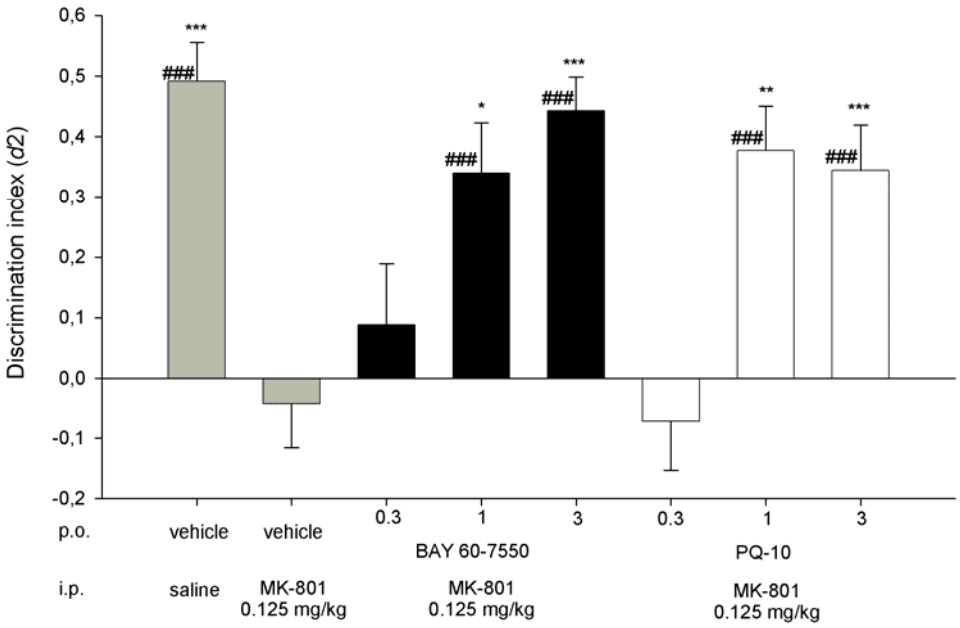

Figure 2 Effects of BAY 60-7550 and PQ-10 on discrimination performance (*d*2) in the ORT (means ± SEM). All drugs were given 30 min before T1; interval was 1h. BAY 60-7550 and PQ-10 completely reversed the MK-801-induced memory deficit. A difference from the vehicle/MK-801 condition is depicted with asterisks (Bonferroni t-tests, *: P < 0.05; **: P < 0.01; ***: P < 0.001). A difference from zero is depicted with # (one sample t-tests, ###: P < 0.001). n=24 per vehicle condition, n=16 per experimental condition

Determination of BAY-60-7550 and PQ-10 levels in plasma and brain samples

The concentrations of BAY 60-7550 or PQ-10 in plasma and brain tissue, 30 min after oral treatment, are summarized in Table 4. BAY 60-7550 concentrations were 0.9 ng/ml in plasma, but were undetectable in brain tissue. PQ-10 concentrations were 77.7 ng/ml in plasma and 51.3 ng/g in the brain. Since the dosage of BAY 60-7550 used in our behavioural experiments (1 mg/kg) resulted in a concentration that was below quantification limit in the brain tissue, we performed an additional experiment with a higher dose of BAY

60-7550 (10 mg/kg) administered at various time intervals (30 min, 60 min and 240 min before sampling) to check whether BAY 60-7550 was able to penetrate the blood-brain barrier (BBB) at all (see Table 5).

Table 4 Concentration of BAY 60-7750 or PQ-10 in plasma or brain tissue. Effects of BAY 60-7550 (1 mg/kg, p.o.) or PQ-10 (1 mg/kg, p.o.) treatment on their concentrations in plasma or brain tissue 30 min after administration (means ± SEM). n = 3 per condition. BQL = below quantification limit

Compound	Plasma (ng/ml)	Brain (ng/g)
BAY 60-7550	0.9 (0.1)	BQL
PQ-10	77.7(23.8)	51.3 (9.0)

Table 5 Concentration of BAY 60-7750 in plasma or brain tissue. Effects of BAY 60-7550 (10 mg/kg, p.o.) treatment on its concentrations in plasma or brain tissue at different time points (30, 60 and 240 min) after administration (means + SEM). n = 3 per condition

Compound	Plasma (ng/ml)	Brain (ng/g)
BAY 60-7550		
t=30 min	72.9 (28.6)	3.6 (1.6)
t=60 min	61.4 (25.5)	3.5 (1.4)
t=240 min	7.5 (2.0)	0.3 (0.3)

DISCUSSION

It was demonstrated that after a 1 h interval, rats in the saline condition still remembered the familiar object whereas scopolamine (0.1 mg/kg) and MK-801 (0.125 mg/kg) conditions disrupted object memory. Furthermore, the scopolamine-induced object memory deficit was reversed by oral co-treatment with either the PDE2-I BAY 60-7550 or the PDE10-I PQ-10, at a minimal effective dose (MED) of 1 and 0.3 mg/kg, respectively. In addition, the MK-801-induced deficit was reversed by both PDE2-I and PDE10-I at a MED of 1 mg/kg. At present, we have no valid explanation why PQ-10 appears to be more potent in the scopolamine model as compared to the MK-801 model.

Scopolamine, MK-801 and BAY 60-7550 had no effect on exploratory activity of the animals whereas PQ-10 increased object exploration time in T1 in the MK-801 model. In general, PDE10-Is are known to have either no effect on exploratory activity, decrease locomotor activity or induce catalepsy (Grauer et al. 2009; Schmidt et al. 2008; Siuciak et al. 2006a). However, MK-801 is known to affect locomotor activity (e.g. Andine et al. 1999; Brosnan-Watters et al. 1996), so the effect on exploratory activity in our study could partly be explained by a possible interaction between MK-801 and PQ-10. Of note, since the *d*2 index is a relative measure of discrimination that corrects for exploration activity, these changes in exploration will not affect the object recognition index.

It was shown that the behaviourally active dose of 1 mg/kg PQ-10 was present in blood plasma and brain tissue 30 min after oral administration, unlike the corresponding dose of 1 mg/kg BAY 60-7550 that was only detectable in the blood. Increasing the dose of BAY 60-7550 up to 10 mg/kg resulted in a brain concentration of 3.6 ng/g. Based on the brain-plasma concentration ratio of 0.05 calculated after administration of 10 mg/kg BAY 60-7550 it can be estimated that the brain concentration 30 min after administration of 1 mg/kg BAY 60-7550 would be 0.045 ng/g. This is below the detection limit of 1 ng/g of our quantification for BAY 60-7550. Of note, the approximate cerebral blood volume relative to total

unperfused brain volume is 0.04 (Hitchcock and Pennington 2006). Considering the averaged brain-plasma concentration of 0.05, this raises the question whether BAY 60-7550 enters the brain substantially. PQ-10 is clearly brain penetrant with a brain-plasma ratio of 0.66. Yet, it can be taken into consideration that PDE2 is the type of PDE with the highest mRNA expression in brain structures implicated in objection recognition memory (Lakics et al. 2010) (see also below), thus probably low brain concentrations could probably be sufficient to have a biological effect. Furthermore, BAY 60-7550 could affect cyclic nucleotide signal cascades by increasing the levels of cAMP and cGMP, which do not necessarily need high levels at the beginning, but may eventually cause biological responses because of signal amplification in the cascades. Nevertheless, the lack of a clear penetration of BAY 60-7550 into the brain raises the question whether an additional or alternative mechanism might be causing the memory improvements after PDE2-I treatment. It might be speculated that an active metabolite penetrates the BBB. In addition, it might be argued that PDE2 inhibition exerts a peripheral (e.g. cardiovascular) effect which improves memory function.

Only a limited number of studies have investigated the effects of PDE2 inhibition on memory. Boess et al. (2004) demonstrated that administration of 3 mg/kg BAY 60-7550 (p.o.) 30 min before the test session reversed a MK-801-induced working memory deficit in the T-maze in mice. Our findings that BAY 60-7550 improved memory processes is in line with previous findings in which the compound was given before the learning trial (Domek-Lopacinska and Strosznajder 2008; van Donkelaar et al. 2008). As such, Domek-Lopacinska and Strosznajder (2008) demonstrated that 0.3 mg/kg BAY 60-7550 (1 h before T1, s.c.) enhanced object memory in 3- and 12-month old rats using a 2 h delay interval after which the animals did not remember the objects presented in T1. Furthermore, Van Donkelaar et al. (2008) showed that administration of this PDE2-I 30 min before T1 (3 mg/kg, p.o.) reversed an ATD-induced STM deficit in the ORT.

The role of PDE10 in learning and memory has been investigated by means of PDE10 knock-out (KO) as well as PDE10-I treated control animals in a variety of tasks. Siuciak and co-workers (2006b; 2008) found that learning and memory in the passive avoidance and water escape task were unaffected in PDE10A KO mice on a DBA1LacJ background. Furthermore, these animals displayed the same conditioned avoidance response as wild-type (WT) mice although they required more training (Siuciak et al. 2006b; Siuciak et al. 2008). However, PDE10A KO mice on a C57BL/6N background were unable to reach the level of performance of the WT animals (Siuciak et al. 2008). Hebb et al. (2008) demonstrated that chronic treatment with the PDE10-I papaverine impaired learning and memory in the water escape task in WT mice and discussed that this could be explained by increased perseveration and impaired locomotor activity. This in contrast to acute treatment with PDE10-Is papaverine (10 and 30 mg/kg, p.o.) and MP-10 (3 mg/kg, p.o.) immediately after training which reversed a MK-801 induced memory deficit (0.1 mg/kg, i.p.) in social odor recognition in CF-1 mice (Grauer et al. 2009). Furthermore, it was shown that papaverine (10 - 30 mg/kg, i.p.) improved memory in the ORT if given 30 min before training in a 48 h delay interval in rats and MP-10 was also effective, though only a strong trend toward significance at the lowest dose tested (0.1 mg/kg, i.p.) (Grauer et al. 2009). These positive findings are supported by our PQ-10 data in the ORT.

The brain regions involved in object recognition are the hippocampus and the rhinal cortices. Although there is some debate about the exact roles of each brain region, the hippocampus is considered as the site where object information from the perirhinal cortex is integrated with contextual information from the parahippocampal (or postrhinal in rats) cortex, thus underlying the formation of episodic memory (e.g. Aggleton and Brown 2006; Eichenbaum et al. 2007; Melichercik et al. 2012; Mumby 2001; Winters and Bussey 2005; Wixted and Squire 2010). It was demonstrated that the levels of PDE2 protein expression are highest in the hippocampus, cortical areas, basal ganglia, substantia nigra, amygdale, interpeduncular nucleus and medial habenula in rats (Stephenson et al. 2009). Furthermore, Van Staveren et al. (2004; 2003) showed that PDE2 mRNA was distributed widely throughout the brain, including the hippocampus, cortex, striatum, amygdale and medial habenula in rodents. In addition, it was demonstrated that incubation of hippocampal slices with PDE2-Is resulted in a dose-dependent increase of cAMP and cGMP (Boess et al. 2004; van Staveren et al. 2001). In addition, PDE2 protein distribution in human, primate, dog and mouse cortex was shown to be similar to that in the rat cortex (Sadhu et al. 1999; Stephenson et al. 2009). In line with these findings, PDE2 mRNA expression was observed in the cortex, hippocampus, caudate, putamen, nucleus accumbens and claustrum to a similar extend in healthy adults, patients with Alzheimer's disease and age matched controls (Lakics et al. 2010; Reyes-Irisarri et al. 2007). The positive effects of PDE2 inhibition on memory performance combined with the protein and mRNA expression data, suggests that it might be a suitable target for cognition enhancement for Alzheimer's disease patients. Additionally, treatment of cognitive deficits in schizophrenia could also be considered since BAY 60-7550 was effective in our glutamatergic MK-801 memory deficit model.

PDE10A localization showed some overlap with the localization of PDE2 although PDE10A is much more pronouncedly expressed in the striatum. Immunohistochemistry showed that PDE10A is expressed predominantly in the nucleus accumbens, caudate nucleus, globus pallidus and substantia nigra, and to a lower extent in the CA regions of the hippocampus, dendate gyrus, cortex, thalamus and cerebellar granule cell layer in macaques, dogs and rodents (Coskran et al. 2006; Seeger et al. 2003). Studies investigating PDE10A mRNA expression in rodents and humans demonstrated similar results (Fujishige et al. 1999; Lakics et al. 2010; Loughney et al. 1999; Soderling et al. 1999) except for the globus pallidus and substantia nigra where PDE10A was barely detected (Seeger et al. 2003). In addition, it was found that administration of PDE10-Is MP-10 and TP-10 dose-dependently increased cAMP and cGMP in the striatum (other brain regions were not investigated) (Grauer et al. 2009; Schmidt et al. 2008), whereas the results for the less selective PDE10-I papaverine vary (Schmidt et al. 2008; Siuciak et al. 2006a). Recently, PDE10 has become a target for treating the positive, negative and cognitive symptoms of schizophrenia. It has been demonstrated that the positive effects of PDE10-Is are not limited to learning and memory, but also includes prepulse inhibition and auditory sensory gating (Grauer et al. 2009; Schmidt et al. 2008), and executive functioning (Rodefer et al. 2005; Rodefer et al. 2012) as well. Furthermore, PDE10 inhibition disrupted conditioned avoidance responding, which is a preclinical model predictive of antipsychotic activity, improved social approach/social avoidance performance and showed less susceptibility to extrapyramidal side effects in rodents (Grauer et al. 2009; Schmidt et al. 2008; Siuciak et al. 2006a). In addition, PQ-10 was effective in a low dose in our cholinergic

scopolamine memory deficit model. This would suggest that PDE10 inhibition could be considered as treatment of cognitive deficits in Alzheimer's disease. Further research using transgenic mouse models of Alzheimer's disease would be needed to confirm this.

Based on the present findings, we conclude that BAY-60-7550 and PQ-10 completely reversed the scopolamine-induced object memory deficit at a dose of 1 – 3 mg/kg and 0.3 - 1 mg/kg, respectively. In addition, both compounds completely reversed the MK-801-induced object memory deficit at a dose of 1 mg/kg onward. It can be argued that increased levels of cAMP and cGMP in the brain underlie the observed object memory improvement after treatment with BAY 60-7550 or PQ-10, although it has to be noted that brain penetration of BAY 60-7550 is poor. Taken together, our findings support the notion that PDE2 and PDE10 inhibition might offer a promising therapeutic tool for memory enhancement in Alzheimer's disease and schizophrenia, respectively.

ACKNOWLEDGEMENTS

Olga Reneerkens was partially supported by Janssen Pharmaceutica (Beerse, Belgium) and a Marie Curie Host Fellowship (MEST-CT 020589). In addition, this work was supported in part by European Fund for Regional Development (EFRE) and by the Free State of Saxony (grant no. SAB12525).

REFERENCES

Aggleton JP, Brown MW (2006) Interleaving brain systems for episodic and recognition memory. *Trends Cogn Sci* 10: 455-63

Akkerman S, Blokland A, Reneerkens O, van Goethem NP, Bollen E, Gijselaers HJ, Lieben CK, Steinbusch HW, Prickaerts J (2012) Object recognition testing: methodological considerations on exploration and discrimination measures. *Behav Brain Res* 232: 335-47

Alderton W, Karran E, Ward S (2009) Current and Future Perspectives in Psychiatric Drug Discovery. *Drug News Perspect* 22: 360-4

Andine P, Widermark N, Axelsson R, Nyberg G, Olofsson U, Martensson E, Sandberg M (1999) Characterization of MK-801-induced behavior as a putative rat model of psychosis. *J Pharmacol Exp Ther* 290: 1393-408

Bailey CH, Bartsch D, Kandel ER (1996) Toward a molecular definition of long-term memory storage. *Proc Natl Acad Sci USA* 93: 13445-52

Bernabeu R, Schmitz P, Faillace MP, Izquierdo I, Medina JH (1996) Hippocampal cGMP and cAMP are differentially involved in memory processing of inhibitory avoidance learning. *Neuroreport* 7: 585-8

Bliss TV, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 361: 31-9

Blokland A, Schreiber R, Prickaerts J (2006) Improving memory: a role for phosphodiesterases. *Curr Pharm Des* 12: 2511-23

Boess FG, Hendrix M, van der Staay FJ, Erb C, Schreiber R, van Staveren W, de Vente J, Prickaerts J, Blokland A, Koenig G (2004) Inhibition of phosphodiesterase 2 increases neuronal cGMP, synaptic plasticity and memory performance. *Neuropharmacology* 47: 1081-92

Brosnan-Watters G, Wozniak DF, Nardi A, Olney JW (1996) Acute behavioral effects of MK-801 in the mouse. *Pharmacol Biochem Behav* 53: 701-11

Chien WL, Liang KC, Teng CM, Kuo SC, Lee FY, Fu WM (2003) Enhancement of long-term potentiation by a potent nitric oxide-guanylyl cyclase activator, 3-(5-hydroxymethyl-2-furyl)-1-benzyl-indazole. *Mol Pharmacol* 63: 1322-8

Coskran TM, Morton D, Menniti FS, Adamowicz WO, Kleiman RJ, Ryan AM, Strick CA, Schmidt CJ, Stephenson DT (2006) Immunohistochemical localization of phosphodiesterase 10A in multiple mammalian species. *J Histochem Cytochem* 54: 1205-13

Domek-Lopacinska K, 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

Egawa T, Mishima K, Matsumoto Y, Iwasaki K, Fujiwara M (1997) Rolipram and its optical isomers, phosphodiesterase 4 inhibitors, attenuated the scopolamine-induced impairments of learning and memory in rats. *Jpn J Pharmacol* 75: 275-81

Eichenbaum H, Yonelinas AP, Ranganath C (2007) The medial temporal lobe and recognition memory. *Annu Rev Neurosci* 30: 123-52

Ennaceur A, Neave N, Aggleton JP (1997) Spontaneous object recognition and object location memory in rats: the effects of lesions in the cingulate cortices, the medial prefrontal cortex, the cingulum bundle and the fornix. *Exp Brain Res* 113: 509-19

Frey U, Huang YY, Kandel ER (1993) Effects of cAMP simulate a late stage of LTP in hippocampal CA1 neurons. *Science* 260: 1661-4

Fujishige K, Kotera J, Michibata H, Yuasa K, Takebayashi S, Okumura K, Omori K (1999) Cloning and characterization of a novel human phosphodiesterase that hydrolyzes both cAMP and cGMP (PDE10A). *J Biol Chem* 274: 18438-45

Ghelardini C, Galeotti N, Gualtieri F, Romanelli MN, Bucherelli C, Baldi E, Bartolini A (2002) DM235 (sunifiram): a novel nootropic with potential as a cognitive enhancer. *Naunyn Schmiedebergs Arch Pharmacol* 365: 419-26

Grauer SM, Pulito VL, Navarra RL, Kelly MP, Kelley C, Graf R, Langen B, Logue S, Brennan J, Jiang L, Charych E, Egerland U, Liu F, Marquis KL, Malamas M, Hage T, Comery TA, Brandon NJ (2009) Phosphodiesterase 10A inhibitor activity in preclinical models of the positive, cognitive, and negative symptoms of schizophrenia. *J Pharmacol Exp Ther* 331: 574-90

Hebb AL, Robertson HA, Denovan-Wright EM (2008) Phosphodiesterase 10A inhibition is associated with locomotor and cognitive deficits and increased anxiety in mice. *Eur Neuropsychopharmacol* 18: 339-63

Hitchcock SA, Pennington LD (2006) Structure-brain exposure relationships. *J Med Chem* 49: 7559-83

Imanishi T, Sawa A, Ichimaru Y, Miyashiro M, Kato S, Yamamoto T, Ueki S (1997) Ameliorating effects of rolipram on experimentally induced impairments of learning and memory in rodents. *Eur J Pharmacol* 321: 273-8

Klinkenberg I, Blokland A (2010) The validity of scopolamine as a pharmacological model for cognitive impairment: a review of animal behavioral studies. *Neurosci Biobehav Rev* 34: 1307-50

Klinkenberg I, Blokland A (2011) A comparison of scopolamine and biperiden as a rodent model for cholinergic cognitive impairment. *Psychopharmacology (Berl)* 215: 549-66

Lakics V, Karran EH, Boess FG (2010) Quantitative comparison of phosphodiesterase mRNA distribution in human brain and peripheral tissues. *Neuropharmacology* 59: 367-74

Loughney K, Snyder PB, Uher L, Rosman GJ, Ferguson K, Florio VA (1999) Isolation and characterization of PDE10A, a novel human 3', 5'-cyclic nucleotide phosphodiesterase. *Gene* 234: 109-17

Matsumoto Y, Unoki S, Aonuma H, Mizunami M (2006) Critical role of nitric oxide-cGMP cascade in the formation of cAMP-dependent long-term memory. *Learn Mem* 13: 35-44

Melichercik AM, Elliott KS, Bianchi C, Ernst SM, Winters BD (2012) Nicotinic receptor activation in perirhinal cortex and hippocampus enhances object memory in rats. *Neuropharmacology* 62: 2096-105

Mumby DG (2001) Perspectives on object-recognition memory following hippocampal damage: lessons from studies in rats. *Behav Brain Res* 127: 159-81

Prickaerts J, de Vente J, Honig W, Steinbusch HW, Blokland A (2002) cGMP, but not cAMP, in rat hippocampus is involved in early stages of object memory consolidation. *Eur J Pharmacol* 436: 83-7

Prickaerts J, Sik A, van Staveren WC, Koopmans G, Steinbusch HW, van der Staay FJ, de Vente J, Blokland A (2004) Phosphodiesterase type 5 inhibition improves early memory consolidation of object information. *Neurochem Int* 45: 915-28

Prickaerts J, Steinbusch HW, Smits JF, de Vente J (1997) Possible role of nitric oxide-cyclic GMP pathway in object recognition memory: effects of 7-nitroindazole and zaprinast. *Eur J Pharmacol* 337: 125-36

Reyes-Irisarri E, Markerink-Van Ittersum M, Mengod G, de Vente J (2007) Expression of the cGMP-specific phosphodiesterases 2 and 9 in normal and Alzheimer's disease human brains. *Eur J Neurosci* 25: 3332-8

Rodefer JS, Murphy ER, Baxter MG (2005) PDE10A inhibition reverses subchronic PCP-induced deficits in attentional set-shifting in rats. *Eur J Neurosci* 21: 1070-6

Rodefer JS, Saland SK, Eckrich SJ (2012) Selective phosphodiesterase inhibitors improve performance on the ED/ID cognitive task in rats. *Neuropharmacology* 62 1182-90

Rutten K, Lieben C, Smits L, Blokland A (2007a) The PDE4 inhibitor rolipram reverses object memory impairment induced by acute tryptophan depletion in the rat. *Psychopharmacology (Berl)* 192: 275-82

Rutten K, Prickaerts J, Blokland A (2006) Rolipram reverses scopolamine-induced and time-dependent memory deficits in object recognition by different mechanisms of action. *Neurobiol Learn Mem* 85: 132-8

Rutten K, Prickaerts J, Hendrix M, van der Staay FJ, Sik A, Blokland A (2007b) 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: 107-12

Sadhu K, Hensley K, Florio VA, Wolda SL (1999) Differential expression of the cyclic GMP-stimulated phosphodiesterase PDE2A in human venous and capillary endothelial cells. *J Histochem Cytochem* 47: 895-906

Schmidt CJ, Chapin DS, Cianfrogna J, Corman ML, Hajos M, Harms JF, Hoffman WE, Lebel LA, McCarthy SA, Nelson FR, Proulx-Lafrance C, Majchrzak MJ, Ramirez AD, Schmidt K, Seymour PA, Siuciak JA, Tingley Iii FD, Williams RD, Verhoest PR, Menniti FS (2008) Preclinical characterization of selective PDE10A inhibitors: A new therapeutic approach to the treatment of schizophrenia. *J Pharmacol Exp Ther* 325: 681-90

Schreiber R, Vivian J, Hedley L, Szczepanski K, Secchi RL, Zuzow M, van Laarhoven S, Moreau JL, Martin JR, Sik A, Blokland A (2007) Effects of the novel 5-HT(6) receptor antagonist RO4368554 in rat models for cognition and sensorimotor gating. *Eur Neuropsychopharmacol* 17: 277-88

Seeger TF, Bartlett B, Coskran TM, Culp JS, James LC, Krull DL, Lanfear J, Ryan AM, Schmidt CJ, Strick CA, Varghese AH, Williams RD, Wylie PG, Menniti FS (2003) Immunohistochemical localization of PDE10A in the rat brain. *Brain Res* 985: 113-26

Sik A, van Nieuwehuyzen P, Prickaerts J, Blokland A (2003) Performance of different mouse strains in an object recognition task. *Behav Brain Res* 147: 49-54

Siuciak JA (2008) The role of phosphodiesterases in schizophrenia : therapeutic implications. *CNS Drugs* 22: 983-93

Siuciak JA, Chapin DS, Harms JF, Lebel LA, McCarthy SA, Chambers L, Shrikhande A, Wong S, Menniti FS, Schmidt CJ (2006a) Inhibition of the striatum-enriched phosphodiesterase PDE10A: a novel approach to the treatment of psychosis. *Neuropharmacology* 51: 386-96

Siuciak JA, McCarthy SA, Chapin DS, Fujiwara RA, James LC, Williams RD, Stock JL, McNeish JD, Strick CA, Menniti FS, Schmidt CJ (2006b) Genetic deletion of the striatum-enriched phosphodiesterase PDE10A: evidence for altered striatal function. *Neuropharmacology* 51: 374-85

Siuciak JA, McCarthy SA, Chapin DS, Martin AN, Harms JF, Schmidt CJ (2008) Behavioral characterization of mice deficient in the phosphodiesterase-10A (PDE10A) enzyme on a C57/Bl6N congenic background. *Neuropharmacology* 54: 417-27

Soderling SH, Bayuga SJ, Beavo JA (1999) Isolation and characterization of a dual-substrate phosphodiesterase gene family: PDE10A. *Proc Natl Acad Sci USA* 96: 7071-6

Son H, Lu YF, Zhuo M, Arancio O, Kandel ER, Hawkins RD (1998) The specific role of cGMP in hippocampal LTP. *Learn Mem* 5: 231-45

Stephenson DT, Coskran TM, Wilhelms MB, Adamowicz WO, O'Donnell MM, Muravnick KB, Menniti FS, Kleiman RJ, Morton D (2009) Immunohistochemical localization of phosphodiesterase 2A in multiple mammalian species. *J Histochem Cytochem* 57: 933-49

Threlfell S, Sammut S, Menniti FS, Schmidt CJ, West AR (2009) Inhibition of Phosphodiesterase 10A Increases the Responsiveness of Striatal Projection Neurons to Cortical Stimulation. *J Pharmacol Exp Ther* 328: 785-95

van der Staay FJ, Rutten K, Barfacker L, Devry J, Erb C, Heckroth H, Karthaus D, Tersteegen A, van Kampen M, Blokland A, Prickaerts J, Reymann KG, Schroder UH, Hendrix M (2008) The novel selective PDE9 inhibitor BAY 73-6691 improves learning and memory in rodents. *Neuropharmacology* 55: 908-18

van der Staay FJ, Rutten K, Erb C, Blokland A (2011) Effects of the cognition impairer MK-801 on learning and memory in mice and rats. *Behav Brain Res* 220: 215-29

van Donkelaar EL, Rutten K, Blokland A, Akkerman S, Steinbusch HW, Prickaerts J (2008) Phosphodiesterase 2 and 5 inhibition attenuates the object memory deficit induced by acute tryptophan depletion. *Eur J Pharmacol* 600: 98-104

van Staveren WC, Markerink-van Ittersum M, Steinbusch HW, de Vente J (2001) The effects of phosphodiesterase inhibition on cyclic GMP and cyclic AMP accumulation in the hippocampus of the rat. *Brain Res* 888: 275-86

van Staveren WC, Steinbusch HW, Markerink-van Ittersum M, Behrends S, de Vente J (2004) Species differences in the localization of cGMP-producing and NO-responsive elements in the mouse and rat hippocampus using cGMP immunocytochemistry. *Eur J Neurosci* 19: 2155-68

Van Staveren WC, Steinbusch HW, Markerink-Van Ittersum M, Repaske DR, Goy MF, Kotera J, Omori K, Beavo JA, De Vente J (2003) mRNA expression patterns of the cGMP-hydrolyzing phosphodiesterases types 2, 5, and 9 during development of the rat brain. *J Comp Neurol* 467: 566-80

Winters BD, Bussey TJ (2005) Transient inactivation of perirhinal cortex disrupts encoding, retrieval, and consolidation of object recognition memory. *J Neurosci* 25: 52-61

Wixted JT, Squire LR (2010) The role of the human hippocampus in familiarity-based and recollection-based recognition memory. *Behav Brain Res* 215: 197-208

Zhang HT, Crissman AM, Dorairaj NR, Chandler LJ, O'Donnell JM (2000) Inhibition of cyclic AMP phosphodiesterase (PDE4) reverses memory deficits associated with NMDA receptor antagonism. *Neuropsychopharmacology* 23: 198-204

Zhang HT, Huang Y, Suvarna NU, Deng C, Crissman AM, Hopper AT, De Vivo M, Rose GM, O'Donnell JM (2005) Effects of the novel PDE4 inhibitors MEM1018 and MEM1091 on memory in the radial-arm maze and inhibitory avoidance tests in rats. *Psychopharmacology (Berl)* 179: 613-9

CHAPTER 5

The PDE5 inhibitor vardenafil does not affect auditory sensory gating in rats and humans

Olga A. H. Reneerkens, Anke Sambeth, Marlies A. van Duinen, Arjan Blokland, Harry W. M. Steinbusch & Jos Prickaerts

Published in Psychopharmacology (2013), 225(2), 303-312

ABSTRACT

Sensory gating is an adaptive mechanism of the brain to prevent overstimulation. Patients suffering from clinical disorders such as Alzheimer's disease or schizophrenia exhibit a deficit in gating, which indicates an impairment in basic information processing that might contribute to the cognitive problems seen in these patients. Phosphodiesterase type 5 inhibitors (PDE5-Is) have been shown to improve cognition in rodents in various behavioural tasks and might consequently be an interesting target for cognition enhancement. However, the effects of PDE5-Is on sensory gating are not known yet. Our objective was to study the effects of PDE5 inhibition on auditory sensory gating in rats and humans. In the rat study vehicle or 0.3-3 mg/kg of the PDE5-I vardenafil was given orally 30 min before testing and electrode locations were the vertex, hippocampus and the striatum. The human subjects received placebo, 10-20 mg vardenafil 85 min before testing and sensory gating was measured at the cortex (Fz, FCz and Cz) electrodes. Significant gating was only found for the N1 component in rats, while all three peaks P1, N1 and P2 showed gating in humans, i.e. the response to the second sound click was decreased as compared to the first for these deflections. Administration of vardenafil did neither have an effect on sensory gating in rats nor in humans. These findings imply that positive effects of PDE5 inhibition on cognition are not mediated by more early phases of information processing.

INTRODUCTION

Sensory gating is an automatic process involved in information processing. More specifically it is an adaptive mechanism of the central nervous system that prevents overstimulation of higher cortical areas and helps filtering sensory information (e.g (Cromwell et al. 2008)). The standard paradigm assessing this mechanism consists of two identical auditory stimuli that are presented with an inter-stimulus interval (ISI) between 0.5-2 s and an inter-trial interval (ITI) of at least 8 s (Cromwell et al. 2008; Hajos 2006). In healthy individuals – humans as well as animals - the response to the second stimulus (S2) will be smaller than the response to the first stimulus (S1). Of note, the duration of the ISI is crucial; if it is shorter than 0.5 s or longer than 2 s, sensory gating will not be elicited. Extensive research has shown that the process of sensory gating is disrupted in patients suffering from clinical disorders including schizophrenia and Alzheimer's disease (e.g. Adler et al. 1982; Ally et al. 2006; Javitt 2009; Jessen et al. 2001).

The responses evoked by this auditory sensory gating paradigm can be assessed using electroencephalographic (EEG) and event-related potential (ERP) measurements. In humans, the P50, also known as P1, is considered to be the main ERP component related to sensory gating (e.g. Chang et al. 2011; Dalecki et al. 2011). In addition, the N100 (N1) and P200 (P2) might also be affected (e.g. Boutros et al. 2009; Lijffijt et al. 2009). There is still a debate about which ERP component in rats is possibly the functional equivalent of the P50 in humans. Some researchers suggest that the P13 (P1) (e.g. Miyazato et al. 1999) is the most suitable candidate, whereas others assume it is the N40 (N1) or P60 (P2) (e.g. Mears et al. 2006; Zhou et al. 2008). It has also been suggested that the entire P1-N1-P2 complex is involved in the auditory sensory gating paradigm in rats just as in humans (e.g. Broberg et al. 2010; Mears et al. 2009).

Recently, phosphodiesterases (PDEs) gained increased attention as a promising target for cognition enhancement. Depending on the enzyme subclass they selectively hydrolyze the second messengers cyclic adenosine monophosphate (cAMP) and/or cyclic guanosine monophosphate (cGMP) (Bender and Beavo 2006). It has been shown that drugs that prevent the breakdown of these PDEs, the so-called PDE inhibitors (PDE-Is), improve cognition in animals in a wide range of behavioural tasks (for review see Chapter 2). Since the cyclic guanosine monophosphate (cGMP) specific phosphodiesterase type 5 inhibitors (PDE5-Is) are clinically approved for treatment of erectile dysfunction, they can be tested in animals as well as humans, which makes them particularly interesting from a translational perspective. It has already been shown that PDE5 inhibition has a positive effect on a variety of cognitive processes in animals, including learning (e.g. Devan et al. 2006; Devan et al. 2007), memory (e.g. Chapter 3 and Prickaerts et al. 2002b; Rutten et al. 2007; van Donkelaar et al. 2008), executive functioning and response inhibition (Rutten et al. 2008). In contrast, only a limited number of studies investigated the effects of PDE5-Is on cognition in humans. Three of those studies did not show any effects of PDE5 inhibition on cognitive performance in healthy adults (Grass et al. 2001; Schultheiss et al. 2001) and patients with schizophrenia (Goff et al. 2009) respectively. Yet, EEG measurements in healthy adults indicated that there might be an effect of treatment with the PDE5-I sildenafil on attention (Schultheiss et al. 2001). Interestingly, it has recently been demonstrated that repeated dosing of the PDE5-I Udenafil improves the cognitive performance of patients suffering from

a erectile dysfunction on a modified version of the mini-mental state examination and an assessment battery addressing frontal executive functioning (Shim et al. 2011).

In our present study, we investigated the effects of PDE5 inhibition on sensory gating in rats and humans. Rats were included because of the extensive learning and memory enhancing effects that have already been reported in rodents while to our knowledge basic auditory information processing has not been studied yet after PDE5 inhibition. Likewise, the effects on sensory gating in humans were studied to gain further insight into the effects of PDE5 inhibition on information processing in humans, but also to see whether the drug effects found in rodents can be translated to the human situation and vice versa. It has indeed been shown that the ERPs of humans and rats show a substantial amount of similarities (e.g. Sambeth et al. 2003). Based on these findings we expect that the effects of drugs on these ERPs are comparable between humans and animals (Maxwell et al. 2004). First, we tested whether our paradigm elicited sensory gating. Next, the effects of the PDE5-I vardenafil on sensory gating were investigated. It was chosen to test 0.3-3 mg/kg vardenafil in rats since this dose range is mostly used in a wide array of behavioural tasks (e.g. Chapter 3 and Prickaerts et al. 2004; Prickaerts et al. 2002b; Rutten et al. 2007; Rutten et al. 2009). We included the vertex, hippocampus and striatum as electrode locations because of their involvement in sensory gating. The vertex was chosen to represent the cortex since the EEG signal at this location is relatively comparable to that at a similar location in humans. We recorded EEG from the Fz, FCz and Cz (vertex) locations in humans (see (Jasper 1958)) and used 10 mg and 20 mg because these are the dosages commonly used in humans.

METHODS AND MATERIALS

Animal study

Animals

All experimental procedures were approved by the local ethical committee for animal experiments of Maastricht University and met governmental guidelines. Thirteen 3-month-old male Wistar rats (Harlan, The Netherlands) were used with average body weights of 385 g (± 12.50). The animals were housed individually in standard Makrolon cages on sawdust bedding in an air-conditioned room (about 20°C). They were kept on a 12/12-h reversed light/dark cycle (lights on from 19.00 to 7.00 h) and had free access to food and water. The rats were housed in the same room as where they were tested. All testing was done between 9.00 and 18.00 h in a shielded Skinnerbox.

Surgery and EEG recordings

The animals received 0.1 ml/kg Temgesic (Schering-Plough B.V., Utrecht, The Netherlands) subcutaneously 30 min before surgery as analgesia. Forene isoflurane (Abbott B.V., Hoofddorp, The Netherlands) was used as a general inhalation anesthetic. After the animal was placed into the stereotactic apparatus and an incision was made to expose the skull, lidocaine was applied as additional local anesthesia. Next, bregma was identified and

the electrodes were placed in the striatum (AP 0.48, ML -3.0, DV -5.0), dorsal hippocampus (AP -2.8, ML -1.8, DV -2.6) and vertex (AP -3.5, ML -1.0, DV -1.0) (Paxinos and Watson 1998). The reference and ground electrodes were both placed in the cerebellum. The electrodes and the connector were fixed to the skull by using three screws and Paladur denture acrylic (Heraeus Kulzer, Hanau, Germany). The animals were given at least two weeks to recover from the surgery.

In the first week after recovery, the animals were handled daily and adapted to the procedure, i.e. they were connected to the EEG set-up and allowed to explore the Skinnerbox in which the recording would take place. In addition the rats were adapted to p.o. administration procedures by saline injections (2 ml/kg). Next, the control condition was tested, i.e. animals were treated with placebo; this was tested twice and averaged for the statistical analysis. Subsequently three doses of the PDE5-I vardenafil were randomly tested (0.3, 1, 3 mg/kg, p.o.). The sensory gating paradigm consisted of 70 pairs of auditory stimuli which were presented with stimulus duration of 10 ms, ISI of 500 ms and ITI 6-10 s. The EEG signal was sampled at 1000 Hz, filtered between 1 - 133.5 Hz and stored on a personal computer. The stimuli were 2500 Hz clicks with a sound intensity of 80 dB. Since the animals were tested in a sound attenuated room with a maximal background noise level of 20 dB, the level of our stimulus salience was approximately 60 dB. After the study was finished, the animals were killed by decapitation and the brains were taken out. The brains were stored in 4% formaldehyde at 4-6 ºC until electrode localization took place.

Treatment

Vardenafil was first dissolved in 1.5 ml ethanol with 2% Tween 80. After extraction of ethanol via vaporization under N2 gas, the compounds were dissolved in 0.5% methylcellulose. The compound was tested at a dose of 0.3-3 mg/kg and administered by oral gavage (2 ml/kg) 30 min before testing. Vardenafil was kindly donated by BAYER (Wuppertal, Germany). The experimenter was blind to the compound and doses tested. All animals were treated with each condition once, except for the control condition (placebo), which was tested twice as part of the training.

Electrode localization

In order to verify the localization of the striatal and hippocampal electrode, coronal slices (50 µm) were made with a vibratome and put on glass slides. Next, a hematoxyline and eosine (HE) staining was applied and the slices were inspected under a microscope. If the localization of the hippocampal or striatal electrode could not be verified and/or the raw data did not show the typical delta and theta waves in the hippocampal EEG, the animal was excluded for that part of the analyses (number of animals mentioned in the results section). Since the vertex electrode measures the EEG signal at the cortical surface, there was no need for localization.

Statistical analysis

Segments between 100ms before until 500ms after stimulus onset were made for each stimulus type (S1 and S2) separately, using the last 100 ms before onset as baseline. High pass (1Hz) and low pass (30 Hz) filters were applied. The segments were visually checked and removed from the data set if a movement artefact occurred within 500 ms after stimulus presentation. Both the grand average (all animals) and the individual data (single animal) were used to determine the auditory evoked potential (AEP) components. In general, P1 was defined as most positive value between 20 and 50 ms after stimulus onset. N1 was the most negative value between 50 – 80 ms for the vertex and between 40 – 70 ms for the striatum and hippocampus. Finally, P2 was defined as most positive value between 65 - 105 ms for the vertex and between 55 – 90 ms for the striatum and hippocampus.

General linear models (GLM) repeated measures were used to analyze the amplitudes of the components. First, the responses to the S1 and S2 were compared for the vehicle condition to see whether sensory gating occurred. Next, the responses to the PDE-Is conditions were compared to placebo condition for each stimulus (treatment) separately as well as for both stimuli together (treatment x stimulus). In case of a statistically reliable effect, comparisons between means of the conditions were analyzed in more detail using post hoc Bonferroni t-tests ($P < 0.05$). Two animals were excluded from the analysis of the vertex and the striatum electrodes because of no reliable EEG signal.

Human study

Participants

All experimental procedures were approved by the independent Ethics Committee of Maastricht University and the Academic Hospital Maastricht (The Netherlands). Eighteen participants (21 ± 0.7 years old, 5 males) were recruited through advertisements at Maastricht University. They had to be willing to sign an informed consent and were paid for their participation.

The subjects' physical and mental health was checked by a physician by means of a standard medical questionnaire and a medical examination. Subjects 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 2 weeks before until the end of the experiment, and any sensory or motor deficit which could reasonably be expected to affect test performance. In addition, subjects who had a first-degree relative with (history of) a psychiatric disorder were excluded as well. The participants could leave the study at any given time without any consequence.

EEG recordings

An EEG cap was used to place a set of 32 EEG electrodes according to the international 10-20 system (Jasper 1958). Only the Fz, FCz and Cz locations were used in the current study since it has been demonstrated previously that midline electrodes show better P50 sensory gating than left/right hemispheric sites, especially the Cz (vertex) and FCz electrodes (Wan et al. 2006). In addition, the Fz electrode has been demonstrated to show a similar amount of P200 gating and was therefore included as well (Wan et al. 2007). 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 and 100 Hz and sampled at 1000 Hz.

The sensory gating paradigm consisted of 60 pairs of identical auditory stimuli with a duration of 3 ms and intensity of 80 dB. Since testing took place in a sound attenuated room with a maximal background noise level of 20 dB, the stimulus salience was approximately 60 dB. The interval between the first (S1) and the second (S2) stimulus was 500 ms; the interval between pairs was randomized between 6 – 10 s. The subjects were familiarized with this test during a training session.

Design and treatment

The study was conducted according to a double-blind, placebo-controlled, 3-way cross-over design. Order of treatments was balanced over three test days and separated by a washout period of at least 7 days. The balancing of the treatment order was be accomplished by counterbalancing.

Treatment consisted of a placebo, 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 (EMEA 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 (EMEA 2008). Since this study was part of a larger experiment consisting of multiple tasks, our sensory gating paradigm was tested 85 minutes after drug treatment. The drugs were ingested orally and combined with a low-fat breakfast, because fatty food might affect the absorption of vardenafil. The experimenter and subjects were blind to the compound and doses tested.

Medical questionnaire

A medical questionnaire was presented to the subjects twice each testing day: directly before ingesting the compound/placebo (baseline) and approximately 100 minutes later (during a short break) (treatment). This questionnaire addressed 31 physical complaints, including headache, nausea, dry mouth, blurred vision and dizziness. Participants could indicate on a four point scale to what extent these items applied to their physical well-being (0 = not present; 3 = extremely present). The difference between the baseline and treatment scores were analysed by using GLM repeated measures.

Statistical analysis

Segments between 100ms before until 500ms after stimulus onset were made for each stimulus type (S1 and S2) separately, using the last 100 ms before onset as baseline. High pass (1Hz) and low pass (30 Hz) filters were applied. The segments were visually checked for EOG activity and other artefacts, and removed from the data set if an artefact occurred during the first 500 ms after stimulus presentation. The grand average was used to determine the AEP components. P1 was defined as most positive value between 60 and 90 ms after stimulus onset, N1 as most negative value between 85 and 150 ms, P2 as most positive value between 140 and 250 ms.

GLM repeated measures were used to analyze the amplitudes of the AEP components at the Fz, FCz and Cz locations (channel). First, the responses to the S1 and S2 were compared for the placebo condition to see whether sensory gating occurred. Next, the responses to the vardenafil conditions were compared to placebo condition for each stimulus separately (treatment x channel) as well as for both stimuli (treatment x stimulus x channel). In case of a statistically reliable effect, comparisons between means of the conditions were analyzed in more detail using post hoc Bonferroni t-tests ($P < 0.05$). One subject was excluded from the analyses because of an incomplete data set.

RESULTS

Animal study

Effects of placebo on sensory gating in rats

The effects of placebo treatment on sensory gating are depicted in Figure 1. GLM repeated measures showed that the N1 peak is less negative in response to S2 than S1 at the vertex ($F(1, 10) = 11.39$, $P < 0.01$). In the hippocampus, the N1 peak was also less negative after the presentation of S2 than S1 ($F(1, 12) = 6.20$, $P < 0.05$).

Effects of PDE5 inhibition on information processing in rats

No effects of vardenafil treatment (0.3-3 mg/kg, p.o. 30 min before testing) on the P1, N1 and P2 were found in the hippocampus and striatum as well as for the P1 and P2 in the vertex. Vardenafil seemed to affect the N1 in the vertex ($F(2, 24) = 3.31$, $P < 0.05$), but further post-hoc analysis revealed no difference between treatment conditions. This is illustrated in Figure 2 showing the results of vardenafil treatment on the peaks and locations that showed sensory gating in the placebo condition (see Figure 1) (N1 vertex: condition*stimulus ($F(1, 14) = 0.33$, n.s.); hippocampus: condition*stimulus ($F(2, 23) = 0.39$, n.s.), condition ($F(2, 24) = 1.87$, n.s.)).

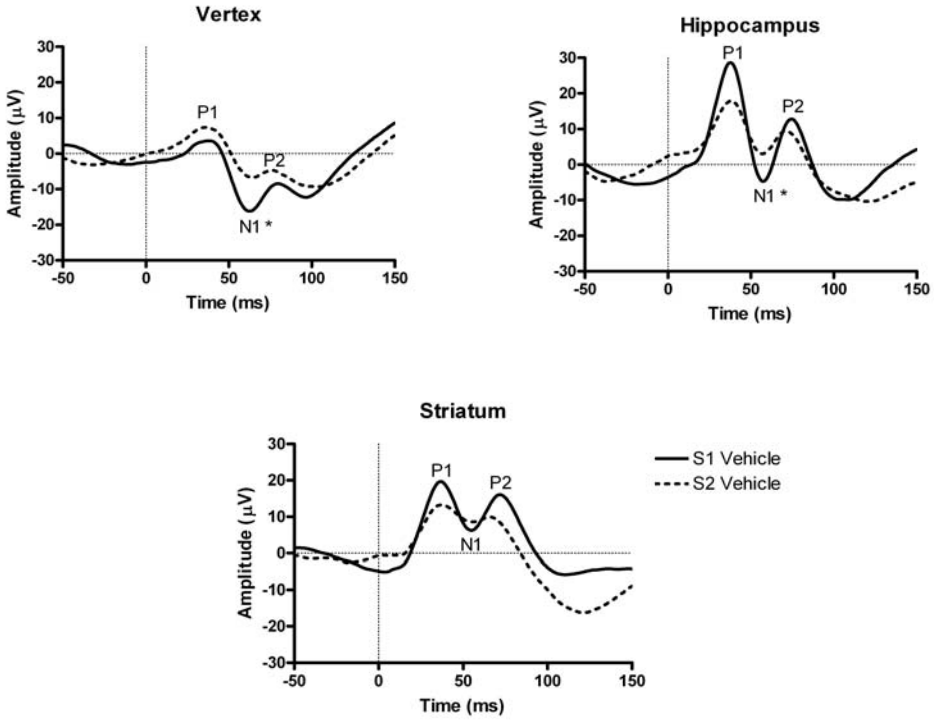

Figure 1 Effects of placebo (vehicle) (p.o. 30 min before testing) on grand average ERPs (P1, N1 and P2 component) after the presentation of S1 and S2; effects on gating are depicted with asterisks (*: P < 0.05). Latencies are shown on the x-axis in milliseconds (ms), amplitudes on the y-axis in microvolts (µV). n_{vertex} = 11; $n_{striatum}$ = 11; $n_{hippocampus}$ = 13

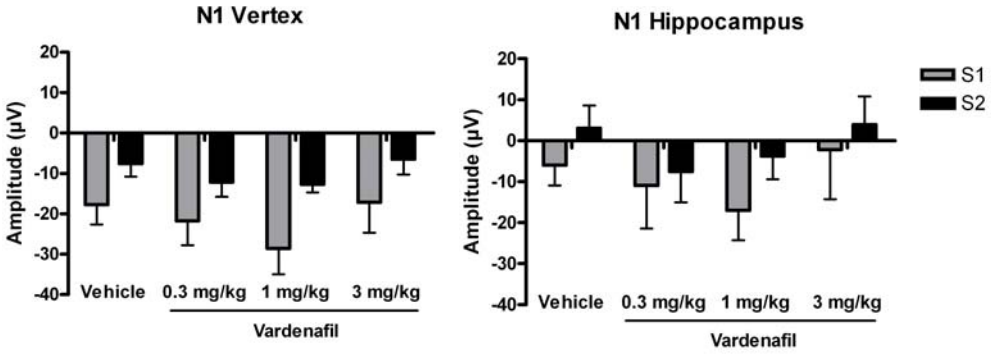

Figure 2 No effects of treatment with the PDE5-I vardenafil on the mean amplitude (±SEM) of N1 in the vertex and N1 in the hippocampus were found (GLM repeated measures). Drugs were given 30 min before testing. Compounds/doses are shown on the x-axis, amplitudes are presented on the y-axis in µV. N_{vertex} = 11, $n_{hippocampus}$ = 13

Human study

Effects of placebo on sensory gating in humans

The effects of placebo treatment on sensory gating are depicted in Figure 3. GLM repeated measures showed that there is an interaction between stimulus and channel for the P1 ($F(1, 17) = 4.60$, $P < 0.05$), N1 ($F(1, 19) = 15.25$, $P < 0.001$) and P2 ($F(1, 19) = 24.61$, $P < 0.001$) peaks. Further analyses for the three channels separately showed that the P1 was less positive after S2 than S1 at the FCz ($F(1, 16) = 5.69$, $P < 0.05$), and Cz ($F(1, 16) = 7.13$, $P < 0.05$). In addition, the N1 was less negative and the P2 less positive after the S2 than S1 at the Fz (N1: $F(1, 16) = 59.55$, $P < 0.001$; P2: $F(1, 16) = 34.94$, $P < 0.001$), FCz (N1: $F(1, 16) = 56.32$, $P < 0.001$; P2: $F(1, 16) = 50.08$, $P < 0.001$) and Cz (N1: $F(1, 16) = 49.48$, $P < 0.001$; P2: $F(1, 16) = 52.73$, $P < 0.001$).

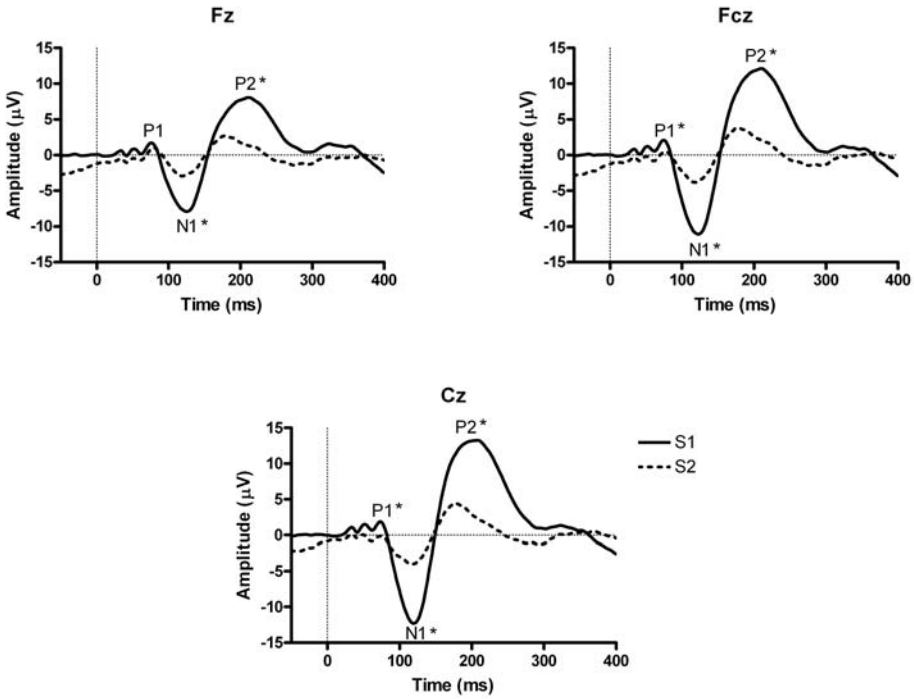

Figure 3 Effects of placebo treatment (orally 85 min before testing) on grand average ERPs (P1, N1 and P2 component) after the presentation of S1 and S2; effects on gating are depicted with asterisks (n = 17). Latencies are shown on the x-axis in milliseconds (ms), amplitudes on the y-axis in microvolts (μV)

Effects of PDE5 inhibition on information processing in humans

The effects of vardenafil (10-20 mg, p.o. 85 min before testing) administration on the ERP components in the placebo condition (see Figure 3) are shown in Figure 4. An interaction effect for the P1 was found for stimulus*treatment*channel ($F(2, 35) = 3.72$, $P < 0.05$). Additional analyses of the differences between S1 and S2 showed an interaction between treatment condition and channel ($F(2, 35) = 3.72$, $P < 0.05$). Post-hoc analyses of each channel separately revealed no further effects. Furthermore, an interaction was detected for the N1 for stimulus*condition ($F(1, 23) = 3.98$, $P < 0.05$); however, Bonferonni post-hoc analysis of the difference between S1 and S2 showed no effect between treatment conditions. No effects of PDE5 inhibition on the P2 peak were found.

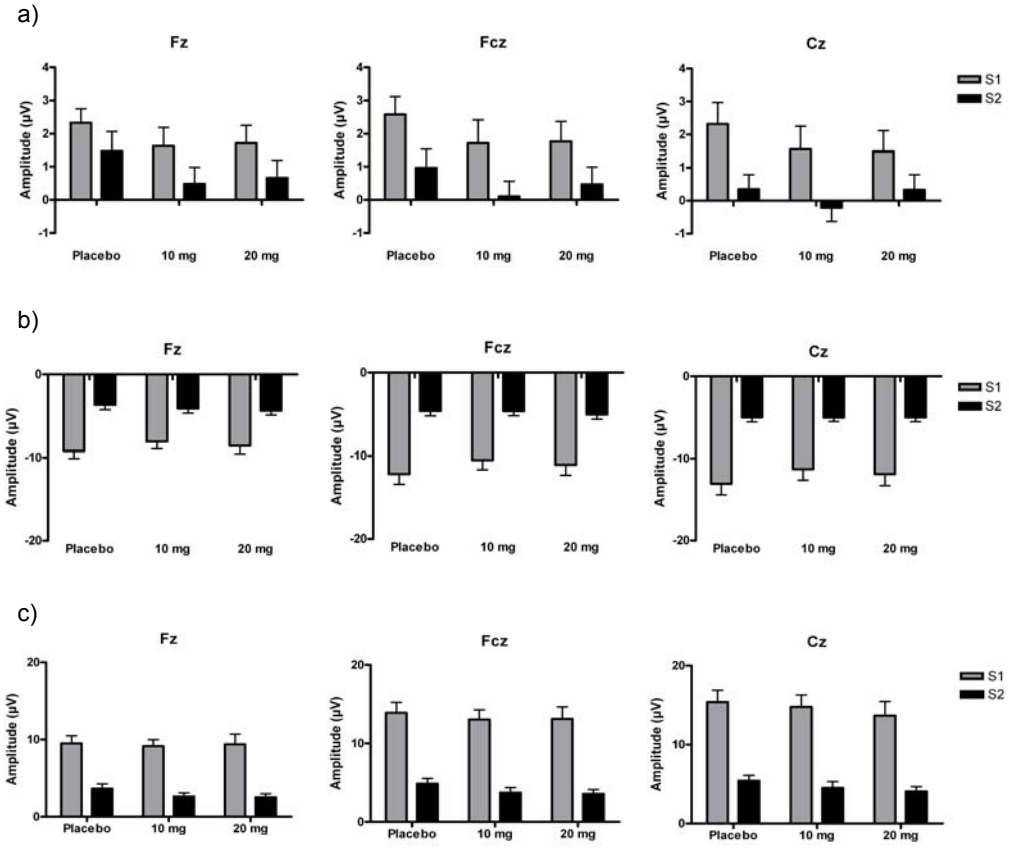

Figure 4 No effects of treatment with the PDE5-I vardenafil on the mean amplitude (±SEM) of a) P1, b) N1 and c) P2 after the presentation of S1 and S2 were found (GLM repeated measures). Drugs were given 85 min before testing; n = 17. Compounds/doses are shown on the x-axis, amplitudes are presented on the y-axis in µV

Medical questionnaire

An effect of treatment was found on the report of headache ($F(2, 28) = 6.34$, $P < 0.01$) and feeling weak ($F(2, 26) = 7.43$, $P < 0.01$). Bonferroni post-hoc analysis revealed that there was an increase after administration of both vardenafil 10 mg and 20 mg compared to the placebo condition.

DISCUSSION

The aim of this study was to test the effects of PDE5 inhibition on auditory sensory gating in rats and humans. It was demonstrated that after treatment with placebo the N1 in the vertex and the hippocampus was more negative after S1 than S2 in rats. Additionally, the response to the S2 was smaller than to the S1 at the P1, N1 and P2 peak in humans in the placebo condition. This indicates that our paradigm elicited sensory gating in the rats as well as in the human subjects. However, neither in rats nor in humans an effect of PDE5 inhibition with vardenafil was found on sensory gating.

In Chapter 3 we showed that vardenafil treatment reversed an MK-801- as well as a scopolamine-induced memory deficit in the object recognition task in rats at a dose of 1 mg/kg and 1-3 mg/kg respectively. Vardenafil was administered orally 30 minutes before testing, similar to our present sensory gating study. To verify the assumption that vardenafil crosses the blood-brain barrier (BBB) in these animals, blood plasma and brain tissue concentration of 3 mg/kg vardenafil were determined 30 min after oral treatment. It was found that vardenafil had a brain-to-plasma ratio (C_b:C_p) of 0.11. The approximate cerebral blood volume relative to total unperfused brain volume is 0.04 (Hitchcock and Pennington 2006), thus a C_b:C_p >0.04 indicated that vardenafil was brain penetrant. In addition, to determine whether there was enough vardenafil present in the brain to be biologically active, we calculated the free brain concentration to meaningfully compare this data to the IC50 value of the compound (0.1 nM). The free brain concentration was calculated using vardenafil's molecular weight and its free fraction in plasma and brain homogenate. The free brain concentration of 3 mg/kg vardenafil was 0.4 nM, which is 4 times its IC50 value. This suggests that the 1 and 3 mg/kg doses of vardenafil used in the present study have sufficient biological activity, i.e. PDE5 inhibition. The dose of 0.3 mg/kg vardenafil would have a relatively low biological activity of 0.4 times the IC50, but apparently still enough to be biologically active and to improve memory function as previously found in the object recognition task (Prickaerts et al. 2002a). It may thus be concluded that the doses of vardenafil we used in this study should have been expected to be active under the conditions tested.

As mentioned before, it has been shown that PDE5-Is improve cognition in a variety of behavioural tasks in rodents and monkeys (for overview see Chapter 2). Yet, although the effects of PDE5 inhibition on cognition (especially learning and memory) have been widely investigated in rodents, little is known about the effects on EEG measurements, including ERPs. However, the results of our current study indicate that the positive effects of PDE5-Is in healthy adult rats might be mediated by affecting higher cognitive processes instead of early basic processes such as sensory gating.

Grass et al. (2001) studied the effects of sildenafil treatment on seven different psychophysical tasks measuring among others short term memory and divided attention in healthy human subjects. Although PDE5 inhibition showed some effects in reaction time tests, no effects on the cognitive tasks were found. Interestingly, in a recent study of Shim et al. (2011) repeated dosing of the PDE5-I udenafil improved performance on the Korean version of the mini-mental state examination and an assessment battery addressing frontal executive functioning in patients suffering from erectile dysfunction. However, another study (Goff et al. 2009) investigating the effects of sildenafil administration in patients with schizophrenia did not show an effect on cognitive performance. The effects of sildenafil on positive and negative symptoms of schizophrenia were also investigated, but no changes in symptoms were found. In contrast, Akhandzadeh et al. (2011) demonstrated that sildenafil when combined with the atypical antipsychotic risperidone increased the latter's effectiveness in reducing the negative symptoms in patients with schizophrenia. Furthermore, it was shown in healthy subjects that although sildenafil treatment did not improve the behavioural response in attention and word recognition tasks, it did have an effect on EEG measurements (Schultheiss et al. 2001). During the auditory selective attention task, sildenafil elicited EEG responses indicative for an improvement of attention. No effects on ERP measurements related to word recognition were found, although a reduction in negativity of these measurements between 150 and 250 ms after stimulus presentation was found in the word recognition task. The role of this negative deflection in word recognition is not clear, but the authors suggest that 'there is an effect of sildenafil on cerebral information processing'. In our current study, we did not find an effect of PDE5 inhibition on a more specific part of information processing, namely sensory gating. However, the participants did report an increase in headache, which is one of the most commonly reported side effects (≥ 10% of the subjects participating in clinical trials) after vardenafil treatment (EMEA 2008), and feeling weak after the administration of 10 – 20 mg vardenafil compared to the placebo condition on a questionnaire about medical complaints. This indicates that vardenafil is at least bioactive at the dosages and time frame used in our sensory gating study. This would also be confirmed by previous pharmacokinetic data (EMEA 2008) which showed that the maximum plasma concentrations of vardenafil after oral dosing are reached within 30 – 120 minutes (median 60 min.); our time point of testing after 85 min is well within this period when also side effects were reported. Additionally, when we take into account the body surface area and the body weight to extrapolate the animal dose to the human dose using the formula of Reagan-Shaw et al. (2008), the doses of 0.3 - 3 mg/kg in rats should be equivalent to 3 - 31 mg in our human participants. This indicates that the 10 mg and 20 mg dosages of vardenafil used in our human experiment are equivalent to the doses, i.e. higher than 1 mg/kg, which have been shown to exert positive effects on cognition in previous animal studies (e.g. Chapter 3 and Prickaerts et al. 2002b) and which we also used in the present animal experiment. Thus, although we did not find an effect of vardenafil on sensory gating, the compound can be assumed to be bioactive. So, the effects of PDE5-Is on EEG measures seem to be task dependent and might affect different parts of information processing as we used a sensory gating paradigm to measure the effects on basic auditory information processing, whereas Schultheiss et al (2001) found the effect in a word recognition task after treatment with 100 mg sildenafil. It can not be ruled out completely that stimulus salience might have had an effect on the ability

to detect drug effects as well, since there are sensory gating studies in which the stimuli did not exceed 15 – 20 dB above background noise levels in animals (Halene and Siegel 2008) and humans (Cadenhead et al. 2005). However, based on previous experiments in our lab (e.g. Sambeth et al. 2007) and a wide variety of sensory gating studies in animals (e.g. Mears et al. 2006; Sambeth et al. 2003; Zhou et al. 2008) as well as humans (Dalecki et al. 2011; Jessen et al. 2001; Lijffijt et al. 2009) which used stimulus parameters similar to ours, it is unlikely that stimulus salience affected our results.

To summarize, the PDE5-I vardenafil did not affect basic auditory information processing tested in a sensory gating paradigm in rats or humans. These findings imply that the positive effects of PDE5 inhibition previously found in both species are possibly the result of positive effects on higher cognitive functions specifically (e.g. memory or attention) instead of on more basic processes involved in a variety of cognitive domains (e.g. basic auditory processing). To further elucidate the effects of PDE5 inhibition on cognition, identical deficit models in animals and humans (e.g. scopolamine or ketamine) should be used in a translational setting. In addition, testing the effects of PDE5-Is on cognitive performance and EEG measurements in a patient population suffering from cognitive dysfunction (e.g. patients with schizophrenia and patients suffering from dementia) is likely to provide further insight into the cognition enhancing potential of PDE5 inhibition.

REFERENCES

Adler LE, Pachtman E, Franks RD, Pecevich M, Waldo MC, Freedman R (1982) Neurophysiological evidence for a defect in neuronal mechanisms involved in sensory gating in schizophrenia. *Biol Psychiatry* 17: 639-54

Akhondzadeh S, Ghayyoumi R, Rezaei F, Salehi B, Modabbernia AH, Maroufi A, Esfandiari GR, Naderi M, Ghebleh F, Tabrizi M, Rezazadeh SA (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: 809-15

Ally BA, Jones GE, Cole JA, Budson AE (2006) Sensory gating in patients with Alzheimer's disease and their biological children. *Am J Alzheimers Dis Other Demen* 21: 439-47

Bender AT, Beavo JA (2006) Cyclic nucleotide phosphodiesterases: molecular regulation to clinical use. *Pharmacol Rev* 58: 488-520

Boutros NN, Brockhaus-Dumke A, Gjini K, Vedeniapin A, Elfakhani M, Burroughs S, Keshavan M (2009) Sensory-gating deficit of the N100 mid-latency auditory evoked potential in medicated schizophrenia patients. *Schizophr Res* 113: 339-46

Broberg BV, Oranje B, Glenthoj BY, Fejgin K, Plath N, Bastlund JF (2010) Assessment of auditory sensory processing in a neurodevelopmental animal model of schizophrenia--gating of auditory-evoked potentials and prepulse inhibition. *Behav Brain Res* 213: 142-7

Cadenhead KS, Light GA, Shafer KM, Braff DL (2005) P50 suppression in individuals at risk for schizophrenia: the convergence of clinical, familial, and vulnerability marker risk assessment. *Biol Psychiatry* 57: 1504-9

Chang WP, Arfken CL, Sangal MP, Boutros NN (2011) Probing the relative contribution of the first and second responses to sensory gating indices: a meta-analysis. *Psychophysiology* 48: 980-92

Cromwell HC, Mears RP, Wan L, Boutros NN (2008) Sensory gating: a translational effort from basic to clinical science. *Clin EEG Neurosci* 39: 69-72

Dalecki A, Croft RJ, Johnstone SJ (2011) An evaluation of P50 paired-click methodologies. *Psychophysiology* 48: 1692-700

Devan BD, Bowker JL, Duffy KB, Bharati IS, Jimenez M, Sierra-Mercado D, Jr., Nelson CM, Spangler EL, Ingram DK (2006) Phosphodiesterase inhibition by sildenafil citrate attenuates a maze learning impairment in rats induced by nitric oxide synthase inhibition. *Psychopharmacology (Berl)* 183: 439-45

Devan BD, Pistell PJ, Daffin LW, Jr., Nelson CM, Duffy KB, Bowker JL, Bharati IS, Sierra-Mercado D, Spangler EL, Ingram DK (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: 134-40

EMEA (2008) European Public Assesment Report; Revision 6. www.emea.europa.eu

Goff DC, Cather C, Freudenreich O, Henderson DC, Evins AE, Culhane MA, Walsh JP (2009) A placebo-controlled study of sildenafil effects on cognition in schizophrenia. *Psychopharmacology (Berl)* 202: 411-7

Grass H, Klotz T, Fathian-Sabet B, Berghaus G, Engelmann U, Kaferstein H (2001) Sildenafil (Viagra): is there an influence on psychological performance? *Int Urol Nephrol* 32: 409-12

Hajos M (2006) Targeting information-processing deficit in schizophrenia: a novel approach to psychotherapeutic drug discovery. *Trends Pharmacol Sci* 27: 391-8

Halene TB, Siegel SJ (2008) Antipsychotic-like properties of PDE4 inhibitors - Evaluation of RO-20-1724 with auditory event related potentials and prepulse inhibition of startle. *J Pharmacol Exp Ther* 326: 230-9

Hitchcock SA, Pennington LD (2006) Structure-brain exposure relationships. J Med Chem 49: 7559-83

Jasper H (1958) The ten-twenty electrode system of the international federation. *Electroencephalogr Clin Neurophysiol* 10: 371-5

Javitt DC (2009) Sensory processing in schizophrenia: neither simple nor intact. *Schizophr Bull* 35: 1059-64

Jessen F, Kucharski C, Fries T, Papassotiropoulos A, Hoenig K, Maier W, Heun R (2001) Sensory gating deficit expressed by a disturbed suppression of the P50 event-related potential in patients with Alzheimer's disease. *Am J Psychiatry* 158: 1319-21

Lijffijt M, Moeller FG, Boutros NN, Burroughs S, Lane SD, Steinberg JL, Swann AC (2009) The Role of Age, Gender, Education, and Intelligence in P50, N100, and P200 Auditory Sensory Gating. *J Psychophysiol* 23: 52-62

Maxwell CR, Liang Y, Weightman BD, Kanes SJ, Abel T, Gur RE, Turetsky BI, Bilker WB, Lenox RH, Siegel SJ (2004) Effects of chronic olanzapine and haloperidol differ on the mouse N1 auditory evoked potential. *Neuropsychopharmacology* 29: 739-46

Mears RP, Boutros NN, Cromwell HC (2009) Reduction of prelimbic inhibitory gating of auditory evoked potentials after fear conditioning. *Behav Neurosci* 123: 315-27

Mears RP, Klein AC, Cromwell HC (2006) Auditory inhibitory gating in medial prefrontal cortex: Single unit and local field potential analysis. *Neuroscience* 141: 47-65

Miyazato H, Skinner RD, Garcia-Rill E (1999) Neurochemical modulation of the P13 midlatency auditory evoked potential in the rat. *Neuroscience* 92: 911-20

Paxinos G, Watson C (1998) The Rat Brain in Stereotaxic Coordinates, 4 edn. Academic Press, Academic Press

Prickaerts J, de Vente J, Honig W, Steinbusch HW, Blokland A (2002a) cGMP, but not cAMP, in rat hippocampus is involved in early stages of object memory consolidation. *Eur J Pharmacol* 436: 83-7

Prickaerts J, Sik A, van Staveren WC, Koopmans G, Steinbusch HW, van der Staay FJ, de Vente J, Blokland A (2004) Phosphodiesterase type 5 inhibition improves early memory consolidation of object information. *Neurochem Int* 45: 915-28

Prickaerts J, van Staveren WC, Sik A, Markerink-van Ittersum M, Niewohner U, van der Staay FJ, Blokland A, de Vente J (2002b) 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: 351-61

Reagan-Shaw S, Nihal M, Ahmad N (2008) Dose translation from animal to human studies revisited. *FASEB J* 22: 659-61

Rutten K, Basile JL, Prickaerts J, Blokland A, Vivian JA (2008) Selective PDE inhibitors rolipram and sildenafil improve object retrieval performance in adult cynomolgus macaques. *Psychopharmacology (Berl)* 196: 643-8

Rutten K, Prickaerts J, Hendrix M, van der Staay FJ, Sik A, Blokland A (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: 107-12

Rutten K, Van Donkelaar EL, Ferrington L, Blokland A, Bollen E, Steinbusch HW, Kelly PA, Prickaerts JH (2009) Phosphodiesterase inhibitors enhance object memory independent of cerebral blood flow and glucose utilization in rats. *Neuropsychopharmacology* 34: 1914-25

Sambeth A, Maes JH, Van Luijtelaar G, Molenkamp IB, Jongsma ML, Van Rijn CM (2003) Auditory event-related potentials in humans and rats: effects of task manipulation. *Psychophysiology* 40: 60-8

Sambeth A, Riedel WJ, Smits LT, Blokland A (2007) Cholinergic drugs affect novel object recognition in rats: relation with hippocampal EEG? *Eur J Pharmacol* 572: 151-9

Schultheiss D, Muller SV, Nager W, Stief CG, Schlote N, Jonas U, Asvestis C, Johannes S, Munte TF (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

Shim YS, Pae CU, Kim SW, Kim HW, Kim JC, Koh JS (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: 109-14

van Donkelaar EL, Rutten K, Blokland A, Akkerman S, Steinbusch HW, Prickaerts J (2008) Phosphodiesterase 2 and 5 inhibition attenuates the object memory deficit induced by acute tryptophan depletion. *Eur J Pharmacol* 600: 98-104

Wan L, Crawford HJ, Boutros N (2006) P50 sensory gating: impact of high vs. low schizotypal personality and smoking status. *Int J Psychophysiol* 60: 1-9

Wan L, Crawford HJ, Boutros N (2007) Early and late auditory sensory gating: moderating influences from schizotypal personality, tobacco smoking status, and acute smoking. *Psychiatry Res* 151: 11-20

Zhou D, Ma Y, Liu N, Chen L, He M, Miao Y (2008) Influence of physical parameters of sound on the sensory gating effects of N40 in rats. *Neurosci Lett* 432: 100-4

CHAPTER 6

PDE2 and PDE10, but not PDE5, inhibition affect basic auditory information processing in rats: a pilot study

Olga A. H. Reneerkens, Anke Sambeth, Arjan Blokland & Jos Prickaerts

Submitted for publication

ABSTRACT

Phosphodiesterase type 2 (PDE2), type 10 (PDE10), and type 5 (PDE5) have been considered as relevant targets for cognition enhancement. Although it is well established that PDE inhibitors improve memory functions in animals, the effects on auditory information processing are less clear. The aim of this study was to test the effects of PDE2 (BAY 60-7550), PDE5 (vardenafil) and PDE10 (PQ-10) inhibition on sensory gating in rats. EEG was recorded from the hippocampus, striatum, and vertex. Sensory gating was found for the N1 in the vertex and hippocampus, as revealed by diminished amplitudes to S2 compared to S1. Administration of PDE-Is did not affect sensory gating. However, PDE2 inhibition increased the P1 peak after presentation of S1 at the vertex and PQ-10 increased the N1 peak in general compared to vehicle treatment at the hippocampus. To summarize, PDE2 and PDE10 inhibition affect auditory information processing in general, whereas PDE5 inhibition has no effect. These findings suggest that the positive effects of PDE5 inhibition on cognition previously found in animals are possibly the results of an effect on higher cognitive functioning specifically whereas the cognition enhancing effects of PDE2 and PDE10 inhibition might also be influenced by effects on earlier stages of information processing.

INTRODUCTION

Phosphodiesterases (PDEs) are enzymes that selectively hydrolyze the second messengers cyclic guanosine monophosphate (cGMP) and/or cyclic adenosine monophosphate (cAMP) by breaking their phosphodiester bond. It has been shown that compounds that inhibit these PDEs, so-called PDE inhibitors (PDE-Is), improve cognitive functioning in a wide variety of behavioural paradigms (for a review see Blokland et al. 2012 and Chapter 2). This has lead to an increased attention for PDEs as a promising target for cognition enhancement. In recent studies we have shown that the cGMP selective PDE5-I vardenafil completely reversed object recognition short-term memory and/or acquisition memory deficits induced by the NMDA antagonist MK-801 or acute tryptophan depletion (Chapter 3 and van Donkelaar et al. 2008). In addition, the PDE2-I BAY 60-7550 and PDE10-I PQ-10, which elevate both cGMP and cAMP, were able to reverse memory impairments caused by MK-801, as well as by the muscarinic antagonist scopolamine (Chapter 4).

Localization studies demonstrated that mRNA expression of PDE2 (Lakics et al. 2010; Stephenson et al. 2012; van Staveren et al. 2004; Van Staveren et al. 2003), PDE5 (Lakics et al. 2010; Loughney et al. 1998; van Staveren et al. 2004) and PDE10 (Fujishige et al. 1999; Lakics et al. 2010; Loughney et al. 1999; Soderling et al. 1999) can all be detected in the cortex and hippocampus of mammals with PDE2 mRNA expression being highest and PDE5 mRNA expression being lowest. In addition, in the striatum predominantly PDE10 and – to a lesser extent – PDE2 mRNA expression was demonstrated as well. Combining the behavioural data in the memory deficit models with these localization data support the notion that these PDE-Is might be a suitable tool to treat memory deficits (Chapter 2 and Blokland et al. 2012; Xu et al. 2011)

Interestingly, the PDE10-I TP-10 was able to reverse d-amphetamine induced deficits in a sensory gating paradigm which assesses basic information processing (Schmidt et al. 2008). A study by Grauer et al. (2009) indicated that PDE10 inhibition has a positive effect on prepulse inhibition (PPI), which also addresses information processing, in rats and mice. This implies that the therapeutic use of a PDE-I and in particular a PDE10-I might not be limited to memory dysfunction, but also extend to early stages of (auditory) information processing.

In the present study we therefore specifically investigated the effects of PDE inhibition on information processing, in particular sensory gating in rats. Sensory gating is an adaptive mechanism that helps to prevent overstimulation of higher cortical areas with sensory information (for review see e.g. Cromwell et al. 2008). This mechanism can be assessed by using a paradigm in which two identical, auditory stimuli are presented with an inter-stimulus interval (ISI) of 500 ms and an inter-trial interval (ITI) of at least 6 s. Normally, the response to the first auditory stimulus (S1) is significantly larger than the response to the second stimulus (S2) reflecting sensory gating. However, this mechanism can be disrupted by e.g. pharmalogical intervention with d-amphetamine (Halene and Siegel 2008; Schmidt et al. 2008) or clinical disorders such as Alzheimer's disease or schizophrenia (Adler et al. 1982; Javitt 2009; Jessen et al. 2001).

In human subjects, the P50 (also known as P1) component of the event-related potential (ERP) is regarded to be the main component in the sensory gating paradigm,

although the N100 (N1) and P200 (P2) seem to be involved as well (e.g. Boutros et al. 2009; Chang et al. 2011; Dalecki et al. 2011; Lijffijt et al. 2009). There is still a debate about which ERP component in rats is possibly the functional equivalent of the P50 in humans. It has been suggested that the P13 (P1), N40 (N1) or even P60 (P2) might be the functional equivalent of the P50 in humans, while it has also been suggested that the entire P1-N1-P2 complex is the most suitable candidate (e.g. Broberg et al. 2010; Mears et al. 2009; Mears et al. 2006; Miyazato et al. 1999; Zhou et al. 2008).

We previously demonstrated that PDE5 inhibition with vardenafil does not affect sensory gating in rats at doses which are normally able to improve memory (Chapter 3 and 5). This led us to conclude that the positive effects of PDE5 inhibition on cognition are mediated by higher cognitive processes and not by more early stages of information processing. In the current study we investigated the effects of a PDE2-I (BAY 60-7550) and PDE10-I (PQ-10) at the dosage that was previously found to improve memory function as well (e.g. Boess et al. 2007 and Chapter 4). For the EEG measurements we included the vertex, hippocampus and striatum as electrode locations. These three locations were included because of their involvement in sensory gating (e.g. Bickford-Wimer et al. 1990; Cromwell et al. 2007; Cromwell et al. 2008). The vertex was also chosen to represent the cortex since the EEG signal at this location can also be measured in humans, thus offering a possible translation of our results to humans. We expected that the cGMP-specific PDE5-I vardenafil would not affect sensory gating as demonstrated previously (Chapter 5). Contrarily, the dual substrate PDE-Is BAY 60-7550 and PQ-10 were expected to have an effect on sensory gating, since it was shown that PDE10 inhibition was able to reverse an amphetamine induced sensory gating impairment (Schmidt et al. 2008).

METHODS AND MATERIALS

Animals

All experimental procedures were approved by the local ethical committee for animal experiments of Maastricht University and met governmental guidelines. Fourteen 3-month-old male Wistar rats (Harlan, The Netherlands) were used with average body weights of 386 g (± 11.66). The animals were housed individually in standard Makrolon cages on sawdust bedding in an air-conditioned room (about 20°C). They were kept on a 12/12-h reversed light/dark cycle (lights on from 19.00 to 7.00 h) and had free access to food and water. The rats were housed in the same room as where they were tested. A radio, which was playing softly, provided background noise in the room. All testing was done between 9.00 and 18.00 h in a shielded Skinnerbox.

Surgery and EEG recordings

The animals received 0.1 ml/kg Temgesic (Schering-Plough B.V., Utrecht, The Netherlands) subcutaneously 30 min before surgery as analgesia. Forene isoflurane (Abbott B.V., Hoofddorp, The Netherlands) was used as a general inhalation anesthetic. After the animal was placed into the stereotactic apparatus and an incision was made to expose the skull, lidocaine was applied as additional local anesthesia. Next, bregma was identified and

the electrodes were unilaterally placed in the striatum (AP 0.48, ML -3.0, DV -5.0), dorsal hippocampus (AP -2.8, ML -1.8, DV -2.6) and at the vertex (AP -3.5, ML -1.0, DV -1.0) (Paxinos and Watson 1998). The reference and ground electrodes were both placed on the cerebellum. The electrodes and the connector were fixed to the skull by using three screws and Paladur denture acrylic (Heraeus Kulzer, Hanau, Germany). The animals were given at least two weeks to recover from the surgery.

In the first week after recovery, the animals were handled daily and adapted to the procedure, i.e. they were connected to the EEG set-up and allowed to explore the Skinnerbox in which the recording would take place. In addition the rats were adapted to p.o. administration procedures by saline injections (2 ml/kg). Next, the control condition was tested, i.e. animals were treated with vehicle; this was tested twice and averaged for the statistical analysis. Subsequently three doses of the PDE5-I vardenafil were randomly tested (0.3, 1, 3 mg/kg, p.o.). The results of this experiment were described in Chapter 5 including only the animals whose dataset was complete and electrode localization was validated for the vehicle treatment and all PDE5-I conditions. Additionally, we randomly tested the PDE2-I BAY 60-7550 and the PDE10-I PQ-10 (both 1 mg/kg p.o.). The sensory gating paradigm consisted of 70 pairs of auditory stimuli which were presented with stimulus duration of 10 ms, ISI of 500 ms and ITI 6-10 s. The EEG signal was sampled at 1000 Hz, filtered between 1 - 133.5 Hz and stored on a personal computer. The stimuli were 2500 Hz clicks with a sound intensity of 80 dB and duration of 10 ms. After the study was finished, the animals were killed by decapitation and the brains were taken out. The brains were stored in 4% formaldehyde at 4-6 ºC until electrode localization took place, which was done as described previously (Chapter 5).

Treatment

BAY 60-7550, vardenafil, and PQ-10 were first dissolved in 1.5 ml ethanol with 2% Tween 80. After extraction of ethanol via vaporization under N2 gas, the compounds were dissolved in 0.5% methylcellulose. It was chosen to test the previously found most effective dose (1 mg/kg, p.o.) of each PDE-I in improving memory in an object recognition task. All compounds were administered by oral gavage (2 ml/kg) 30 min before testing. BAY 60-7550 and vardenafil were a gift from BAYER (Wuppertal, Germany); PQ-10 was kindly donated by Johnson & Johnson (Beerse, Belgium). All animals were treated with each condition once, except for the control condition (vehicle), which was tested twice as part of the training.

Statistical analysis

Segments between 100ms before until 500ms after stimulus onset were made for each stimulus type (S1 and S2) separately, using the last 100 ms before onset as baseline. High pass (1Hz) and low pass (30 Hz) filters were applied. The segments were visually checked and removed from the data set if a movement artefact occurred within 500 ms after stimulus presentation. Both the grand average (all animals) and the individual data (single animal) were used to determine the auditory evoked potential (AEP) components. In general, P1 was defined as the most positive value between 20 and 55 ms after stimulus onset, N1 as

the most negative value between 35 and 75 ms, P2 as the most positive value between 55 and 100 ms.

General linear models (GLM) repeated measures were used to analyze the amplitudes of the components. First, the responses to the S1 and S2 were compared for the vehicle condition to see whether sensory gating occurred. Next, the responses to the PDE-Is conditions were compared to placebo condition for each stimulus (treatment: vehicle and PDE-I) separately as well as for both stimuli together (Treatment x Stimulus). One animal was excluded from the vertex and two animals from the striatum electrodes because of no reliable EEG signal at the corresponding electrode.

RESULTS

Effects of vehicle on sensory gating

The effects of vehicle treatment on sensory gating are depicted in Figure 1. GLM repeated measures showed that the N1 peak was less negative after S2 than S1 in the vehicle condition ($F(1, 12) = 6.12$, $P < 0.05$) at the vertex and hippocampus ($F(1, 13) = 6.20$, $P < 0.05$), whereas no effects on the P1 and P2 were found. In the striatum, no effects were found ($F < 4.36$). Thus, sensory gating was generally found in the hippocampus and the vertex.

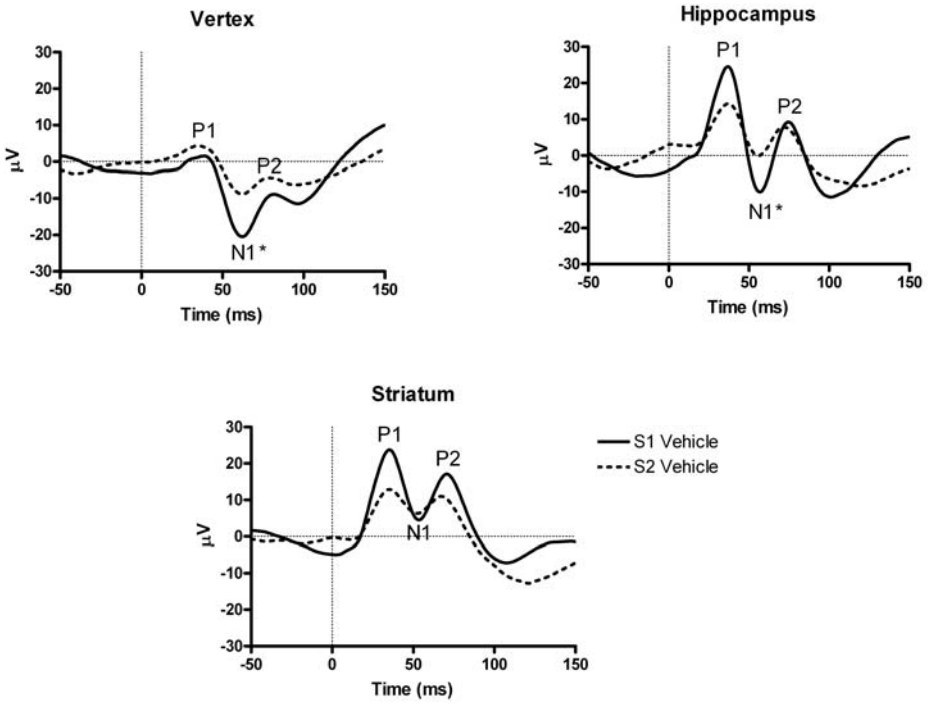

Figure 1 Effects of vehicle on grand average ERPs after the presentation of S1 and S2. N_{vertex} = 13; $N_{hippocampus}$ = 14; $N_{striatum}$ = 12. Effects on gating are depicted with asterisks in the saline condition (*: $P < 0.05$)

Effects of PDE inhibition on information processing

Vertex

The PDE2-I BAY 60-7550 did not have an effect on the ERP components of the S2 or sensory gating. However, GLM repeated measures showed that the P1 peak of the S1 was more positive after BAY 60-7550 than vehicle treatment ($F(1, 10) = 7.59$, $P < 0.05$) (see Figure 2). No other effects of PDE2 inhibition were found. In addition, treatment with the PDE5-I vardenafil or the PDE10-I PQ-10 did not affect S1, S2 or sensory gating (data not shown, $F < 2.84$).

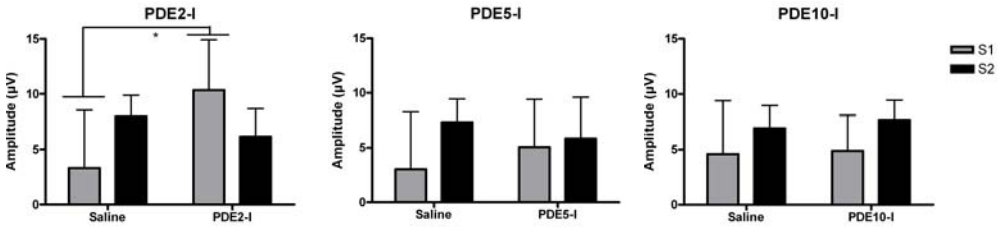

Figure 2 Effects of treatment with a PDE2-I (BAY 60-7550), PDE5-I (vardenafil) or PDE10-I (PQ-10) on the mean amplitude (±SEM) of the P1 in the vertex after the presentation of S1 and S2. $N_{PDE2} = 11$; $N_{PDE5} = 11$; $N_{PDE10} = 12$

Hippocampus

No effects of PDE2 or PDE5 inhibition on the ERP components of the S1, S2 or sensory gating were found (data not shown, $F < 2.69$). The PDE10-I PQ-10 did not affect S1 or S2 separately either. Yet, the GLM repeated measures with treatment as well as stimulus as a within subject variable showed that PQ-10 treatment increased the overall N1 compared to vehicle treatment ($F(1, 13) = 4.71$, $P < 0.05$). This is also illustrated in Figure 3 showing the effects of all PDE-Is on their hippocampal N1 peaks that showed sensory gating in the placebo condition (see Figure 1).

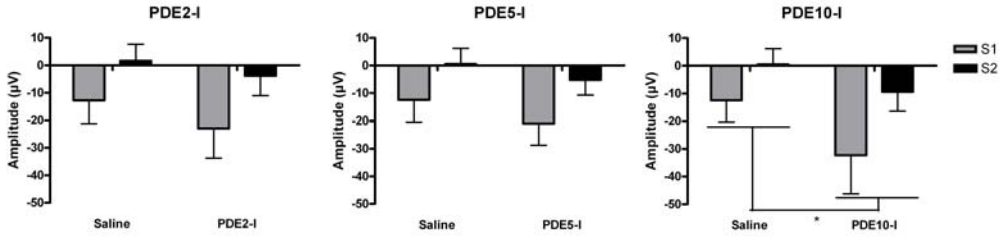

Figure 3 Effects of treatment with a PDE2-I (BAY 60-7550), PDE5-I (vardenafil) or PDE10-I (PQ-10) on the mean amplitude (±SEM) of the N1 in the hippocampus after the presentation of S1 and S2. $N_{PDE2} = 13$; $N_{PDE5} = 14$; $N_{PDE10} = 14$

Striatum

GLM repeated measures demonstrated no effects of PDE2 (n=11), PDE5 (n=11) or PDE10 (n=12) inhibition on the ERP components of S1, S2 or sensory gating (data not shown, $F < 2.98$).

DISCUSSION

The present study showed that after treatment with vehicle the N1 in the vertex and hippocampus was less negative after S2 than after S1 indicating sensory gating in both electrode locations. However, in the striatum, no sensory gating was found. None of the PDE-Is affected sensory gating directly as no Stimulus x Treatment interaction was found. Yet, it was demonstrated that the PDE2-I BAY 60-7550 increased the P1 peak after S1 compared to vehicle in the vertex. In addition, the PDE10-I PQ-10 affected the N1 in general, with this peak being more negative after PQ-10 than vehicle treatment in the hippocampus.

Administration of the PDE5-I vardenafil did not have an effect on sensory gating as we already recently showed in rats and humans (Chapter 5). Although the effect of PDE5 inhibition on learning and memory has been widely investigated (for review see e.g. Chapter 2 and Prickaerts et al. 2004), little is known about the effects on ERPs. A study by Schultheiss et al (2001) investigated the effects of PDE5 inhibition on ERPs in humans. They showed that sildenafil (100 mg p.o., 1 h before testing) reduced N150/250 and increased P300 activity related to the improvement in attention in healthy human adults. Additionally, to our knowledge only one study has thus far examined the effects of PDE5 inhibition on ERPs and information processing in animals (Chapter 5). Our results indicate that the effects of PDE5 inhibition during memory tasks using the same dosage range (Chapter 3 and Baratti and Boccia 1999; Devan et al. 2006; Devan et al. 2007; Devan et al. 2004; Singh and Parle 2003) are predominantly mediated by effects on higher cognitive processes and not on early stages of information processing, since the early ERP components were not affected by PDE5 inhibition.

The PDE2-I BAY 60-7550 and PDE10-I PQ-10 did not affect sensory gating either. Schmidt et al (2008) previously showed that the PDE10-I TP-10 (3 mg/kg) reversed sensory gating deficits in the hippocampus induced by d-amphetamine. However, this study was performed in anesthetized rats while we used freely moving animals in the present study. In addition, we did not use a deficit model here, which clearly could explain the difference between both studies. Furthermore, based on the IC50 values it could be argued that PQ-10 is about 10 times less potent than TP-10 (Siuciak 2008), yet we tested PQ-10 at a dose (1 mg/kg) known to improve memory processes (Chapter 4). Finally, the routes of administration differed between both studies (intravenously versus orally), which might also have an effect. In contrast to auditory sensory gating, the TP-10 compound did not have an effect on sensorimotor gating in PPI paradigms (Schmidt et al. 2008). However, the PDE10-Is papaverine and MP-10, approximately 5 times less potent and 10 times more potent than PQ-10 respectively (Alderton et al. 2009; Siuciak 2008), reversed MK-801 induced PPI deficits in rats and improved PPI-linked sensorimotor gating in healthy mice (Grauer et al. 2009). This suggests that it would be interesting to test PQ-10 in a PPI paradigm as well.

Nevertheless, although we presently did not find a direct effect of PDE10 inhibition with PQ-10 nor of PDE2 inhibition with BAY 60-7550 on auditory sensory gating, PQ-10 as well as BAY 60-7550 showed effects on information processing. Administration of BAY 60-7550 increased the P1 peak after presentation of S1 at the vertex, which might indicate an arousal effect, and PQ-10 increased the N1 peak at the hippocampus in general compared to vehicle treatment, which might point to a general enhancing effect on auditory information processing. However, it has to be noted that the SEM and the number of statistical tests were quite large in our study, which might have led to a false positive and should be investigated further in future research.

Interestingly, the PDE-Is that have an effect on information processing both affect cGMP and cAMP, whereas PDE5 is cGMP specific (e.g. Bender and Beavo 2006). The fact that we only found an effect of the dual substrate PDE-Is suggests that information processing might be influenced by cAMP. This would also be in line with previous findings of Maxwell et al. (2004) who demonstrated that the cAMP-specific phosphodiesterase type 4 inhibitor (PDE4-I) rolipram enhanced the amplitude of the P20 (P1) and N40 (N1) peaks after S1 in the hippocampus in non-anesthetized mice in a sensory gating paradigm. In addition, this compound reversed an amphetamine-induced deficit at these peaks after S1. However, no effects on sensory gating were found for the P20 and N40. Another study (Halene and Siegel 2008) showed that the PDE4-I Ro-20-1724 increased the response to S1 at the P20 (P1) and N40 (N1) peaks in the hippocampus in mice in a sensory gating paradigm as well. In addition, they found that this PDE4-I reversed a decreased N40 after S1 and a N40 gating deficit induced by d-amphetamine. Interestingly, they did not find an effect of PDE4 inhibition on a d-amphetamine induced PPI deficit in mice. These results imply that dual substrate as well as cAMP specific PDE-Is are able to affect auditory information processing in a sensory gating paradigm, but that the results differ when it comes to other paradigms such as PPI.

To summarize, the PDE5-I vardenafil did not have an effect on basic auditory information processing examined in a sensory gating paradigm. This in contrast to administration of the PDE2-I BAY 60-7550 or PDE10-I PQ-10 which affected auditory information processing in general. These results imply that the positive effects of PDE5 inhibition on cognition previously found in animals are possibly the results of an effect on higher cognitive functioning specifically whereas the cognition enhancing effects of PDE2 and PDE10 inhibition might also be influenced by effects on earlier cognitive processes such as information processing. To further elucidate the effects of PDE inhibition on information processing deficit models (e.g. d-amphetamine) and other paradigms (e.g. PPI) could be employed.

REFERENCES

Adler LE, Pachtman E, Franks RD, Pecevich M, Waldo MC, Freedman R (1982) Neurophysiological evidence for a defect in neuronal mechanisms involved in sensory gating in schizophrenia. *Biol Psychiatry* 17: 639-54

Alderton W, Karran E, Ward S (2009) Current and Future Perspectives in Psychiatric Drug Discovery. *Drug News Perspect* 22: 360-4

Baratti CM, Boccia MM (1999) Effects of sildenafil on long-term retention of an inhibitory avoidance response in mice. *Behav Pharmacol* 10: 731-7

Bender AT, Beavo JA (2006) Cyclic nucleotide phosphodiesterases: molecular regulation to clinical use. *Pharmacol Rev* 58: 488-520

Bickford-Wimer PC, Nagamoto H, Johnson R, Adler LE, Egan M, Rose GM, Freedman R (1990) Auditory sensory gating in hippocampal neurons: a model system in the rat. *Biol Psychiatry* 27: 183-92

Blokland A, Menniti FS, Prickaerts J (2012) PDE inhibition and cognition enhancement. *Expert Opin Ther Pat* 22: 349-54

Boess FG, De Vry J, Erb C, Flessner T, Hendrix M, Luithle J, Methfessel C, Riedl B, Schnizler K, van der Staay FJ, van Kampen M, Wiese WB, Koenig G (2007) The novel alpha7 nicotinic acetylcholine receptor agonist N-[(3R)-1-azabicyclo[2.2.2]oct-3-yl]-7-[2-(methoxy)phenyl]-1-benzofuran-2- carboxamide improves working and recognition memory in rodents. *J Pharmacol Exp Ther* 321: 716-25

Boutros NN, Brockhaus-Dumke A, Gjini K, Vedeniapin A, Elfakhani M, Burroughs S, Keshavan M (2009) Sensory-gating deficit of the N100 mid-latency auditory evoked potential in medicated schizophrenia patients. *Schizophr Res* 113: 339-46

Broberg BV, Oranje B, Glenthoj BY, Fejgin K, Plath N, Bastlund JF (2010) Assessment of auditory sensory processing in a neurodevelopmental animal model of schizophrenia--gating of auditory-evoked potentials and prepulse inhibition. *Behav Brain Res* 213: 142-7

Chang WP, Arfken CL, Sangal MP, Boutros NN (2011) Probing the relative contribution of the first and second responses to sensory gating indices: a meta-analysis. *Psychophysiology* 48: 980-92

Cromwell HC, Klein A, Mears RP (2007) Single unit and population responses during inhibitory gating of striatal activity in freely moving rats. *Neuroscience* 146: 69-85

Cromwell HC, Mears RP, Wan L, Boutros NN (2008) Sensory gating: a translational effort from basic to clinical science. *Clin EEG Neurosci* 39: 69-72

Dalecki A, Croft RJ, Johnstone SJ (2011) An evaluation of P50 paired-click methodologies. *Psychophysiology* 48: 1692-700

Devan BD, Bowker JL, Duffy KB, Bharati IS, Jimenez M, Sierra-Mercado D, Jr., Nelson CM, Spangler EL, Ingram DK (2006) Phosphodiesterase inhibition by sildenafil citrate attenuates a maze learning impairment in rats induced by nitric oxide synthase inhibition. *Psychopharmacology (Berl)* 183: 439-45

Devan BD, Pistell PJ, Daffin LW, Jr., Nelson CM, Duffy KB, Bowker JL, Bharati IS, Sierra-Mercado D, Spangler EL, Ingram DK (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: 134-40

Devan BD, Sierra-Mercado D, Jr., Jimenez M, Bowker JL, Duffy KB, Spangler EL, Ingram DK (2004) Phosphodiesterase inhibition by sildenafil citrate attenuates the learning impairment induced by blockade of cholinergic muscarinic receptors in rats. *Pharmacol Biochem Behav* 79: 691-9

Fujishige K, Kotera J, Michibata H, Yuasa K, Takebayashi S, Okumura K, Omori K (1999) Cloning and characterization of a novel human phosphodiesterase that hydrolyzes both cAMP and cGMP (PDE10A). *J Biol Chem* 274: 18438-45

Grauer SM, Pulito VL, Navarra RL, Kelly MP, Kelley C, Graf R, Langen B, Logue S, Brennan J, Jiang L, Charych E, Egerland U, Liu F, Marquis KL, Malamas M, Hage T, Comery TA, Brandon NJ (2009) Phosphodiesterase 10A inhibitor activity in preclinical models of the positive, cognitive, and negative symptoms of schizophrenia. *J Pharmacol Exp Ther* 331: 574-90

Halene TB, Siegel SJ (2008) Antipsychotic-like properties of PDE4 inhibitors - Evaluation of RO-20-1724 with auditory event related potentials and prepulse inhibition of startle. *J Pharmacol Exp Ther* 326: 230-9

Javitt DC (2009) Sensory processing in schizophrenia: neither simple nor intact. *Schizophr Bull* 35: 1059-64

Jessen F, Kucharski C, Fries T, Papassotiropoulos A, Hoenig K, Maier W, Heun R (2001) Sensory gating deficit expressed by a disturbed suppression of the P50 event-related potential in patients with Alzheimer's disease. *Am J Psychiatry* 158: 1319-21

Lakics V, Karran EH, Boess FG (2010) Quantitative comparison of phosphodiesterase mRNA distribution in human brain and peripheral tissues. *Neuropharmacology* 59: 367-74

Lijffijt M, Moeller FG, Boutros NN, Burroughs S, Lane SD, Steinberg JL, Swann AC (2009) The Role of Age, Gender, Education, and Intelligence in P50, N100, and P200 Auditory Sensory Gating. *J Psychophysiol* 23: 52-62

Loughney K, Hill TR, Florio VA, Uher L, Rosman GJ, Wolda SL, Jones BA, Howard ML, McAllister-Lucas LM, Sonnenburg WK, Francis SH, Corbin JD, Beavo JA, Ferguson K (1998) Isolation and characterization of cDNAs encoding PDE5A, a human cGMP-binding, cGMP-specific 3',5'-cyclic nucleotide phosphodiesterase. *Gene* 216: 139-47

Loughney K, Snyder PB, Uher L, Rosman GJ, Ferguson K, Florio VA (1999) Isolation and characterization of PDE10A, a novel human 3', 5'-cyclic nucleotide phosphodiesterase. *Gene* 234: 109-17

Maxwell CR, Kanes SJ, Abel T, Siegel SJ (2004) Phosphodiesterase inhibitors: a novel mechanism for receptor-independent antipsychotic medications. *Neuroscience* 129: 101-7

Mears RP, Boutros NN, Cromwell HC (2009) Reduction of prelimbic inhibitory gating of auditory evoked potentials after fear conditioning. *Behav Neurosci* 123: 315-27

Mears RP, Klein AC, Cromwell HC (2006) Auditory inhibitory gating in medial prefrontal cortex: Single unit and local field potential analysis. *Neuroscience* 141: 47-65

Miyazato H, Skinner RD, Garcia-Rill E (1999) Neurochemical modulation of the P13 midlatency auditory evoked potential in the rat. *Neuroscience* 92: 911-20

Paxinos G, Watson C (1998) The Rat Brain in Stereotaxic Coordinates, 4 edn. Academic Press, Academic Press

Prickaerts J, Sik A, van Staveren WC, Koopmans G, Steinbusch HW, van der Staay FJ, de Vente J, Blokland A (2004) Phosphodiesterase type 5 inhibition improves early memory consolidation of object information. *Neurochem Int* 45: 915-28

Schmidt CJ, Chapin DS, Cianfrogna J, Corman ML, Hajos M, Harms JF, Hoffman WE, Lebel LA, McCarthy SA, Nelson FR, Proulx-Lafrance C, Majchrzak MJ, Ramirez AD, Schmidt K, Seymour PA, Siuciak JA, Tingley Iii FD, Williams RD, Verhoest PR, Menniti FS (2008) Preclinical characterization of selective PDE10A inhibitors: A new therapeutic approach to the treatment of schizophrenia. *J Pharmacol Exp Ther* 325: 681-90

Schultheiss D, Muller SV, Nager W, Stief CG, Schlote N, Jonas U, Asvestis C, Johannes S, Munte TF (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

Singh N, Parle M (2003) Sildenafil improves acquisition and retention of memory in mice. *Indian J Physiol Pharmacol* 47: 318-24

Siuciak JA (2008) The role of phosphodiesterases in schizophrenia : therapeutic implications. *CNS Drugs* 22: 983-93

Soderling SH, Bayuga SJ, Beavo JA (1999) Isolation and characterization of a dual-substrate phosphodiesterase gene family: PDE10A. *Proc Natl Acad Sci USA* 96: 7071-6

Stephenson DT, Coskran TM, Kelly MP, Kleiman RJ, Morton D, O'Neill SM, Schmidt CJ, Weinberg RJ, Menniti FS (2012) The distribution of phosphodiesterase 2A in the rat brain. *Neuroscience* 226: 145-55

van Donkelaar EL, Rutten K, Blokland A, Akkerman S, Steinbusch HW, Prickaerts J (2008) Phosphodiesterase 2 and 5 inhibition attenuates the object memory deficit induced by acute tryptophan depletion. *Eur J Pharmacol* 600: 98-104

van Staveren WC, Steinbusch HW, Markerink-van Ittersum M, Behrends S, de Vente J (2004) Species differences in the localization of cGMP-producing and NO-responsive elements in the mouse and rat hippocampus using cGMP immunocytochemistry. *Eur J Neurosci* 19: 2155-68

Van Staveren WC, Steinbusch HW, Markerink-Van Ittersum M, Repaske DR, Goy MF, Kotera J, Omori K, Beavo JA, De Vente J (2003) mRNA expression patterns of the cGMP-hydrolyzing phosphodiesterases types 2, 5, and 9 during development of the rat brain. *J Comp Neurol* 467: 566-80

Xu Y, Zhang HT, O'Donnell JM (2011) Phosphodiesterases in the central nervous system: implications in mood and cognitive disorders. *Handb Exp Pharmacol*: 447-85

Zhou D, Ma Y, Liu N, Chen L, He M, Miao Y (2008) Influence of physical parameters of sound on the sensory gating effects of N40 in rats. *Neurosci Lett* 432: 100-4

CHAPTER 7

The effects of the PDE5 inhibitor vardenafil on cognitive performance in healthy adults: a behavioural-EEG study

Olga A.H. Reneerkens*, Anke Sambeth*, Jan G. Ramaekers, Harry W.M. Steinbusch, Arjan Blokland & Jos Prickaerts

* Authors contributed equally

Accepted for publication in Journal of Psychopharmacology

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 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.

INTRODUCTION

Phosphodiesterase type 5 inhibitors (PDE5-Is) such as sildenafil (also known as Viagra), tadalafil (Cialis) and vardenafil (Levitra) are often used 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). Because of 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 behavioural tasks in animals (for review see Chapter 2). 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 (Chapter 3 and Devan et al. 2007) 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 behavioural 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 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 (Chapter 5). 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 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 behavioural models (e.g. Chapter 3 and Domek-Lopacinska and Strosznajder 2008; Patil et al. 2004; Prickaerts et al. 2002; 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 in Chapter 5.

The aim of this study was to examine the effects of vardenafil on cognition, in particular memory, but also executive function and attention, and the electrophysiological correlates, i.e. ERPs, of information processing in healthy volunteers. Cognitive performances were assessed while simultaneously recording brain activity. 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). The participants were recruited through advertisements at Maastricht University. They had to be willing to sign an informed consent 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 1) likely to show better performance on the test the next time (regression to the mean) and 2) 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, 5 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 2 weeks before 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 (history of) a 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, 3-way cross-over design. Order of treatments was balanced over three test days and separated by a

washout period of at least 7 days. The balancing of the treatment order was accomplished by counterbalancing.

Treatment consisted of a placebo, 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 (EMEA 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 (EMEA 2008). Therefore, we started the measurement 45 minutes 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 assessing 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 minutes 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 latter has already been reported as part of a translational study (Chapter 5). 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 minutes we started testing again; first they performed the tower of London (TOL), then the Stroop task, a reaction time (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 & Lader visual analogue scale (VAS)

Subjective evaluations of alertness were assessed by using an adjusted series of 9 visual analogue scales (100 mm), which provided summary scores for alertness (Bond and Lader 1974).

Questionnaire 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 are 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 second; the total inter-trial interval (ITI) was 3 seconds. 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 seconds (total ITI of 3 seconds) 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 were calculated for the analyses, for the recognition test reaction times 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 seconds. 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 reaction time 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 (Kessels et al. 1999; Sambeth et al. 2009)) that consisted of two parts; immediate and delayed recognition.

The immediate recognition comprises 6 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 seconds. 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 reaction time 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 ones 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 6 steps was excluded from the analysis). Each condition was randomly presented 10 times, expect for the 6 steps condition which only occurred 4 times. Different versions of this test were balanced over test days. Reaction times 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, colour names (in Dutch) were printed in coloured ink and presented with an ITI between 2.5-3.5 seconds; in the congruent category, the colour name and the colour of the ink were the same, in the incongruent category they were not. The participants had to name the colour of the ink, not the words themselves. Because of the urge to read the printed words (even if one is asked to ignore them) interference occurs. Since the printed words and ink colour 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 colours used in this task were blue, red, green and yellow. The colour of the ink had to be named by pressing one out of four buttons, which each represented one of the colours. Each colour was randomly presented 20 times in the congruent as well as the incongruent condition which brings the total amount of stimuli in the congruent as well as the incongruent condition at 80. The main performance measures were the reaction times 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 impair 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 reaction time 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 and 100 Hz and sampled at 1000 Hz. The EEG responses were recorded during the VLT, CRMT, SMT and Stroop task.

The EEG data was analysed using Vision Analyzer 2 (Brain Products, Gilching, Germany) software. Epoch files were made from 100ms before stimulus onset until 1000ms after onset, using the last 100 ms before stimulus onset as baseline. High pass (1Hz) 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 most negative value between 70 and 140 ms after stimulus onset, P150 as most positive value between 130 and 210, N200 as most negative value between 140-310 ms, P300 as most positive value between 255 and 380 ms, N400 as most negative value between 365 and 520 ms (not detected in the CRMT and Stroop) and P600 (not detected in the SMT encoding phase) as most positive value between 380 and 700 ms.

Statistical analysis

General linear model (GLM) repeated measures were used to analyse 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 minutes later) for further analysis. Treatment (three levels: placebo, vardenafil 10 mg, vardenafil 20 mg) was used as a within subject 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 analysed 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 behavioural response to the Stroop and the RT task all 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-behaviour

Immediate recall No effects of vardenafil treatment were found with regard to words recalled correctly ($F(2, 32) = 0.38$, 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 mentioned double ($F(2, 32) = 0.08$, n.s.).

Delayed recognition An effect for the reaction time 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 reaction time ($F(2, 32) = 1.72$, 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. Further analyses showed that, the P300 was decreased after vardenafil 20 mg compared to placebo at the Fz, Cz and CPz, after both vardenafil conditions compared to placebo treatment at the FCz and Pz, and after vardenafil 20 mg compared to 10 mg treatment at the FCz and Pz (see also Figure 1).

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.

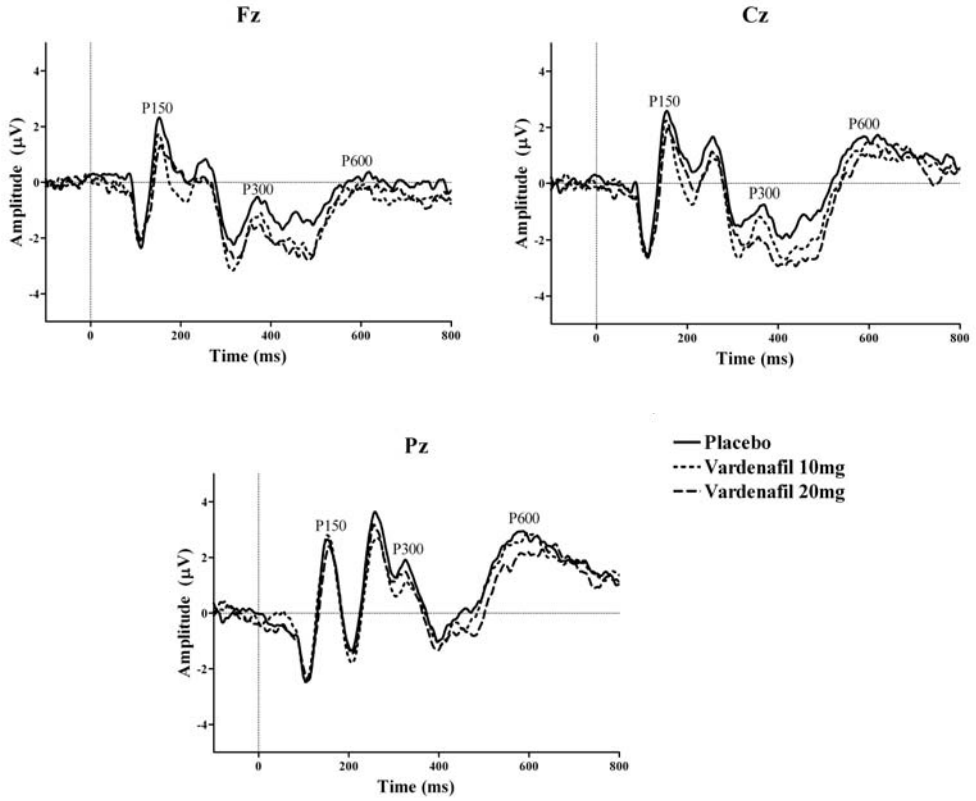

Figure 1 Effects of vardenafil treatment on grand average ERPs during encoding in the VLT; generally the P300 was decreased after vardenafil treatment compared to placebo. Latencies are shown on the x-axis in milliseconds (ms), amplitudes on the y-axis in microvolts (μV)

CRMT- behaviour

The overall reaction time 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 reaction times ($F(2, 32) = 1.51$, 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 on 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 reaction time ($F(2, 32) = 1.49$, n.s.) was found. However, the interval between the pictures had an effect on reaction time ($F(2, 32) = 5.41$, $P < 0.05$), with the interval of 10 causing a slower reaction time than the interval of 3 stimuli.

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, see also Fig. 2). 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-behaviour

Immediate recognition No effects of vardenafil treatment were found with regard to the reaction time ($F(2, 32) = 0.32$, 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 reaction time ($F(2, 32) = 0.31$, n.s.) and number of correct responses ($F(2, 32) = 0.69$, n.s.) in the delayed recognition.

Table 1 No effects of vardenafil treatment on the behavioural performance on the memory tasks (VLT, CRMT and SMT) were found (mean values (±SEM)). Reaction times are presented in milliseconds (ms). n = 17

			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	Reaction time	846.16 (28.82)	796.67 (24.06)	848.50 (31.40)
	recognition old	Correct	12.94 (0.49)	13.59 (0.40)	13.35 (0.35)
	Delayed	Reaction time	907.69 (36.20)	862.45 (26.83)	875.58 (26.06)
	recognition new	Correct	13.47 (0.45)	13.35 (0.42)	13.41 (0.41)
CRMT	Immediate	Reaction time	703.46 (19.33)	719.71 (16.65)	721.33 (19.22)
	recognition new	Correct	56.94 (0.75)	56.24 (0.94)	56.94 (0.73)
	Immediate	Reaction time	668.70 (20.46)	690.65 (21.61)	692.12 (21.56)
	recognition old	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	Reaction time	853.40 (42.71)	820.03 (46.31)	816.81 (51.85)
	recognition	Correct	52.06 (1.03)	51.06 (1.13)	50.18 (1.14)
	Delayed	Reaction time	899.56 (62.37)	863.99 (67.18)	894.55 (59.51)
	recognition	Correct	44.29 (1.22)	45.47 (1.10)	45.35 (1.23)

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 reaction time increased ($F(3, 48) = 135.39$, $P < 0.001$) and the number 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 reaction time ($F(2, 32) = 0.29$, 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 also Table 2).

Stroop-behaviour

The reaction time 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 (reaction time ($F(2, 30) = 0.27$, 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).

Table 2 No effects of vardenafil treatment on the behavioural performance on the TOL and Stroop were found (mean values (±SEM)). Reaction times are presented in seconds (s) for the TOL and ms for the Stroop. n_{TOL} = 17, n_{Stroop} = 16

			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)
	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)
Stroop	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)

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

RT task

GLM repeated measures showed an effect of hand side on the reaction time ($F(1, 15) = 4.98$, $P < 0.05$). More specifically, the reaction time was higher when the participants had to respond with their right hand compared to the left hand. Furthermore, there was a treatment x side interaction for the correct responses ($F(2, 30) = 6.84$, $P < 0.01$). This effect was further analysed 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 & Lader visual analogue scale

The participants reported no effect of vardenafil treatment on alertness ($F(2, 32) = 2.71$, n.s.).

Questionnaire 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 the PDE5-I vardenafil on cognition and ERP measurements in healthy volunteers. No effects of vardenafil treatment were found on any of the behavioural 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 the 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 reaction time 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 behavioural 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 found a decrease of the P300 after vardenafil treatment during the encoding of the words whereas behavioural 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 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 possible 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, behavioural 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 behavioural response at 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 pharmalogical studies whereas no effect on behavioural 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 they did not have to execute an explicit behavioural 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 EEG is more sensitive to pick up changes as compared to behaviour.

Although no effects of vardenafil administration on the POMS and the Bond & Lader were found, the questionnaire addressing medical complaints showed that vardenafil 10 mg and 20 mg increased the report of feeling weak and headache. The latter is one of the most commonly reported side effects after vardenafil treatment (reported by more than 10% of the participants from the clinical trials (EMEA 2008)). Together with the fact that maximum plasma concentrations of vardenafil after oral administration are reached within 30 to 120 minutes (EMEA 2008), this finding 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 (per os) which have been found to enhance memory function in rats (e.g. Chapter 3 and 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 (Chapter 3). Since vardenafil was presently found to affect ERPs it might have entered the brain and could be biologically active there. Nevertheless, it can not 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 on its own. 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 not (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 Chapter 2) and the task 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 of 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 whether the first 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 cognitive 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 the behavioural 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 need further investigation in the future, e.g. by using deficits 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 contradicting effects on the electrophysiological correlates of cognition.

ACKNOWLEDGEMENTS

The authors would like to thank Floriaan Demeester, Ili Ma, Moos Peeters, Conny Quaedflieg en Saranne Jacobs for their technical assistance.

REFERENCES

Akhondzadeh S, Ghayyoumi R, Rezaei F, Salehi B, Modabbernia AH, Maroufi A, Esfandiari GR, Naderi M, Ghebleh F, Tabrizi M, Rezazadeh SA (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: 809-15

Baratti CM, Boccia MM (1999) Effects of sildenafil on long-term retention of an inhibitory avoidance response in mice. *Behav Pharmacol* 10: 731-7

Bender AT, Beavo JA (2006) Cyclic nucleotide phosphodiesterases: molecular regulation to clinical use. *Pharmacol Rev* 58: 488-520

Bond A, 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, Zuurman L, Jansma JM, Mehta MA, van Gerven JM, Kahn RS, Ramsey NF (2012) Effects of Delta9-tetrahydrocannabinol administration on human encoding and recall memory function: a pharmacological FMRI study. *J Cogn Neurosci* 24: 588-99

Cacioppo JT, Tassinary LG, Berntson GG (2000) Handbook of Psychophysiology, 2nd edn. Cambridge University Press, Cambridge University Press

Curran HV, Pooviboonsuk P, Dalton JA, Lader MH (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, Carter G, Mallard T, Whyte I (2012) Cognitive impairment in patients clinically recovered from central nervous system depressant drug overdose. *J Clin Psychopharmacol* 32: 503-10

Devan BD, Pistell PJ, Daffin LW, Jr., Nelson CM, Duffy KB, Bowker JL, Bharati IS, Sierra-Mercado D, Spangler EL, Ingram DK (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: 134-40

Domek-Lopacinska K, 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

EMEA (2008) European Public Assesment Report; Revision 6. www.emea.europa.eu

Federmeier KD, 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. Academic Press, Burlington, pp 1-44

Gazzaniga MS, Ivry RB, Mangun GR (2002) Cognitive neuroscience: The biology of the mind, 2nd edn. W.W. Norton & Company, Inc., W.W. Norton & Company, Inc.

Goff DC, Cather C, Freudenreich O, Henderson DC, Evins AE, Culhane MA, Walsh JP (2009) A placebo-controlled study of sildenafil effects on cognition in schizophrenia. *Psychopharmacology (Berl)* 202: 411-7

Grass H, Klotz T, Fathian-Sabet B, Berghaus G, Engelmann U, Kaferstein H (2001) Sildenafil (Viagra): is there an influence on psychological performance? *Int Urol Nephrol* 32: 409-12

Jasper H (1958) The ten-twenty electrode system of the international federation. *Electroencephalogr Clin Neurophysiol* 10: 371-5

Kessels RP, Postma A, 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: 423-8

Lakics V, Karran EH, Boess FG (2010) Quantitative comparison of phosphodiesterase mRNA distribution in human brain and peripheral tissues. *Neuropharmacology* 59: 367-74

Langtry HD, Markham A (1999) Sildenafil: a review of its use in erectile dysfunction. *Drugs* 57: 967-89

Lezak MD (1995) Neuropsychological Assesment, 3rd edn. Oxford University Press, Oxford University Press

Linssen AM, Riedel WJ, Sambeth A (2011) Effects of tyrosine/phenylalanine depletion on electrophysiological correlates of memory in healthy volunteers. *J Psychopharmacol* 25: 230-8

Loughney K, Hill TR, Florio VA, Uher L, Rosman GJ, Wolda SL, Jones BA, Howard ML, McAllister-Lucas LM, Sonnenburg WK, Francis SH, Corbin JD, Beavo JA, Ferguson K (1998) Isolation and characterization of cDNAs encoding PDE5A, a human cGMP-binding, cGMP-specific 3',5'-cyclic nucleotide phosphodiesterase. *Gene* 216: 139-47

Luck SJ (2005) An introduction to the Event-Related Potential Technique. MIT press, MIT press

McNair DM, Lorr M, Droppleman LF (1971) Manual for the profile of mood states. Educational and Industrial Testing Service

Patil CS, Jain NK, Singh VP, Kulkarni SK (2004) Differential Effect of the PDE5 Inhibitors, Sildenafil and Zaprinast, in Aging- and Lippolysaccharide-Induced Cognitive Dysfunction in Mice. *Drug Dev Res* 63: 66-75

Prickaerts J, Steinbusch HW, Smits JF, de Vente J (1997) Possible role of nitric oxide-cyclic GMP pathway in object recognition memory: effects of 7-nitroindazole and zaprinast. *Eur J Pharmacol* 337: 125-36

Prickaerts J, van Staveren WC, Sik A, Markerink-van Ittersum M, Niewohner U, van der Staay FJ, Blokland A, de Vente J (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: 351-61

Puzzo D, Staniszewski A, Deng SX, Privitera L, Leznik E, Liu S, Zhang H, Feng Y, Palmeri A, Landry DW, Arancio O (2009) Phosphodiesterase 5 inhibition improves synaptic function, memory, and amyloid-beta load in an Alzheimer's disease mouse model. *J Neurosci* 29: 8075-86

Reagan-Shaw S, Nihal M, Ahmad N (2008) Dose translation from animal to human studies revisited. FASEB J 22: 659-61

Reyes-Irisarri E, Markerink-Van Ittersum M, Mengod G, de Vente J (2007) Expression of the cGMP-specific phosphodiesterases 2 and 9 in normal and Alzheimer's disease human brains. *Eur J Neurosci* 25: 3332-8

Riedel WJ, Klaassen T, Deutz NE, van Someren A, van Praag HM (1999) Tryptophan depletion in normal volunteers produces selective impairment in memory consolidation. *Psychopharmacology (Berl)* 141: 362-9

Rodefer JS, Saland SK, Eckrich SJ (2012) Selective phosphodiesterase inhibitors improve performance on the ED/ID cognitive task in rats. *Neuropharmacology* 62 1182-90

Rutten K, Basile JL, Prickaerts J, Blokland A, Vivian JA (2008) Selective PDE inhibitors rolipram and sildenafil improve object retrieval performance in adult cynomolgus macaques. *Psychopharmacology (Berl)* 196: 643-8

Rutten K, Prickaerts J, Hendrix M, van der Staay FJ, Sik A, Blokland A (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: 107-12

Rutten K, Vente JD, Sik A, Ittersum MM, Prickaerts J, Blokland A (2005) The selective PDE5 inhibitor, sildenafil, improves object memory in Swiss mice and increases cGMP levels in hippocampal slices. *Behav Brain Res* 164: 11-6

Sambeth A, Riedel WJ, Tillie DE, Blokland A, Postma A, Schmitt JA (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, Markus CR, Deutz NE, Riedel WJ (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: 375-84

Schultheiss D, Muller SV, Nager W, Stief CG, Schlote N, Jonas U, Asvestis C, Johannes S, Munte TF (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, Campbell RK, Baker DE (2005) Phosphodiesterase 5 inhibitors for erectile dysfunction. *Ann Pharmacother* 39: 1286-95

Shallice T (1982) Specific impairments of planning. Philos Trans R Soc Lond B Biol Sci 298: 199-209

Shim YS, Pae CU, Kim SW, Kim HW, Kim JC, Koh JS (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: 109-14

van Donkelaar EL, Rutten K, Blokland A, Akkerman S, Steinbusch HW, Prickaerts J (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, Franken IH (2007) Electrophysiological correlates of word repetition spacing: ERP and induced band power old/new effects with massed and spaced repetitions. *Int J Psychophysiol* 66: 205-14

CHAPTER 8

General discussion

The aim of this thesis was to investigate the potential of PDE inhibition to improve cognition in a translational setting. In short, we studied the effects of PDE2, 5 and 10 inhibition on memory function in rats with the use of scopolamine and MK-801 induced deficit models. Additionally, we investigated whether a PDE2-I or PDE10-I could affect sensory gating in rats, and whether a PDE5-I could affect sensory gating in both rats and humans, i.e. healthy volunteers. Finally, the effects of PDE5 inhibition on cognition in healthy volunteers were addressed by using behavioural tasks as well as EEG measurements.

OVERVIEW MAIN FINDINGS OF THESIS

PDE inhibition and object memory function in rats

In Chapter 3 and 4 we studied the effects of PDE2, 5 and 10 inhibition on object memory in rats. In **Chapter 3**, we used two different PDE5-Is to investigate these effects; vardenafil and UK-343,664. The first was shown to cross the BBB, the latter did not. Vardenafil reversed memory deficits induced by the NMDA antagonist MK-801 and improved memory function decay over time in the object recognition task, whereas UK-343,664 did not have an effect. This would imply that there is PDE5 present within the brain that mediates the effects vardenafil on object recognition. However, the memory deficits induced by the muscarinic antagonist scopolamine were reversed by vardenafil as well as UK-343,664. Co-administration of scopolamine with vardenafil or UK-343,664 did not alter the brain penetration of the compounds, which suggest that the integrity of the BBB is not compromised. This is in line with the literature in which no evidence was found that scopolamine administration affects the BBB integrity. Thus, the positive effects of UK-343,664 in the scopolamine-induced memory deficit could be mediated by systemic PDE5. Nevertheless, since UK-343,664 did not improve memory decay over time, nor reversed MK-801 induced deficits, it is most likely that the memory enhancing effects of UK-343,664 in the scopolamine model can be attributed to its combination with central and/or peripheral effects of scopolamine.

We further investigated the effects of PDE2 and PDE10 inhibition in a MK-801- and scopolamine-induced memory deficit model in **Chapter 4**. It was found that the PDE2-I BAY 60-7550 as well as the PDE10-I PQ-10 were able to reverse the object memory deficits induced by MK-801 and scopolamine. Additionally, it was demonstrated that PQ-10 was clearly brain penetrant at the behaviourally active dose. Contrarily, BAY 60-7550 was only detected in the brain at higher dosages although the brain-plasma ratio was still quite low. This suggests that low concentrations are probably sufficient to have a biological effect. This might be caused by a high expression of PDE2 in brain structures that are implicated in object recognition or by an effect on cyclic nucleotides signal cascades which does not necessarily need a high level at the beginning, but can lead to a biological response due to signal amplification. Additionally, it might be speculated that an active metabolite crosses the BBB. Finally, it could be the case that PDE2 inhibition exerts not only a central, but a peripheral effect as well.

PDE inhibition and sensory gating in rats and humans

In Chapter 5 and 6 we studied the effects of PDE inhibition on information processing in an auditory sensory gating paradigm. We demonstrated in **Chapter 5** that rats and humans both showed sensory gating in the placebo condition. In rats, only the N1 peak was less negative after the S2 than S1 in the vertex and the hippocampus. The human volunteers showed sensory gating at all peaks (P1, N1 and P2) at the Fz, FCz and Cz electrodes. However, neither in rats nor in humans an effect of vardenafil was found on basic auditory information processing. Since the dosages used in the animals were able to improve memory function in animals (see Chapter 3), these results imply that the positive effects of cGMP-specific PDE5 inhibition on cognition are predominantly mediated by effects on higher cognitive processes and not on information processing.

In the follow-up study described in **Chapter 6**, we investigated the effects of PDE2 and PDE10 inhibition in the same paradigm in rats. Although auditory sensory gating was found in the placebo condition at the N1 peak in the hippocampus and vertex, it was not affected by administration of the PDE2-I BAY 60-7550 or the PDE10-I PQ-10. However, it was shown that BAY 60-7550 increased the P1 peak after S1 in the vertex. Furthermore, PQ-10 affected the N1 peak in the hippocampus in general, with this peak being more negative in the PQ-10 than placebo condition. The dosage we used in this study showed memory improving effects in Chapter 4. Therefore, these findings indicate that the positive effects of the dual substrate PDE2-I and PDE10-I on object memory might partly be affected on more early phases of information processing when given before the learning trial. However, it still needs to be determined whether these effects are similar when given immediate after the learning trial or before the recognition trial.

PDE inhibition and cognition in humans

The effects of vardenafil on cognition and ERP measurements in healthy humans were looked into in **Chapter 7**. No effects were found on any of the behavioural performances in the cognitive tasks, more specifically addressing memory function and executive functioning. Yet there was an effect on EEG as vardenafil treatment decreased the P300 peak after the immediate recall part of the VLT. There are several possible explanations for the apparent discrepancy between finding an effect on an ERP component and finding nothing on behavioural performance. EEG is more sensitive to pick up effects as compared to behaviour. Thus, PDE5 inhibition might have had an effect on encoding without eliciting an effect on the free recall of the words the participants had to learn. In addition, some compensatory mechanism might also have counteracted or compensated the effects of PDE5 inhibition, resulting in no change in behavioural performance. Unfortunately, since we did not find an effect of vardenafil treatment on the behavioural response on the VLT, it is difficult to pinpoint the exact meaning of this effect.

METHODOLOGICAL CONSIDERATIONS

In the animals, we found that PDE2, PDE5 and PDE10 inhibition all had a positive effect on memory function. However, we did not find any effect of PDE5 inhibition on the

behavioural performance on a variety of cognitive tasks in our human volunteers. There might be several explanations for this, including type of treatment, reversal of cognitive deficits versus improvement of normal functioning, the environment in which the animals or participant reside and the type of tasks that are used in an experiment.

Treatment

Since we did not find an effect of PDE5 inhibition on cognition in healthy volunteers, it could be argued that the dosages we used in our study might not be in the optimal dose-range for humans to find cognition-enhancing effects. It has been demonstrated in this thesis (Chapter 3) as well as in other studies (Prickaerts et al. 2002; Rutten et al. 2007; van Donkelaar et al. 2008), that 1-3 mg/kg vardenafil administered orally improves memory function in rats. To extrapolate these dosages used in animals to the dosages needed in humans, we used the Reagan-Shaw formula (2008): Human dose equivalent (mg/kg) = animal dose (mg/kg) * (animal Km / human Km). Given the Km value of 6 for rats and 37 for adult humans, the 1-3 mg/kg in animals would be equivalent to 10-31 mg in humans (given the average body weight of 64 kg of the volunteers that participated in our study). Thus, the dosages we used in our human study (10 mg and 20 mg vardenafil) were well within this range and should be appropriate translational doses.

Another point that could be raised is the time point and route of administration. We started the behavioural as well as EEG measurements 45 minutes after vardenafil (or placebo) administration. Previous studies on pharmacokinetic properties of vardenafil by the EMEA (2008) have shown that peak plasma levels were reached between 30 and 120 minutes after a single dose of 20 mg vardenafil and that the terminal half-life was around 4-5 h. Additionally, in our own study we found an increased report of feeling weak and headache after vardenafil treatment. The latter was reported by more than 10% of the participants in previous clinical trials (EMEA 2008), which makes it one of the most reported side effects after vardenafil intake. Combined with the pharmacokinetic properties of vardenafil, this indicates that vardenafil was very likely bioactive during our testing period.

In order to exert an effect its target, PDE5, has to be available. As the literature shows, the levels of PDE5 are rather low compared to other PDEs (Lakics et al. 2010; Loughney et al. 1998) and decline even further as people age (Reyes-Irisarri et al. 2007). Since we demonstrated in Chapter 7 that vardenafil affected ERPs, it probably entered the brain, which was the case in rats as shown in Chapter 3. Nevertheless, it cannot be ruled out that PDE5 levels are not high enough or PDE5-Is, including vardenafil, do not penetrate the BBB to an extent that is sufficient to exert behavioural effects on cognitive tasks in humans. However, Shim et al. (2011) found a positive effect of chronic treatment with the PDE5-I udenafil on the performance on the Korean version of the mini-mental state exam and an assessment battery addressing executive functioning. Although they used people suffering from erectile dysfunction instead of healthy volunteers, the use of chronic treatment might be an interesting alternative to the acute treatment as used in our study.

Reversal of deficits versus improvement of normal function

Except for the 24 h delay interval in the ORT as used in Chapter 3, we only used deficit models to study the effects of PDE-Is in rats, whereas we used healthy volunteers for our human study. Subsequently, another explanation for the different results of PDE inhibition on cognition between rats and humans might be the fact that we predominantly studied the reversal of deficits in animals versus the improvement of normal functioning in humans. In the literature we found that PDE2, PDE5 as well as PDE10 inhibition improved cognition in unimpaired rats in a variety of behavioural tasks (e.g. Boess et al. 2004; Domek-Lopacinska and Strosznajder 2008; Grauer et al. 2009; Rutten et al. 2009; Singh and Parle 2003). However, when looking at the – rather limited – amount of literature on the effects of PDE inhibition on cognition in humans, we found that PDE5 inhibition had an effect on ERP measurements during the auditory attention and word recognition (Schultheiss et al. 2001), but did not affect cognitive performance in unimpaired adults (Grass et al. 2001; Schultheiss et al. 2001). Goff et al. (2009) did not find an effect of sildenafil treatment on cognition in patients suffering from schizophrenia either. However, in people suffering from erectile dysfunction PDE5 inhibition improved cognition as mentioned above (Shim et al. 2011). So although the cognition enhancing effects can be found in impaired as well as unimpaired rats, it might be the case that the future of PDE inhibition as cognition enhancer in humans is limited to people demonstrating impaired cognitive functioning. This assumption needs to be investigated further by using deficit models in healthy volunteers.

Environment

One of the major differences in the daily life experiences of the humans and animals participating in our studies are the environments in which they reside. The rats were housed in standard, laboratory housing conditions, while humans generally live in conditions that may be considered as an enriched environment. Although this difference seems trivial, preliminary data of a recent study by Blokland et al. (2012) might prove otherwise. In this study the memory performance of rats that were housed in either standard housing or enriched environment was tested. The enriched environment animals performed similar to the standard housed rats in the 1 h interval, but outperformed them on the 24 h interval. PDE5 inhibition with vardenafil only improved object memory in the standard housed animals. However, in the enriched environment animals PDE5 inhibition did not enhance memory function. These results do not only raise questions about the housing of animals and the effect on their performance in experiments investigating possible cognition enhancers, but also suggest that the enriched environment in which our healthy human volunteers reside might leave little room for improvement. It would therefore be interesting to repeat (part of our) animal studies with rats that were housed in an enriched environment.

Cognitive tasks

Finally, the lack of findings in the human volunteers might also be explained by the type of cognitive tasks we used in our experiments. In our sensory gating studies for example, the task setup and testing conditions were quite similar between rats and humans and,

possibly as a result of this, the results were comparable as well. However, the sensory gating paradigm addresses an automatic process for which no special task instructions are needed and in which the sensory preferences of a certain species play a role, as is for example the case in memory tasks. Although all memory tasks used in our experiments address explicit memory function, the human volunteers got specific instructions concerning the tasks they had to perform before testing began, whereas the rats were simply introduced into ORT set-up after which the measurements started. Additionally, the memory tasks for the humans focused on visual input, while the ORT predominantly relied on tactile information. These task differences can unintentionally introduce confounding factors when translating the results from humans to rats or vice versa.

On the other hand, the wide variety of cognitive tasks and animals species in which cognition enhancing effects of PDE inhibition were demonstrated (for review see Chapter 2) would provide us with ample evidence to expect effects of PDE inhibition on cognitive performance anyway. Especially since the tasks we used in the human study, i.e. the VLT, TOL and Stroop tasks, are well established tasks to investigate cognitive processes such as memory performance and executive functioning. However, an interesting alternative might be the use of operant tasks in which the task differences between animals and humans can be kept to a minimum and EEG responses could be measured as well.

CLINICAL IMPLICATIONS AND FUTURE DIRECTIONS

Does PDE inhibition enhance cognition? Unfortunately, we did not find any effects of PDE5 inhibition on cognitive performance in healthy humans (Chapter 7). However, as demonstrated in Chapter 2, 3 and 4, PDE-Is, including PDE5-Is, can enhance cognition in animals, especially in rodents. The reversal of memory deficits in pharmacological models that mimic cognitive problems affiliated to e.g. schizophrenia and Alzheimer's disease (see Chapter 3 and 4), as well as the memory improving effects in transgenic mouse models of Alzheimer's disease (Cuadrado-Tejedor et al. 2011; Puzzo et al. 2009; Sierksma et al. 2012), suggest that these compounds could have a promising future as cognition enhancers for people suffering from neurodegenerative and psychiatric disorders. It has indeed been shown that chronic administration of PDE5-Is is able to improve cognitive function in people suffering from erectile dysfunction (Shim et al. 2011) and even reduce positive and negative symptoms when used as an adjunctive therapy with risperidone in patients with schizophrenia. Interestingly, in contrast to PDE5 inhibition, PDE2 and PDE10 inhibition influenced early information processing in rats (Chapter 5 and 6). This implies that these compounds might be especially appealing for patients who suffer from (early) information processing deficits as e.g. in patients suffering from schizophrenia (McCarley et al. 1991; Turetsky et al. 2009).

Further, preclinical research should focus on behavioural tasks that can be used in a translational setting. In addition, clinical testing should consider the use of cognition deficit models as is done more often in animals. Furthermore, it is interesting to not only test the effects of acute administration of the PDE-Is, but to include chronic administration as well. It is also important to shed more light on the possibly interfering effects of housing conditions of the animals, i.e. can our results be replicated in animals in enriched environments. Finally, it is essential to explore the effects of different types of PDE-Is in more detail, in

order to gain further insight into the potential of PDEs as a target for cognition enhancement. For example, we already demonstrated that the dual substrate PDE-Is BAY 60-7550 and PQ-10 affected early auditory information processing, whereas the cGMP-specific PDE5-I vardenafil did not. This implies that the each type of PDE-I could have a more specific use to target the cognition impairments related to a particular disorder. In conclusion, specific PDE-Is can improve cognition and the potential of these PDE-Is as cognition enhancers needs to be elucidated in future translational research.

REFERENCES

Blokland A, Akkerman S, Prickaerts J (2012) Environmental enrichment: differential effects on drugs in object recognition memory in rats Society for Neuroscience. Neuroscience Meeting Planner, New Orleans

Boess FG, Hendrix M, van der Staay FJ, Erb C, Schreiber R, van Staveren W, de Vente J, Prickaerts J, Blokland A, Koenig G (2004) Inhibition of phosphodiesterase 2 increases neuronal cGMP, synaptic plasticity and memory performance. *Neuropharmacology* 47: 1081-92

Cuadrado-Tejedor M, Hervias I, Ricobaraza A, Puerta E, Perez-Roldan JM, Garcia-Barroso C, Franco R, Aguirre N, Garcia-Osta A (2011) Sildenafil restores cognitive function without affecting beta-amyloid burden in a mouse model of Alzheimer's disease. *Br J Pharmacol* 164: 2029-41

Domek-Lopacinska K, 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

EMEA (2008) European Public Assesment Report; Revision 6. www.emea.europa.eu

Goff DC, Cather C, Freudenreich O, Henderson DC, Evins AE, Culhane MA, Walsh JP (2009) A placebo-controlled study of sildenafil effects on cognition in schizophrenia. *Psychopharmacology (Berl)* 202: 411-7

Grass H, Klotz T, Fathian-Sabet B, Berghaus G, Engelmann U, Kaferstein H (2001) Sildenafil (Viagra): is there an influence on psychological performance? *Int Urol Nephrol* 32: 409-12

Grauer SM, Pulito VL, Navarra RL, Kelly MP, Kelley C, Graf R, Langen B, Logue S, Brennan J, Jiang L, Charych E, Egerland U, Liu F, Marquis KL, Malamas M, Hage T, Comery TA, Brandon NJ (2009) Phosphodiesterase 10A inhibitor activity in preclinical models of the positive, cognitive, and negative symptoms of schizophrenia. *J Pharmacol Exp Ther* 331: 574-90

Lakics V, Karran EH, Boess FG (2010) Quantitative comparison of phosphodiesterase mRNA distribution in human brain and peripheral tissues. *Neuropharmacology* 59: 367-74

Loughney K, Hill TR, Florio VA, Uher L, Rosman GJ, Wolda SL, Jones BA, Howard ML, McAllister-Lucas LM, Sonnenburg WK, Francis SH, Corbin JD, Beavo JA, Ferguson K (1998) Isolation and characterization of cDNAs encoding PDE5A, a human cGMP-binding, cGMP-specific 3',5'-cyclic nucleotide phosphodiesterase. *Gene* 216: 139-47

McCarley RW, Faux SF, Shenton ME, Nestor PG, Adams J (1991) Event-related potentials in schizophrenia: their biological and clinical correlates and a new model of schizophrenic pathophysiology. *Schizophr Res* 4: 209-31

Prickaerts J, van Staveren WC, Sik A, Markerink-van Ittersum M, Niewohner U, van der Staay FJ, Blokland A, de Vente J (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: 351-61

Puzzo D, Staniszewski A, Deng SX, Privitera L, Leznik E, Liu S, Zhang H, Feng Y, Palmeri A, Landry DW, Arancio O (2009) Phosphodiesterase 5 inhibition improves synaptic function, memory, and amyloid-beta load in an Alzheimer's disease mouse model. *J Neurosci* 29: 8075-86

Reagan-Shaw S, Nihal M, Ahmad N (2008) Dose translation from animal to human studies revisited. *FASEB J* 22: 659-61

Reyes-Irisarri E, Markerink-Van Ittersum M, Mengod G, de Vente J (2007) Expression of the cGMP-specific phosphodiesterases 2 and 9 in normal and Alzheimer's disease human brains. *Eur J Neurosci* 25: 3332-8

Rutten K, Prickaerts J, Hendrix M, van der Staay FJ, Sik A, Blokland A (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: 107-12

Rutten K, Van Donkelaar EL, Ferrington L, Blokland A, Bollen E, Steinbusch HW, Kelly PA, Prickaerts JH (2009) Phosphodiesterase inhibitors enhance object memory independent of cerebral blood flow and glucose utilization in rats. *Neuropsychopharmacology* 34: 1914-25

Schultheiss D, Muller SV, Nager W, Stief CG, Schlote N, Jonas U, Asvestis C, Johannes S, Munte TF (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

Shim YS, Pae CU, Kim SW, Kim HW, Kim JC, Koh JS (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: 109-14

Sierksma AS, Rutten K, Sydlik S, Rostamian S, Steinbusch HW, van den Hove DL, Prickaerts J (2012) Chronic phosphodiesterase type 2 inhibition improves memory in the APPswe/PS1dE9 mouse model of Alzheimer's disease. *Neuropharmacology* 64: 124-36

Singh N, Parle M (2003) Sildenafil improves acquisition and retention of memory in mice. *Indian J Physiol Pharmacol* 47: 318-24

Turetsky BI, Bilker WB, Siegel SJ, Kohler CG, Gur RE (2009) Profile of auditory information-processing deficits in schizophrenia. *Psychiatry Res* 165: 27-37

van Donkelaar EL, Rutten K, Blokland A, Akkerman S, Steinbusch HW, Prickaerts J (2008) Phosphodiesterase 2 and 5 inhibition attenuates the object memory deficit induced by acute tryptophan depletion. *Eur J Pharmacol* 600: 98-104

Summary

The aim of this thesis was to investigate whether PDE inhibition can improve cognition by using a translational approach. The general introduction (**Chapter 1**) described the rationale of this thesis and the aims of the studies we conducted. In **Chapter 2** we reviewed and discussed the literature on the effects of PDE-Is on cognition across species.

In **Chapter 3** we examined the effects of PDE5 inhibition on memory in the ORT and determined whether compound penetration of the BBB is required for this activity. Vardenafil was shown to cross the BBB, but UK-343,664 did not. Vardenafil improved time-dependent memory decay and reversed the MK-801-induced memory deficit whereas UK-343,664 had no effect. However, both PDE5-Is attenuated the memory impairment induced by scopolamine. Co-administration of UK-343,664 and scopolamine did not alter the brain partitioning of either molecule which suggests that the positive effect of UK-343,664 on scopolamine-induced memory decay might arise from peripheral PDE5 inhibition. The results imply that there may be multiple mechanisms that mediate the efficacy of PDE5 inhibition to improve memory performance in tasks such as the ORT and that these involve PDE5 located both within and outside of the brain.

In **Chapter 4** we presented the effects of PDE2 and PDE10 inhibition on memory function in the ORT using the scopolamine- and MK-801-induced memory deficit model. Both PDE2-I BAY 60-7550 and PDE10-I PQ-10 attenuated the scopolamine-induced as well as the MK-801-induced memory deficits. PQ-10 was highly brain penetrant, whereas 60-7550 levels in the brain were very low after oral treatment. We concluded that since BAY 60-7550 and PQ-10 reversed both scopolamine- and MK-801-induced memory deficits, this supports the notion that dual substrate PDE inhibitors might be suitable candidates for cognition enhancement.

In **Chapter 5** we explored the effects of PDE5 inhibition on auditory sensory gating in rats and humans. Significant gating was only found for the N1 component in rats, while all three peaks P1, N1 and P2 showed gating in humans, i.e. the response to the second sound click was decreased as compared to the first for these deflections. Administration of vardenafil did neither have an effect on sensory gating in rats nor in humans. These results imply that the positive effects of PDE5 inhibition on cognition are not mediated by more early phases of information processing.

In **Chapter 6** we focused on the effects of PDE2 (BAY 60-7550), PDE5 (vardenafil) and PDE10 (PQ-10) inhibition on sensory gating in rats. EEG was recorded from the hippocampus, striatum, and vertex. Sensory gating was found for the N1 in the vertex and hippocampus, as revealed by diminished amplitudes to S2 compared to S1. Administration of PDE-Is did not affect sensory gating. However, PDE2 inhibition increased the P1 peak after presentation of S1 at the vertex and PQ-10 increased the N1 peak in general compared to vehicle treatment at the hippocampus. These findings suggest that the positive effects of PDE5 inhibition on cognition previously found in animals are possibly the result of an effect on higher cognitive functioning specifically whereas the cognition enhancing effects of PDE2 and PDE10 inhibition might also be influenced by effects on earlier stages of information processing.

In **Chapter 7** we assessed whether the PDE5-I vardenafil improves memory and executive functioning and affects EEG in healthy young adults. 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 and 20 mg 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.

In **Chapter 8** we evaluated the main findings of this thesis and addressed several methodological considerations. In addition, clinical implications and suggestions for future research were discussed. We concluded that specific PDE-Is can improve cognition and the potential of these PDE-Is as cognition enhancers needs to be elucidated in future translational research.

Samenvatting

Het doel van dit proefschrift was om met behulp van een translationele aanpak te onderzoeken of fosfodiesterase (phosphodiesterase: PDE) remming cognitie kan verbeteren. De algemene introductie (**Hoofdstuk 1**) beschreef de motivering van dit proefschrift en de doelen van de experimenten die we hebben uitgevoerd. In **Hoofdstuk 2** gaven we een overzicht van de literatuur over de effecten van PDE remmers op cognitie in verschillende diersoorten.

In **Hoofdstuk 3** onderzochten we de effecten van PDE remming op geheugen in de object herkenningstaak (object recognition task: ORT) en bepaalden we of de PDE remmers de bloed-hersenbarrière moeten passeren om een effect uit te oefenen. We lieten zien dat vardenal de bloed-hersenbarrière passeerde, maar dat UK-343,664 dat niet deed. Vardenafil verbeterde tijdsafhankelijk verval van het geheugen en maakte de geheugengebreken die veroorzaakt werden door MK-801 ongedaan. UK-343,664 had geen effect. Beide PDE5 remmers wisten echter de verslechtering in geheugen die opgewekt werd door scopolamine ongedaan te maken. Gelijktijdige toediening van UK-343,664 en scopolamine had geen effect op de mate waarin deze stoffen in het brein kwamen, wat erop duidt dat de positieve effecten van UK-343,664 op geheugenproblemen geïnduceerd door scopolamine mogelijk veroorzaakt worden door perifere PDE5 remming. Deze resultaten impliceren dat er mogelijk meerdere mechanismen zijn die de werkzaamheid van PDE remming wat betreft geheugen verbetering op taken zoals de ORT kunnen beïnvloeden en dat deze zowel PDE5 in als buiten het brein omvatten.

In **Hoofdstuk 4** presenteerden we de effecten van PDE2 en PDE10 remming op geheugen in de ORT waarbij het geheugen werd verslechterd door scopolamine of MK-801. De PDE2 remmer BAY 60-7550 en PDE10 remmer PQ-10 maakten beide de geheugenproblemen die veroorzaakt werden door zowel scopolamine als MK-801 toediening ongedaan. De hoeveelheid PQ-10 in het brein was hoog na orale toediening, terwijl de hoeveelheid BAY 60-7550 erg laag was. We concludeerden dat PDE remmers die zowel effect hebben op cAMP als cGMP geschikte kandidaten zijn om cognitie te verbeteren.

In **Hoofdstuk 5** exploreerden we de effecten van PDE5 remming op auditieve sensorische filtering in ratten en mensen. Significante filtering werd alleen gevonden voor de N1 component in ratten, terwijl bij mensen alledrie de pieken, P1, N1 en P2, filtering lieten zien, dat wil zeggen dat de reactie op de tweede geluidsstimulus afgenomen was ten opzichte van de eerste voor deze pieken. Toediening van vardenafil had geen effect op sensorische filtering in ratten, noch in mensen. Deze resultaten doen vermoeden dat de positieve effecten van PDE5 inhibitie op cognitie niet gemediëerd worden door vroegere fases van informatieverwerking.

In **Hoofdstuk 6** hebben we ons gericht op de effecten van PDE2 (BAY 60-7550), PDE5 (vardenafil) en PDE10 (PQ-10) remming op sensorische filtering in ratten. EEG activiteit werd opgenomen van de hippocampus, het striatum en de vertex. Sensorische filtering werd gevonden voor de N1 in de vertex en hippocampus, zoals verminderde amplitudes na de S2 ten opzichten van de S1 lieten zien. Toediening van PDE remmers had geen invloed op

deze sensorische filtering. PDE2 remming vergrootte echter wel de P1 component van de vertex na de presentatie van S1 en PQ-10 vergrootte de N1 piek van de hippocampus in zijn geheel in vergelijking tot vehicle behandeling. Deze bevindingen duiden erop dat de positieve effecten van PDE5 inhibitie op cognitie die eerder gevonden zijn in dieren waarschijnlijk specifiek het resultaat zijn van een effect op hoger cognitief functioneren, terwijl de cognitie-verbeterende effecten van PDE2 en PDE10 remming mogelijk ook beïnvloed worden door effecten op eerdere fases van informatieverwerking.

In **Hoofdstuk 7** hebben we onderzocht of de PDE5 remmer vardenafil het geheugen en executief functioneren verbetert en EEG beïnvloedt in gezonde jongvolwassenen. Er werden geen prominente effecten van vardenafil op cognitie gevonden; deelnemers maakten alleen meer fouten op een reactietaak na inname van 20 mg vardenafil. Tijdens het coderen van woorden was de P300 over het algemeen kleiner na behandeling met vardenafil. Verder was de N400 van de Fz in de spatiële geheugentaak groter na toediening van 10 mg vardenafil dan placebo en 20 mg. Tenslotte werd er vaker melding gemaakt van hoofdpijn en gevoel van zwakte na behandeling met vardenafil. Vardenafil had geen effect op de cognitieve prestatie van gezonde volwassenen en liet alleen enkele incidentele effecten op ERPs zien. Deze resultaten in mensen zijn niet in lijn met de cognitie-verbeterende effecten van PDE remmers in gezonde dieren.

In **Hoofdstuk 8** evalueerden we de belangrijkste bevindingen van dit proefschrift en schonken we aandacht aan enkele methodologische overwegingen. Verder werden klinische implicaties en voorstellen voor toekomstig onderzoek besproken. We concludeerden dat specifieke PDE remmers cognitie kunnen verbeteren en dat het potentieel van deze PDE remmers als cognitie verbeteraars verder bekeken moet worden in toekomstig translationeel onderzoek.

Dankwoord

‘Dit is mijn dag, ik lach om zwaartekracht. Genoeg gewacht, ik lach om zwaartekracht. En niemand haalt mij ooit nog neer.’ (Wicked). Net als de meeste musicals, kent een promotie traject doorgaans een aarzelend, doch enthousiast begin, een middenstuk met pieken en dalen, een dramatische wending vlak voor het eind en tot slot een happy end ;). En zoals een musical niet alleen bestaat uit de grote naam op het affiche, heeft ook een AiO project nog een heel team van mensen die een bijdrage hebben geleverd. Dus hoogste tijd voor het meest gelezen onderdeel van een proefschrift; dames en heren, het dankwoord!

Prof. dr. Harry Steinbusch, mijn promotor. Bedankt dat ik deel mocht uitmaken van de School for Mental Health and Neuroscience en dat u me de kans heeft gegeven te promoveren.

Dr. Jos Prickaerts, mijn co-promotor. Beste Jos, bedankt voor je grenzeloze vertrouwen, niet alleen in het project, maar zeker ook in mij. Jouw uitspraak ‘het kan niet meer misgaan’, die je vlak voor de kerstvakantie in 2010 deed, is onbedoeld legendarisch geworden en al helemaal niet op de manier die je waarschijnlijk voor ogen had ;). Maar twee jaar - en de nodige huisbezoekjes - later is het proefschrift is af, de overgrote meerderheid zelfs gepubliceerd, oftewel, het is allemaal toch nog goedgekomen! (al mijd ik sneeuw en gladheid zoveel mogelijk tot en met de dag van de verdediging, je weet maar nooit, haha)

Dr. Thomas Steckler, mein Chef in dem Jahr bei Johnson & Johnson. Thomas, sie haben mir wirklich viel beigebracht und es war sehr interessant um ein Jahr in der Industrie zu arbeiten. Die Idee eines translationalem Stroop Testes war sehr aussichtsreich; schade, dass die Tierchen so verdammt intelligent waren! Vielleicht hätten wir ihnen tatsächlich Schokolade und Wein geben sollen zur Motivation, Zeit für ein neues Experiment?

Dr. Anke Sambeth. Anke, zonder jou had dit boekje er toch wel wat anders uitgezien. Jouw liefde voor het vak is bewonderingswaardig en ik wil je bedanken voor je eindeloze geduld met mij en de hulp met de EEG studies (rat en humaan). Wanneer ik Guus op de radio hoor, krijg ik nog regelmatig een flashback naar ons in de OK!

Alle neuroscience collega’s van de UNS50 en de psychofarmacologen van de UNS40, bedankt voor de leuke tijd op de uni. In het bijzonder (oud) kamergenoten: Marjo, Laura, Linda, Kris (ondanks dat je niet meer in het PDE veld zit, toch nog altijd heel geïnteresseerd, top!), Ayhan, Jochen, Eva, Sven, Eva, de urologen-club, Annerieke, Marisela en natuurlijk Marlies. En uiteraard de rest van de MC crew waaronder Nick, Jérôme, Tim, Gunther, Daan (warme choco met slagroom consulten ftw ;)), Caroline, Rianne, Caroline en Lena. Bedankt voor alle late/vroege (afhankelijk van hoe je het bekijkt) stapavonden, ontspannende koffiepauzes, luisterende oren inclusief peptalks indien nodig, assistentie bij experimenten, gezelligheid binnen en buiten het kantoor, en zo kan ik nog wel even doorgaan ;). En uiteraard dr. Blokland, door wie ik ooit bij Jos terecht ben gekomen, Arjan, bedankt!
De dames van het secretariaat Mirèse, Akke, Sandra, Anouk, Marie-Therèse en Lisa die de adminstratieve zaken al die jaren in goede banen hebben geleid. Nicole, bedankt voor het geregel omtrent mijn verblijf bij JnJ en voor je goede adviezen!

De analisten, Hellen, Marjanne, Denise en Marjan, voor het eindeloze afwegen van alle stofjes, Cees voor het keuren van alle proefpersonen en natuurlijk de CPV'ers voor alle hulp omtrent de levering en huisvesting van de dieren.

JnJ collega's voor het toffe en leerzame jaar in Beerse. Bureaugenotes Ann en Wieteke voor het aangename gezelschap, de lol en de wisselende voorrraad snoep. Hilde voor de altijd vriendelijke hulp op het secretariaat, ook bij de zoveelste vraag of papierhandel. Darrel thanks for all the help, varying from arranging a lab 'till sharing knowledge about previous experiments. Hansfried voor het ontwerpen en leveren van de test apparatus van de Stroop en Sigrid, Roland, Luc en Pim voor de PPI experimenten. De mensen van het LAM, met name Willy, Werner en Liselotte, voor het verzorgen van de beestjes.
And last but not least the JnJ rockers John – US 'let's rock 'n roll' – T., John – UK 'the shoes we shall not talk about'– A. and of course my all-time favorite chef: Laetitia. My stay at JnJ wouldn't have been this legen… wait for it … dary without you guys!

De studenten die hebben bijgedragen aan de uitvoer/analyse van de humane studie: Ili, Agnieszka, Moos, Connie en Saranne. Een speciale vermelding voor Floriaan die de boel draaiende heeft gehouden toen ik naar JnJ vertrok, maar de humane studie nog niet helemaal was afgerond; top gedaan!

De leden van de leescommissie en de corona, bedankt voor de tijd die jullie hebben gestoken in het lezen van mijn proefschrift en de aanwezigheid bij de verdediging hiervan.

En uiteraard alle pluizebollen en proefpersonen die hebben deelgenomen aan de studies; zonder jullie geen onderzoek.

Het is bijna onmogelijk alle vrienden, familie en kennissen te bedanken die me de afgelopen jaren hebben gesteund in de goede en iets minder goede tijden; (nogmaals) bedankt voor jullie interesse en steun tijdens het hele traject en niet in de laatste plaats voor de lawine aan kaarten, mails, smsjes, telefoontjes, bezoek en cadeautjes de afgelopen twee jaar; het is onmogelijk in woorden uit te drukken hoeveel dit voor me betekend heeft :). Al zijn er toch een aantal mensen die ik nog even persoonlijk wil bedanken, dus daar gaan we!

De mannen van Drumschool Maastricht: Pieter, Wouter en Michel die altijd voor een leuke drumles wisten/weten te zorgen en zo mijn stressniveau (tijdelijk) tot 0 wisten te reduceren. Met een motorisch gestoorde leerling soms best een opgave ;).

De gezondheidszorg is vaak een onderwerp van eindeloos geklaag en gezeur. Maar laten we niet vergeten dat we er zonder in 99,9% van de gevallen nog heel wat slechter af zouden zijn. Dus in het bijzonder een woord van dank voor Ludo, René, orthopedie en revalidatie dankzij wie ik er de 18^{e} april letterlijk kan staan (sorry voor de hakken ;)).

Dees, van de Veldmuiskes tot Les Couscous en van het Jeanne d'Arc tot de scouting; soms zijn er mensen die altijd al in je leven lijken te zijn geweest. En ook al kamperen we

tegenwoordig niet meer in tuinen en maken we geen tekeningen meer in het AKO gebouw, samen lachen en lekker eten kunnen we nog steeds als de besten!

Suus, het begon ooit allemaal in B1F met twee brugmuggen die zich bezighielden met dingen als een gft klooster, het griezelen over (het borsthaar van) enge docenten en eindeloze telefoongesprekken. Bijna twintig jaar later is het via talloze logeerpartijen, idolen als Queen en The Boss en iets serieuzere opleidingen, uiteindelijk toch nog goed gekomen met ons! Es, in het begin vooral 'het jongere zusje van', maar dat ligt alweer jaren achter ons. Met 'nne echte Sjeng (ok, vleugje exotisch Brabo-schap ;)) kan het eigenlijk alleen maar gezellig zijn; om over onze zeer productieve lunches natuurlijk maar te zwijgen! En Geleen is natuurlijk een voorstad van Sjengenland, niets aan de hand ;).

De Belgiqueskes van de psychologie opleiding. We hebben met ons groepje vele hilarische, maar helaas ook enkele trieste momenten beleefd. Het is fijn te weten dat we er in beide gevallen voor elkaar zijn. Ankiebol, mijn meest Nederlandse Belg met uitzondering van de charmante IE en UU klanken. Al zijn we ieder onze eigen weg gegaan na de studie, wanneer we samen zijn is het net alsof we ons gisteren nog gezien hebben. Pat, onze drukke Belg die altijd wel tijd weet te maken voor (het helpen van) andere mensen en waar de deur altijd wagenwijd openstaat. Ow, en perfect de militaire voertuigen in Transformers weet te duiden! Na al die jaren blijft het rustig bijkletsen aan jouw keukentafel in Hoeselt onder het genot van een steak peperroomsaus altijd een van mijn favoriete momenten. Dr. Peke, mijn mede-altijdgelijkhebbende, afdeling Belg(ië); ik ben blij dat je achter me staat op een dag als vandaag. Van 'meezingen' met Rammstein als we terugkwamen van een tentamen tot het ongeduldig wachten op de volgende aflevering van Buffy en later True Blood. Ondanks dat jouw leven er totaal anders uitziet dan het mijne, ben ik blij dat we elkaar regelmatig zien om eindeloos te ouwehoeren en de Oscarwaardige verhaallijnen van onze series te bediscussiëren ;).

De kerels, Alex, Michiel en Ron. Bedankt voor fantasy wereld die ik dankzij jullie heb leren kennen. Een paar jaar terug waren comics, rpgs, fantasy evenementen en zelfs Chtulhu totaal onbekend voor me en nu zou ik niet meer weten hoe het er zonder was ;). Ow, en dat er zoiets bestaat als een 30 seconden regel en dat tentstokken & uitgaanscentrum moeilijke woorden zijn, waren toch wel het grootste leermomenten op Malta. We moeten jullie allleen wel nog in een pèkske zien te krijgen hè :P.

Manuel, ook per mail kun je iemand supporteren hebben we gemerkt ;). Bedankt voor de vele peptalks, al moet ik nog werken aan m'n beestachtige brul *grrrrrrrrrr*!

De weerwolven van Wakkerdam / pubquiz crew, en dan vooral Lieke & Martijn, Caroline & Joeri, Daniëlle & Robbert, bedankt voor de extra bijnaam Olgjeweerwolfje en de vele top drie notaties bij de quiz. Dat we nog maar vaak mogen winnen (en lol hebben, want winnen is uiteráárd niet het belangrijkste (aangezien de eerste prijs niet te zuipen is :P)).

Dr. Inge, op het moment dat ik dit schrijf, ben ik net jouw paranimf geweest en tegen de tijd dat jij dit leest, is het bijna jouw beurt :). Volgens mij ben ik sneller klaar als ik de interesses

en gedachtegangen opnoem die we niet delen; ‘Get out of my head Zoekeh!’ is niet voor niets een van onze meest gebruikte uitspraken! Bedankt voor je vastberadenheid me de afgelopen twee jaar gewoon overal mee naartoe te slepen, al is het in een rolstoel over het hellende Pinkpop terrein met 30+ graden celsius of met een rollator plus bagage in een NS trein die toch niet zo rollatorvriendelijk bleek te zijn ;). En als we het carrière-technisch echt niet meer weten, worden we gewoon de nieuwe Beavis & Butthead (of Waldorf & Statler), hahaha.

Sandra, twee keer per week baantjes trekken in onze Hierder chloorbak is veel leuker in het gezelschap van ’n aander Hierder mèidske! En die zich als het even kan vooral niet schaamt als ze naast iemand zwemt die een veel te glimmende glitterdouchemuts als ideaal zwemaccessoire ziet ;). Mijn gruwelijke hekel aan baantjes trekken wordt aanzienlijk gereduceerd door jouw vrolijke gezelschap en de eindeloze voorraad gespreksonderwerpen, variërend van aubergines tot Elvis (de kat natuurlijk) en van piratenrokken tot Luik!

Nicky, de buurvrouw die eigenlijk niet mijn buurvrouw is :P. Naast pratend wandelen (of was het wandelend praten?), houden we ons uiteraard ook bezig met goede doelen, zoals het steunen van de lokale lunchroom! En als iemand de mening deelt dat warme chocomelk 365,25 dagen per jaar kan, dus ook bij 25+ graden, en de TLC pulp stiekem ook wel leuk vindt, sja, zou bijna zeggen ‘kan niet misgaa...’ :P.

Tot slot wil ik natuurlijk m’n familie bedanken; we hebben geen grote familie maar de belangstelling van de (oud)ooms/tantes (Hans, bedankt voor het geduld en de creativiteit mbt de cover; simpel is niet altijd makkelijk ;)), (achter)neven/nichten en uiteraard oma’s (oma Leny, bedankt voor de extra support :)) was altijd aanwezig. En uiteraard het thuisfront, de (meestal) stressverlagende huisdieren - de jongens Fidel & Nigra, Visje(s) en de ‘kuusje’ Bientje & Jimmy - en, de laatste maar zeker niet de minste, pap & mam. Bedankt voor het luisteren naar mijn oneindige blijheid over de dingen die goed gingen en vooral ook naar het hardgrondig geknotter als het weer ’s niet ging zoals ik het voor ogen had ;). Maar ook bedankt voor alle ondersteuning de afgelopen jaren, niet alleen figuurlijk, maar na de beruchte 3e kerstdag ook letterlijk. De eindeloze blokjes om door Heer, de talloze ziekenhuisbezoeken en de lange kampeervakantie in de woonkamer waren toch net wel beter te verhapstukken met goed gezelschap :). Maargoed, daar gaan we verder geen bomen meer aan verspillen; het is achter de rug, het proefschrift is afgevinkt, tijd voor de volgende punten op de lijst!

Curriculum Vitae

Olga Antonia Hendrika Reneerkens werd geboren op 29 juni 1982 in Maastricht (Nederland) waar zij in 2000 het VWO (Lyceum) diploma behaalde aan het Jeanne d'Arc College. Datzelfde jaar begon ze haar studie psychologie aan de Universiteit Maastricht. Tijdens haar studie heeft ze hier tevens gewerkt als student-assistent en statistiek tutor. In 2005 is ze afgestudeerd als biologisch psycholoog met als afstudeervarianten cognitive neuroscience en neuropsychologie. Vervolgens is ze aangesteld als onderzoeksassistente op de afdeling Psychiatrie en Neuropsychologie van de Universiteit Maastricht onder begeleiding van Dr. Jos Prickaerts. Na het behalen van een Kootstra fellowship, werd deze aanstelling in december 2007 omgezet in een PhD-project. Het tweede jaar van dit project kreeg zij dankzij een Marie-Curie fellowship de mogelijkheid om een jaar bij Johnson & Johnson te Beerse (België) te kunnen werken onder supervisie van Dr. Thomas Steckler. Hierna is zij teruggekeerd naar de Universiteit Maastricht waar zij onder begeleiding van Dr. Jos Prickaerts en Prof. Dr. Harry Steinbusch haar PhD programma voortzette en dit proefschrift afrondde.

Olga Antonia Hendrika Reneerkens was born on June 29th 1982 in Maastricht (the Netherlands) where she completed her secondary education (Lyceum/VWO) at the Jeanne d'Arc College in 2000. In that same year she started her study Psychology at Maastricht University. During this period she worked as a student-assistant and engaged in teaching statistics. In 2005, she graduaded in biological psychology with a specialty in cognitive neuroscience and neuropsychology. Next, she was appointed as a research assistant at the department of Psychiatry and Neuropsychology at Maastricht University under supervision of Dr. Jos Prickaerts. After she obtained a Kootstra fellowship grant, this appointment was changed to a PhD studentship in December 2007. During the second year of this project a Marie-Curie fellowship granted her the opportunity to work at Johnson & Johnson in Beerse (Belgium) for a year under supervision of Dr. Thomas Steckler. After this year in Belgium she returned to Maastricht University to continue her PhD program and finish this thesis under supervision of Dr. Jos Prickaerts and Prof. Dr. Harry Steinbusch.

Publications

Peer reviewed publications – First author

Reneerkens, O.A.H., Rutten, K., Steinbusch, H.M.W., Blokland, A. & Prickaerts, J. (2009). Selective phosphodiesterase inhibitors: a promising target for cognition enhancement. Psychopharmacology, 202, 419-443.

Reneerkens, O.A.H., Rutten, K., Akkerman, S., Blokland, A., Menniti, F., Steinbusch, H.W.M. & Prickaerts, J. (2012). Phosphodiesterase type 5 (PDE5) inhibition improves object recognition memory: Indications for central and peripheral mechanisms. Neurobiology of Learning and Memory, 97(4), 370-379.

Reneerkens, O.A.H., Sambeth, A., Van Duinen, M.A., Blokland, A., Steinbusch, H.W.M. & Prickaerts, J. (2013). The PDE5 inhibitor vardenafil does not affect auditory sensory gating in rats and humans. Psychopharmacology, 225(2), 303-312.

Reneerkens, O.A.H., Rutten, K., Bollen, E., Hage, T., Blokland, Steinbusch, H.W.M. & Prickaerts, J. (2013). Inhibition of phoshodiesterase type 2 or type 10 reverses object memory deficits induced by scopolamine or MK-801. Behavioural Brain Research, 236(1), 16-22.

Reneerkens, O.A.H., Sambeth, A., Ramaekers, J., Steinbusch, H.W.M., Blokland, A. & Prickaerts, J. The effects of the PDE5 inhibitor vardenafil on cognitive performance in healthy adults: a behavioural-EEG study (accepted for publication). Journal of Psychopharmacology.

Peer reviewed publications – Co-author

Rutten, K., **Reneerkens, O.A.H.**, Hamers, H., Sik, A., McGregor, I., Prickaerts, J. & Blokland, A. (2008). Automatic scoring of novel object recognition in rats. Journal of Neuroscience Methods, 171, 72-77.

De Bruin, N.M., Prickaerts, J., Van Loevezijn, A., Venhorst, J., De Groote, L., Houba, P., **Reneerkens, O.A.H.**, Akkerman, S. & Kruse, C.G. (2011) Two novel 5-HT6 receptor antagonists ameliorate scopolamine-induced memory deficits in the object recognition and object location tasks in Wistar rats. Neurobiology of Learning and Memory, 96, 392-402.

Prickaerts, J., Van Goethem, N.P., Chesworth, R., Shapiro, G., Boess, F.G., Methfessel, C., **Reneerkens, O.A.H.**, Flood, D.G., Hilt, D., Gawryl, M., Bertrand, S., Bertrand, D. & König, G. (2011). EVP-6124, a novel and selective α7 nicotinic acetylcholine receptor partial agonist, improves memory performance by potentiating the acetylcholine response of α7 nicotinic acetylcholine receptors. Neuropharmacology, 62(2), 1099-1110.

Akkerman, S., Blokland, A., **Reneerkens, O.A.H.**, Van Goethem N.P., Bollen, E., Gijselaers, H.J., Lieben, C.K., Steinbusch, H.W. & Prickaerts, J. (2012). Object recognition testing: Methodological considerations on exploration and discrimination measures. Behavioural Brain Research, 232(2), 335-347.

Book chapter

Bollen, E., Rutten, K., **Reneerkens, O.A.H.**, Steinbusch, H.W.M. & Prickaerts, J. (In Press). Beyond erectile dysfunction: Understanding PDE5 activity in the central nervous system. In: Cyclic-Nucleotide Phosphodiesterases in the Central Nervous System: From Biology to Drug Discovery (Eds. Brandon N and West A). John Wiley and Sons.

Publications in progress

Reneerkens, O.A.H., Sambeth, A., Steinbusch, H.W.M. & Prickaerts, J. PDE2 and PDE10, but not PDE5, inhibition affect basic auditory information processing in rats (Submitted).

Abbreviations

AEP:	auditory evoked potential
BAY:	BAY 60-7550
BBB:	blood-brain barrier
BQL:	below quantification limit
cAMP:	cyclic adenosine monophosphate
C_b:	brain concentration
C_b:C_p:	brain-to-plasma ratio
cGMP	cyclic guanosine monophosphate
C_p:	plasma concentration
CRMT:	continuous recognition memory task
*d*2:	(relative) measure of discrimination between the new and familiar objects
*e*1:	measure of the time spent in exploring both objects in T1
*e*2:	measure of the time spent in exploring both objects in T2
EEG:	electroencephalography
ERP:	event-related potential
GLM:	general linear model
i.p.:	intraperitoneal
ISI:	interstimulus interval
ITI:	intertrial interval
LTP:	long-term potentiation
MK:	MK-801
NMDA:	N-Methyl-D-aspartate
n.s.:	not significant
ORT:	object recognition task
PDE:	phosphodiesterase
PDE-I:	phosphodiesterase inhibitor
p.o.:	per os, orally
POMS:	profile of mood states
PPI:	prepulse inhibition
PQ:	PQ-10
RT:	reaction time
S1:	first stimulus
S2:	second stimulus
s.c.:	subcutaneous
scop:	scopolamine
SMT:	spatial memory task
T1:	trial 1
T2:	trial 2
TOL:	tower of London
UK:	UK-343,664
Var:	vardenafil
VAS:	visual analogue scale
VLT:	verbal learning task