Targeting phosphodiesterase type 4 for improving cognitive fronto-striatal functioning

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a translational approach

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Contents

List of abbreviations

6-OHDA	=	6-hydroxydopamine
AC	=	adenylyl cyclase
ACC	=	anterior cingulate cortex
ADHD	=	attention deficit hyperactivity disorder
ACC	=	anterior cingulate cortex
AD	=	Alzheimer's disease
AEP	=	auditory evoked potentials
AKAP	=	A-kinase anchoring protein
ALS	=	amyotrophic lateral sclerosis
AMPA	=	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ASR	=	acoustic startle response
ATP	=	adenosine triphosphate
BBB	=	blood-brain barrier
BDNF	=	brain-derived neurotrophic factor
BID	=	*bis in die* (twice a day)
CA	=	cornu ammonis (of the hippocampal formation)
CAAT	=	conditioned avoidance attention task
Ca^{2+}	=	calcium
CaM	=	calmodulin
CaMK	=	Ca^{2+}/calmodulin-dependent protein kinase
cAMP	=	cyclic adenosine monophosphate
cGMP	=	cyclic guanosine monophosphate
CNGC	=	cyclic nucleotide-gated channels
CNS	=	central nervous system
COPD	=	chronic obstructive pulmonary disease
CREB	=	cAMP response element binding protein
CSRTT	=	choice serial reaction time task
CS	=	conditioned stimulus
D1	=	dopamine type 1 receptor
D2	=	dopamine type 2 receptor
DA	=	dopamine
DARPP-32	=	dopamine- and cAMP-regulated phosphoprotein MR 32 kDa

DISC-1	=	disrupted in schizophrenia 1
dlPFC	=	dorsolateral prefrontal cortex
DOPAC	=	3,4-dihydroxyphenylacetic acid
ECoG	=	electrocorticogram
ED	=	erectile dysfunction
EEG	=	electroencephalography
EPAC	=	exchange factor directly activated by cAMP
ERK	=	extracellular receptor kinase
ERP	=	event-related potential
FDA	=	US food and drug administration
GABA	=	γ-aminobutyric acid
GC	=	guanylyl cyclase
GP_e	=	globus pallidus pars externa
GP_i	=	globus pallidus pars interna
HDGEC	=	Huntington's disease gene expansion carriers
IBMX	=	isobutylmethylxanthine
IKK	=	IkB kinase
IL	=	infralimbic cortex
ISI	=	inter stimulus interval
ITI	=	inter trial interval
KO	=	knockout
LI	=	latent inhibition
LID	=	levodopa-induced dyskinesia
LTD	=	long-term depression
LTP	=	long-term potentiation
MCI	=	mild cognitive impairment
MMSE	=	mini-mental state examination
mPFC	=	medial prefrontal cortex
mRNA	=	messenger ribonucleic acid
MSN	=	medium spiny neuron
NAc	=	nucleus accumbens
NE	=	norepinephrine
NMDA	=	N-methyl-D-aspartate
NO	=	nitric oxide
NOS	=	nitric oxide synthase

OCD	=	obsessive-compulsive disorder
ORDT	=	object retrieval detour task
PANNS	=	positive and negative syndrome scale
PCP	=	phencyclidine
pCREB	=	phosphorylated CREB
PD	=	Parkinson's disease
PDE	=	phosphodiesterase
PDE-I	=	phosphodiesterase inhibitor
PET	=	positron emission tomography
PKA	=	protein kinase A
PKG	=	protein kinase G
PnC	=	pontine reticular nucleus
PP-1	=	protein phosphatase-1
PPI	=	prepulse inhibition
PPTg	=	pedunculopontine tegmental nucleus
PSTH	=	peristimulus-time histogram
RACK1	=	receptor for activated C kinase 1
rCBF	=	regional cerebral blood flow
SN_c	=	substantia nigra pars compacta
SN_r	=	substantia nigra pars reticulata
STN	=	subthalamic nucleus
TH	=	tyrosine hydroxylase
TrkB	=	tropomyosin-related kinase B
US	=	United States
US	=	unconditioned stimulus
VTA	=	ventral tegmental area
WAIS	=	Wechsler adult intelligence scale

Chapter 1

General introduction

Acting means doing something for a particular purpose or to solve a problem (Cambridge dictionary, 2017). It starts with early information processing steps leading all the way up to the execution of a response. Acting involves motor, cognitive and limbic components. Acting includes bottom-up processes like sensation, perception, evaluation of different possible actions and context analysis. Additionally, top-down processes including inhibitory control, motivation, emotional state and experience congregate and shape the bottom-up processing of information resulting in action selection and execution. Sometimes, we act before adequately sampling and evaluating available information (impulsivity). However, our actions have an optimal effect when they are well prepared and when the context is evaluated properly. In order to achieve this goal, evolution has shaped the brain constructing a complex network in which all the information is brought together, known as the fronto-striatal circuits.

The fronto-striatal circuits

The fronto-striatal circuits are parallel organized circuits that run from the frontal cortex, through the basal ganglia, to the thalamus from where they project back to the frontal cortex, closing the circuits (Alexander et al., 1986; Alexander et al., 1990). Therefore, fronto-striatal circuits are also known as cortico-striatal-thalamic loops. The fronto-striatal circuits consist of motor, associative/cognitive and limbic circuits (Alexander et al., 1986; Alexander et al., 1990). Each circuit originates in a different part of the frontal cortex. Similarly, different parts of the basal ganglia structures and thalamus participate in these parallel segregated circuits (Yeterian and Pandya, 1991; Slattery et al., 2001). The different circuits are oriented dorsal to ventral and, even though they are called fronto-striatal circuits, the ventral limbic circuits originate not only from the frontal cortex (anterior cingulate cortex (ACC)) but also from the hippocampus and amygdala (Temel et al., 2005). At least five circuits can be distinguished: the oculo-motor circuit (eye movement), motor circuit (motor functioning), dorsolateral prefrontal circuit (cognitive functioning), the orbitofrontal circuit (cognitive functioning) and anterior cingulate circuit (limbic functioning).

The fronto-striatal circuits start with glutamatergic cortical efferents to the striatum (caudate nucleus, putamen and nucleus accumbens (NAc)). Within the basal ganglia all projections are γ-aminobutyric acid (GABA)-ergic except for output from the subthalamic nucleus (STN) which is glutamatergic. In the basal ganglia each fronto-striatal circuit splits into a direct and an indirect pathway. Cortical glutamatergic activation of a striatal direct pathway stimulates the release of GABA, having an inhibitory effect in the globus pallidus pars interna (GPi) and substantia nigra pars reticulata (SNr). As the GPi and SNr inhibit the thalamus, which in turn stimulates the cortex, activation of the direct pathway causes disinhibition of the thalamus leading to increased excitatory output of the neural

network, and thereby activation of behavior (either motor, cognitive or limbic). The indirect pathway has the opposite effect. Activation of the indirect pathway induces GABA release in the globus pallidus pars externa (GPe) which normally inhibits the release of GABA to the STN. The STN is thus disinhibited and increases stimulation of the GPi/SNr, which in turn inhibits the thalamic stimulation back to the cortex and results in inhibition of behavior. Additionally, the hyperdirect pathway consists of cortical glutamatergic projections to the STN, thereby completely circumventing the striatum. This STN activation increases stimulation of the GPi/SNr, resulting in inhibition of the thalamic stimulation to the cortex. Without cortical stimulation the GPi and the SNr function like autonomous pacemakers, tonically inhibiting the thalamus, thereby preventing cortical stimulation, i.e. behavioral output (for a more extensive discussion (see Haber and Rauch, 2010; Gerfen and Surmeier, 2011; Surmeier et al., 2011; Calabresi et al., 2014)).

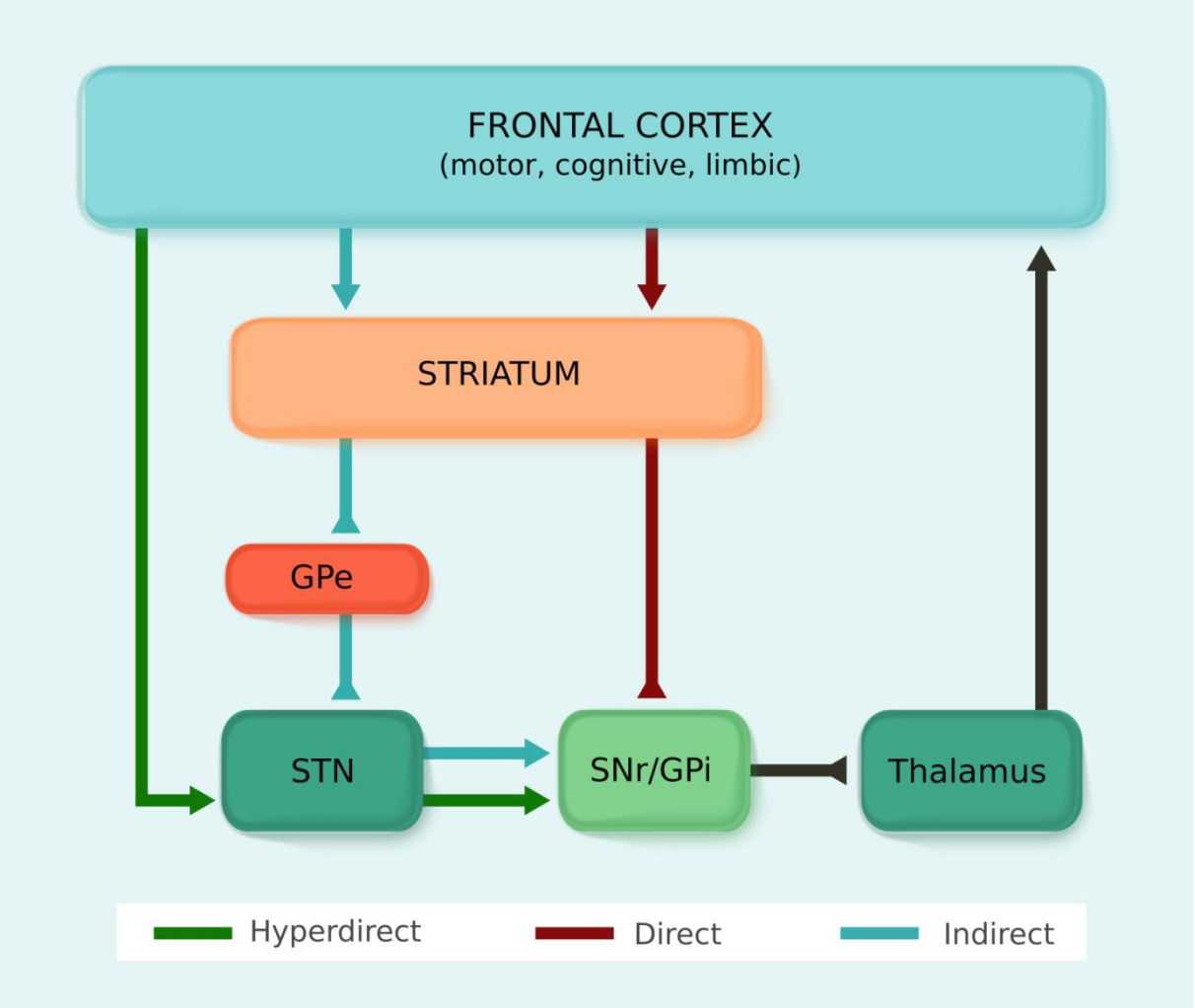

Figure 1. Hyperdirect, direct and indirect pathways of the fronto-striatal circuit. Hyperdirect pathway: frontal cortex-STN-SNr/GPi-thalamus. Direct pathway: striatum-SNr/GPi-thalamus. Indirect pathway: striatum-GPe-STN-SNr/GPi-thalamus. STN=subthalamic nucleus, SNr=substantia nigra pars reticulata, GPi=globus pallidus pars

interna, GPe=globus pallidus pars externa. Sharp arrow heads represent excitatory connections; blunted arrow heads represent inhibitory connections.

Dopaminergic modulation

At the level of the basal ganglia the fronto-striatal system is modulated by various GABAergic and cholinergic interneurons (Calabresi et al., 2014). Additionally, every circuit is modulated by dopamine (Surmeier et al., 2007; Surmeier et al., 2011). Dopaminergic cells from substantia nigra pars compacta (SNc) and ventral tegmental area (VTA) project to almost every structure within the circuits (e.g. nigrostriatal, mesolimbic, mesocortical and thalamic dopamine projections). As a result, dopaminergic receptors are strongly expressed throughout the fronto-striatal circuits (Gerfen and Surmeier, 2011; Nishi et al., 2011; Kuroiwa et al., 2012). Unsurprisingly, dopaminergic medication has proven effective as treatment for several disorders related to dysfunctional fronto-striatal circuits (e.g. ADHD, schizophrenia, Parkinson's disease). Dopamine released from SNc and/or VTA binds to both dopamine type1 (D1) receptors and dopamine type2 (D2) receptors on medium spiny neurons (MSNs) in the striatum (Gerfen and Surmeier, 2011). D1 receptors are mainly found on MSNs of the direct pathway and D2 receptors are mainly found on MSNs of the indirect pathway where they establish antagonistic interactions with adenosine A_{2A} receptors (Gerfen et al., 1990; Ferre et al., 2011). D1 receptors activate the $G_{s/olf}$ family of G proteins to stimulate cyclic adenosine monophosphate (cAMP) production and thereby the direct pathway in the striatum (Sibley et al., 1993; Beaulieu and Gainetdinov, 2011). In contrast, the D2 receptors couple to the $G_{i/o}$ family of G proteins and thus induce inhibition of cAMP production, thereby inhibiting the indirect pathway which eventually leads to disinhibition of the frontal cortex. Actions of the dopamine receptors in both pathways can be viewed as synergistically or complementary.

Several neuropsychiatric disorders, including neurodegenerative disorders like Parkinson's disease and Huntington's disease, psychiatric illnesses such as schizophrenia, bipolar disorder and obsessive-compulsive disorder, and pervasive developmental disorders like attention deficit hyperactivity disorder (ADHD) and autism spectrum disorder, all share the fronto-striatal circuits as their neurobiological basis (Alexander et al., 1986; Haber and Rauch, 2010; Gunaydin and Kreitzer, 2015). Dysfunction of these circuits produces a wide range of motor, cognitive and affective symptoms observed in these neuropsychiatric disorders (Chudasama and Robbins, 2006).

The need to develop strategies (e.g. pharmacotherapy, gene-therapy, immunotherapy) that treat neuropsychiatric disorders is high since the accompanying symptoms and discomfort have a large impact on the quality of life of the patients and constitute a major burden to society. As a result,

several strategies have been described targeting a wide range of domains. Research has focused on both environmental and genetic factors that cause neuronal dysfunction and death, or on enhancement of the ability of neurons to adapt (e.g. Mattson et al., 2002). Despite the large effort that has been put in these strategies, currently available treatment shows often low efficacy and/or high rates of adverse events. Therefore, the need for strategies that counteract the detrimental processes involved in the decline of motor, cognitive and affective functioning (efficacy) with better side-effect profiles (adverse events) remains high.

Phosphodiesterases

Recently, phosphodiesterases (PDEs) are receiving increased attention as possible pharmacotherapeutic target for treatment of disorders characterized by motor, cognitive and affective symptoms related to fronto-striatal circuit dysfunction. There are eleven subfamilies of PDE comprising about 21 different genes, each containing several splice variants and isoforms making up more than a hundred specific human PDEs (Bender and Beavo, 2006) (see Table 1) and each having a specific localization in the human body (Rentero et al., 2003; Wang et al., 2003; Esposito et al., 2009; Lakics et al., 2010). PDEs degrade the second messengers cAMP and/or cyclic guanosine monophosphate (cGMP). PDE1, PDE2, PDE3, PDE10 and PDE11 are dual-substrate PDEs. PDE4, PDE7 and PDE8 are cAMP-specific, whereas PDE5, PDE6 and PDE9 are cGMP-specific (Beavo, 1995). A PDE inhibitor is a pharmacological compound blocking one or more isoforms of PDEs, thus increasing second messengers and improving intra- and intercellular signaling (Bender and Beavo, 2006).

Table 1

Overview of different PDE families, properties, substrates and compounds

Type	Property	Substrate	Clinically evaluated inhibitors
PDE1	Ca^{2+}-CaM-stimulated	cAMP/cGMP	Vinpocetine, ITI-214
PDE2	cGMP-stimulated	cAMP/cGMP	-
PDE3	cGMP-inhibited	cAMP/cGMP	Cilostazol
PDE4	cAMP-specific	cAMP	Rolipram, ND1251, MK-0952, MEM1414, HT-0712, roflumilast, Denbufylline

PDE5	cGMP-specific	cGMP	Sildenafil, udenafil, vardenafil
PDE6	Photoreceptor	cGMP	-
PDE7	cAMP high affinity	cAMP	-
PDE8	cAMP high affinity	cAMP	-
PDE9	cGMP high affinity	cGMP	PF-04447943, BI 409306
PDE10	cAMP-inhibited	cAMP/cGMP	MP-10, TAK-063, RO5545965, AMG 579, OMS824
PDE11	Dual substrate	cAMP/cGMP	-

With regard to cognition enhancement, initially, memory deficits related to Alzheimer's disease were the focus of PDE inhibitor research (e.g. Blokland et al., 2006; Heckman et al., 2015a; Wang et al., 2015). However, nowadays PDE inhibitors are also investigated as cognition enhancers in for example depression (e.g. Wong et al., 2006; Esposito et al., 2009; Zhang, 2009; Fujita et al., 2012; O'Donnell and Xu, 2012) and schizophrenia (Menniti et al., 2007; Siuciak, 2008; Zhang, 2010; Duinen et al., 2015; Heckman et al., 2015b).

With regard to the potential of PDE inhibitors as a treatment for motor dysfunctions, Parkinson's disease (Belmaker et al., 1978; Volicer et al., 1986; Nishino et al., 1993; Sancesario et al., 2004) and Huntington's disease (DeMarch et al., 2007; DeMarch et al., 2008; Puerta et al., 2010) are the main researched disorders. Additionally, cyclic nucleotide regulation by PDE inhibitors has been related to tardive dyskinesia induced by antipsychotic treatment, or levodopa-induced dyskinesias (Sasaki et al., 1995; Yamashita et al., 1997b; Yamashita et al., 1997a; Sharma et al., 2013; Wilson and Brandon, 2015).

Finally, PDE inhibitors have also been linked to affect and emotion in disorders like depression, schizophrenia (negative symptoms) and anxiety disorders (Brink et al., 2008; Hebb et al., 2008; Schmidt et al., 2008; Zinn et al., 2009; Reierson et al., 2011; Smith et al., 2013; Ding et al., 2014; Rutter et al., 2014; Plattner et al., 2015; Zhang et al., 2015).

Phosphodiesterases in the fronto-striatal circuits

Within the fronto-striatal circuits, cAMP influences both presynaptic neurotransmitter release and postsynaptic intracellular pathways. The former might be mediated via a presynaptic calcium (Ca^{2+})/calmodulin-dependent protein kinase (CaMK)/cAMP/cAMP-dependent protein kinase (PKA) cascade and elevation of cAMP has been found to result in the synthesis and/or release of several neurotransmitters including two main players in the fronto-striatal circuits: glutamate and dopamine (Schoffelmeer et al., 1985; Imanishi et al., 1997; Rodriguez-Moreno and Sihra, 2013). The influence on postsynaptic intracellular pathways occurs through activation of postsynaptic PKA by cAMP produced by AC stimulated by either glutamatergic-induced Ca2+ influx or dopamine receptor-stimulated G_s. After PKA activation, two subsequent pathways play an important function in the fronto-striatal circuits: cAMP response element binding protein (CREB) pathway (Mayr and Montminy, 2001) and the Dopamine- and cAMP-Regulated PhosphoProtein MR 32 kDa (DARPP-32) pathway (Greengard, 2001; Svenningsson et al., 2004). In the first pathway, glutamate or dopamine activated PKA signaling subsequently phosphorylates CREB (pCREB). pCREB is an activated transcription factor, which initiates transcription of specific genes influencing neuroplasticity. The latter includes genes for neurotransmitter receptors such as ionotropic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors or growth factors as brain-derived neurotrophic factor (BDNF) (Scott Bitner, 2012). The dopamine receptor/cAMP/PKA/DARPP-32 cascade constitutes the second pathway. DARPP-32 is phosphorylated at Thr34 in both striatal and frontal neurons converting into a potent inhibitor of protein phosphatase-1 (PP-1), whereas phosphorylation at Thr75 by Cdk5 converts DARPP-32 into an inhibitor of PKA. DARPP-32 has therefore the unique property of being a dual-function protein, acting either as an inhibitor of PP-1 or PKA, respectively stimulating or inhibiting neuroplasticity, respectively (Svenningsson et al., 2004). Of note, the DARPP-32 signaling cascade is also linked to the CREB pathway (Greengard et al., 1999).

In addition to the backbone formed by MSNs and their dopaminergic modulation, the importance of interneurons in physiological and pathological fronto-striatal functioning is becoming increasingly apparent. Several types of interneurons can be found in the striatum, like cholinergic and different GABAergic interneurons (Gerfen and Surmeier, 2011). In particular, nitric oxide synthase (NOS) containing GABAergic interneurons I would like to highlight. These nitric oxide (NO)-producing interneurons play an important role in fronto-striatal functioning (West and Tseng, 2011). NO diffuses into dendrites of MSNs which contain high levels of guanylate cyclase (GC), which, when activated, lead to the synthesis of cGMP. In the intact striatum, transient elevations in intracellular cGMP primarily act to increase neuronal excitability and to facilitate glutamatergic fronto-striatal transmission (West and Tseng, 2011; Threlfell and West, 2013). Although the main focus in the

fronto-striatal system has been on cAMP signaling, several PDE inhibitors (also) target cGMP and may exert their effects largely dependent on cGMP signaling cascade (Padovan-Neto et al., 2015).

Since the focus of the dissertation is on fronto-striatal dopaminergic regulation, PDE1B, PDE2A, PDE4, PDE7B, PDE9A, and PDE10A in particular are of special interest (Lakics et al., 2010). PDE1B, PDE7B, and PDE10A are highest enriched in striatum and/or frontal cortex. PDE2A, PDE4 (A, B, D), and PDE9A are more widely distributed but are also expressed in striatum and/or frontal cortex. There are only very limited preclinical data on PDE2A, PDE7A, and PDE9A inhibition. To date, most research has been devoted to the potential of PDE1B, PDE4, and PDE10A for regulation of dopaminergic fronto-striatal signaling, and therefore these subtypes will be predominantly discussed in the present thesis with an emphasis on PDE4.

In addition to their well-known activating effect on PKA and PKG, cyclic nucleotides bind to cyclic nucleotide-gated channels (CNGCs) and 'exchange factor directly activated by cAMP' 1 and 2 (EPAC1 and EPAC2) (Keravis and Lugnier, 2012). Because the activities of several of these effectors can be altered simultaneously in response to increases in cellular cAMP or cGMP, PDE inhibitors can activate several cellular signaling events that yield a series of finely-tuned 'read-outs' and that markedly affect the numerous cellular processes (Conti and Beavo, 2007; Francis et al., 2011). In the fronto-striatal circuits, the main cellular processes are believed to be those related to neuroplasticity and neuroprotection through previously mentioned activation of CREB and DARPP-32 pathways. We consider both the latter to be the general mechanisms of action of PDE inhibition in the fronto-striatal circuits. However, known effects of PDE inhibitors on neurodegeneration, neuroinflammation and cytokine-mediated responses may play additional roles, yet are outside the scope of the present thesis (Hebb and Robertson, 2008; Wilson and Brandon, 2015).

The integration of individual PDEs into specific groups of proteins involved in the regulation of protein degradation, so called signalosomes, within regionally-restricted, subcellular compartments of neurons of the fronto-striatal circuits, determines the functional roles of these individual PDEs and their respective isoforms (Jurevicius and Fischmeister, 1996; Zaccolo et al., 2000; Mongillo et al., 2006; Maurice, 2011; Stangherlin et al., 2011; Stangherlin and Zaccolo, 2012). In addition, different PDE isoforms can integrate multiple distinct cellular inputs and allow crosstalk between cyclic nucleotides and other signaling networks and systems (Dodge-Kafka et al., 2005; Mongillo et al., 2006; Houslay et al., 2007; Stangherlin et al., 2011; Wilson et al., 2011; Kritzer et al., 2012). Together, this is considered the main determinant of the target PDE isoform within a specific neuropsychiatric disorder related to the fronto-striatal circuits.

Targeting PDEs for inhibition

Increased PDE activity is assumed to reduce cAMP signaling in pathways important for brain plasticity and neuroprotection and is therefore considered to be causal, while a decrease in PDE activity might be considered as compensatory (Bollen and Prickaerts, 2012; Gurney et al., 2015). Additionally, PDE expression is assumed to decrease with aging. Whether this is an age-related decrease or a compensatory mechanism is not known (Richter et al., 2013).

From a therapeutic perspective, the response of a biological system to an endogenous or exogenous molecule depends upon the dose. Therefore, it is crucial to perform what is termed 'dose-response curves' (often inverted-U shaped). In the current thesis, these dose-response curves explain optimal levels of performance by cAMP/PKA activity and bioavailability. Pharmacological agents thus aim to induce optimal levels of cAMP thereby enhancing performance (cognition enhancement). Aging and mental illness can increase or decrease levels of cAMP/PKA. This change in cyclic nucleotide activity and bioavailability can be compensated by pharmacological agents returning them to optimal levels. Regarding PDEs and their respective inhibitors, it appears most promising to target PDEs with increased expression. This way, cognition and plasticity deficits resulting from impaired cAMP/PKA signaling might be improved by inhibiting specific PDE isoforms. However, PDE inhibition might have negative effects on cognition and plasticity when PDEs are already downregulated and cAMP levels and PKA activity are high which results in shifting the dose-response curve to the left. In this scenario, elevating cAMP levels might go over a physiological level and disrupt signaling. Along this line, high doses of rolipram impaired prefrontal cognitive function in aged, but not young monkeys, likely due to overstimulation of the already disinhibited cAMP/PKA signaling pathway in the aged prefrontal cortex (Ramos et al., 2003; Arnsten et al., 2005). This argues to specifically target PDEs that are overexpressed. Consequently, the inhibition of, in particular, PDE4 could be a translational tool to modulate the intracellular cyclic nucleotide signaling cascades, thereby improving cognitive fronto-striatal functioning

Aim and outline of the thesis

As outlined above, PDE4 might be a relevant target for the treatment of cognitive dysfunctions in disorders related to the fronto-striatal circuits. Therefore, the main aim of the current dissertation is to further investigate the function of PDE4 in cognitive fronto-striatal circuits. The focus is set on cognition, opposed to motor and limbic functions of the fronto-striatal circuits, and includes cognitive functions such as attention, sensory gating, sensorimotor gating and impulsivity. This

requires a thorough review on the existing literature to understand the current status in this field. In the first chapter, it will be evaluated how PDE inhibition can lead to changes in the functions of the fronto-striatal circuit. This chapter provides thus an overview of the efficacy of PDE inhibitors, especially inhibitors of PDE4, as modulators of the fronto-striatal circuits (Chapter 2).

Chapter 3 provides an overview of the current status of the knowledge on the role of PDE1, PDE4 and PDE10 in the regulation of dopaminergic modulation of fronto-striatal circuits. Dopamine is one of the key neurotransmitters in the fronto-striatal-thalamic circuitry. Further, recent studies suggest that PDE inhibition may modulate dopamine release. In contrast to the previous chapter, this chapter discusses the relation between PDEs and dopamine in relation to the cognitive functions in more detail. In addition to a discussion on the neurobiological mechanisms of PDE inhibition, a clinical perspective for PDE inhibition will be provided.

The next chapter (Chapter 4) describes the effects of the PDE4 inhibitor roflumilast in the sensory gating paradigm tested in healthy humans. Sensory gating is a process involved in early information processing which prevents overstimulation of the brain. It is especially affected in patients suffering from schizophrenia, Alzheimer's disease and ADHD. Sensory gating is believed to be induced by inhibitory interneurons of the auditory cortex and the thalamic 'gate', frontal inhibitory output neurons or in the interneurons that locally release inhibitory neurotransmitter in any other brain area capable of eliciting sensory gating. The neurobiology of sensory gating can thus mainly be found in the fronto-striatal circuits. It is known that PDE4 is highly expressed in fronto-striatal areas related to sensory gating including the thalamus and frontal cortex (Lakics et al., 2010). Therefore, we investigated the effects of roflumilast on sensory gating in healthy human participants (Chapter 4).

In Chapter 5 we examine the functional output of the fronto-striatal circuit to the thalamus at an electrophysiological level. More specifically, the aim of this study was to better understand the role of PDE4 in the fronto-striatal pathways. This will be accomplished by studying the distinctive effects of PDE4 inhibition on the three basal ganglia pathways: the hyperdirect, direct and indirect pathway. The effects of roflumilast on the three pathways will be studied via the tri-phasic (excitation-inhibition-excitation) response of the SNr after infralimbic cortex stimulation. The SNr is the output module of the basal ganglia and the location where all three pathways come together. The infralimbic cortex is stimulated because the cognitive fronto-striatal circuits in the rat originate within the medial prefrontal cortex. This study will reveal which pathway is sensitive to PDE4 inhibition and may contribute to a better understanding how PDE4 inhibition may affect functions in the cortico-striatal system (Chapter 5).

In Chapter 6, the mediating role of PDE4 in the dopaminergic modulation of premature responding (motor impulsivity) will be investigated. Response inhibition, which includes action restraint, finds its neurobiological origin in fronto-striatal circuitry and can be modulated by dopamine. Intracellularly, the effect of dopamine is largely mediated through the cAMP/PKA signaling cascade. It has been suggested that areas in the prefrontal cortex are very sensitive to their neurochemical environment, including catecholamine levels (Arnsten, 2009). Both high and low catecholamine release in the prefrontal cortex impairs prefrontal cortex function. As a result, we are interested in the effects of PDE4 inhibition on premature responding in a hypo, normal and hyper dopaminergic state of the brain. As a hypo dopaminergic model we will induce a 6-OHDA lesion in the prefrontal cortex, more specifically the infralimbic cortex. This is a well-known animal model for the induction of ADHD symptoms including motor impulsivity related to action restraint (Freund et al., 2014; Lukkes et al., 2016). For the hyperdopaminergic state we also turn to a well-established model of impaired action restraint, namely the systemic administration of d-amphetamine, which has proven to robustly increase premature responding (Winstanley et al., 2006). Taken together, we investigate the effects of a PDE4 inhibitor on premature responding in a choice reaction time task in a hypo, normal and hyper dopaminergic state of the fronto-striatal circuitry.

To conclude, the main aim of the dissertation is to investigate the role of PDE4 in the fronto-striatal circuits with an emphasis on cognition. This was investigated in both a physiological (Chapter 4 and Chapter 5) as well as a pathophysiological (Chapter 6) condition of the fronto-striatal circuit. The main findings of the current dissertation are summarized and discussed in Chapter 7.

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Chapter 2

PDE and cognitive processing: beyond the memory domain

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Abstract

Phosphodiesterase inhibitors (PDE-Is) enhance cAMP and/or cGMP signaling via reducing the degradation of these cyclic nucleotides. Both cAMP and cGMP signaling are essential for a variety of cellular functions and exert their effects both pre- and post-synaptically. Either of these second messengers relays and amplifies incoming signals at receptors on the cell surface making them important elements in signal transduction cascades and essential in cellular signaling in a variety of cell functions including neurotransmitter release and neuroprotection. Consequently, these processes can be influenced by PDE-Is as they increase cAMP and/or cGMP concentrations. PDE-Is have been considered as possible therapeutic agents to treat impaired memory function linked to several brain disorders, including depression, schizophrenia and Alzheimer´s disease (AD). This review will, however, focus on the possible role of phosphodiesterases (PDEs) in cognitive decline beyond the memory domain. Here we will discuss the involvement of PDEs on three related domains: attention, information filtering (sensory- and sensorimotor gating) and response inhibition (drug-induced hyperlocomotion). Currently, these are emerging cognitive domains in the field of PDE research. Here we discuss experimental studies and the potential beneficial effects of PDE-I drugs on these cognitive domains, as effects of PDE-Is on these domains could potentially influence effects on memory performance. Overall, PDE4 seems to be the most promising target for all domains discussed in this review.

Keywords: cAMP, cGMP, PDE, attention, gating, response inhibition

1. Phosphodiesterases and cognition

1.1 Cognitive decline

One of the problems many people come to face with increasing age is a decline in cognitive functions (Mattson et al., 2002). The loss of cognitive functioning is even more serious in patients suffering from neuropathological conditions such as Alzheimer's disease (AD), depression and schizophrenia (Blaney, 1986; Frith, 1996). The need to develop drugs that counteract cognitive decline is of major importance since these deficits have a large impact on the quality of life of these patients. So far, several strategies have been described which could slow down cognitive decline. Research has focused on environmental and genetic factors that cause neuronal dysfunction and death or on enhancement of the ability of neurons to adapt (Mattson et al., 2002). Despite these strategies, there is still a great need for drugs that counteract the processes involved in the decline of cognitive functioning (Reneerkens et al., 2009).

For cognition enhancement or reversal of cognitive deficits, several drug targets, like the serotonergic, cholinergic, glutamatergic and monoaminergic neurotransmitter systems, have been suggested. All these neurotransmitter systems have shown to be involved in cognition. Furthermore, cognitive performance can be improved via numerous biological targets 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, 2005; Reneerkens et al., 2009). There has been an increasing interest in the drugs that inhibit the phosphodiesterase enzyme to improve cognitive performance in animals and man (Blokland et al., 2012; Bollen and Prickaerts, 2012; Reneerkens et al., 2009).

1.2 Phosphodiesterases

Phosphodiesterases (PDEs) are enzymes that play a major role in cell signaling by hydrolyzing the second messengers cyclic adenosine monophosphate (cAMP) and/or cyclic guanosine monophosphate (cGMP) throughout the body and brain (Beavo, 1995; Lugnier, 2006). Phosphodiesterase inhibitors (PDE-Is) reduce the degradation of cAMP and/or cGMP, leading to increased concentration of cAMP and/or cGMP (Beavo, 1995). These signals will then have a prolonged effect on the signaling pathways in neurons.

Currently, PDEs constitute 11 gene-related families of isozymes (see Table 1). In total, there are estimated to be over 100 specific human PDEs (Bender and Beavo, 2006). Different PDE families can be distinguished based on their affinity for the second messengers cAMP, cGMP or both (see Table 1) (Beavo, 1995).

Table 1. Overview of the different PDE families, the related genes, property and the substrate of the PDE (Bender and Beavo, 2006).

Type	Genes	Property	Substrate
PDE1	A, B, C	Ca^{2+}-CaM-stimulated	cAMP/cGMP
PDE2	A	cGMP-stimulated	cAMP/cGMP
PDE3	A, B	cGMP-inhibited	cAMP/cGMP
PDE4	A, B, C, D	cAMP-specific	cAMP
PDE5	A	cGMP-specific	cGMP
PDE6	A, B, C	Photoreceptor	cGMP
PDE7	A, B	cAMP high affinity	cAMP
PDE8	A, B	cAMP high affinity	cAMP
PDE9	A	cGMP high affinity	cGMP
PDE10	A	cAMP-inhibited	cAMP/cGMP
PDE11	A	Dual substrate	cAMP/cGMP

1.3 Second messengers

Second messengers relay and amplify incoming signals at receptors on the cell surface making them important elements in signal transduction cascades. The second messengers cAMP and cGMP are essential in cellular signaling in a variety of cellular functions including neurotransmitter release, neuroplasticity and neuroprotection, as described below (Francis et al., 2011). Consequently, these processes can be influenced by PDE-Is as they increase cAMP and/or cGMP concentrations.

cAMP is synthesized from adenosine triphosphate (ATP) by adenylyl cyclase (AC). The latter is activated by calmodulin-dependent protein kinase II (CaMKII) after Ca^{2+} influx or directly by an activated G-coupled protein receptor. cAMP can activate protein kinase A (PKA) which phosphorylates the cAMP response element-binding protein (CREB), and can thereby affect transcription of genes related to synaptic plasticity and neurogenesis, like brain-derived neurotrophic factor (BDNF) (Scott Bitner, 2012). On the other hand, cGMP is derived from guanosine triphosphate (GTP) by guanylyl cyclase (GC), which in turn is activated by nitric oxide (NO). NO is produced by NO

synthase (NOS) after Ca^{2+} influx. The NO/cGMP pathway activates protein kinase G (PKG), which can also induce CREB phosphorylation (Lu et al., 1999), likely via modulation of the cAMP/PKA pathway (Bollen et al., 2014)

Since PDE-Is postsynaptically affect transcription of genes related to synaptic plasticity and neurogenesis, these effects are long-lasting and probably not involved in the short-lasting actions related to cognitive domains to be discussed in this review. As such, plasticity effects are more likely to play an important role in long-term potentiation (LTP), which is assumed to be the substrate of memory (Sarvey et al., 1989), and the formation of memories. Though, the enzyme AC is also present presynaptically and elevation of cAMP has been found to result in the synthesis and/or release of several neurotransmitters including glutamate and dopamine (DA) (Imanishi et al., 1985). This might be mediated via a presynaptic CaMK/cAMP/PKA cascade and hence signal transduction is influenced. Also, NO is known to act as a retrograde messenger and can thus stimulate presynaptic GC. Linked to this, via a cGMP/PKG cascade, the synthesis and/or release of neurotransmitters including glutamate and DA can also be influenced (Arancio et al., 1995; Sanchez et al., 2002), and thus again signal transduction. Figure 1 provides a schematic overview of the pre- and postsynaptic cellular processes related to the second messengers cAMP and cGMP involved in signal transduction.

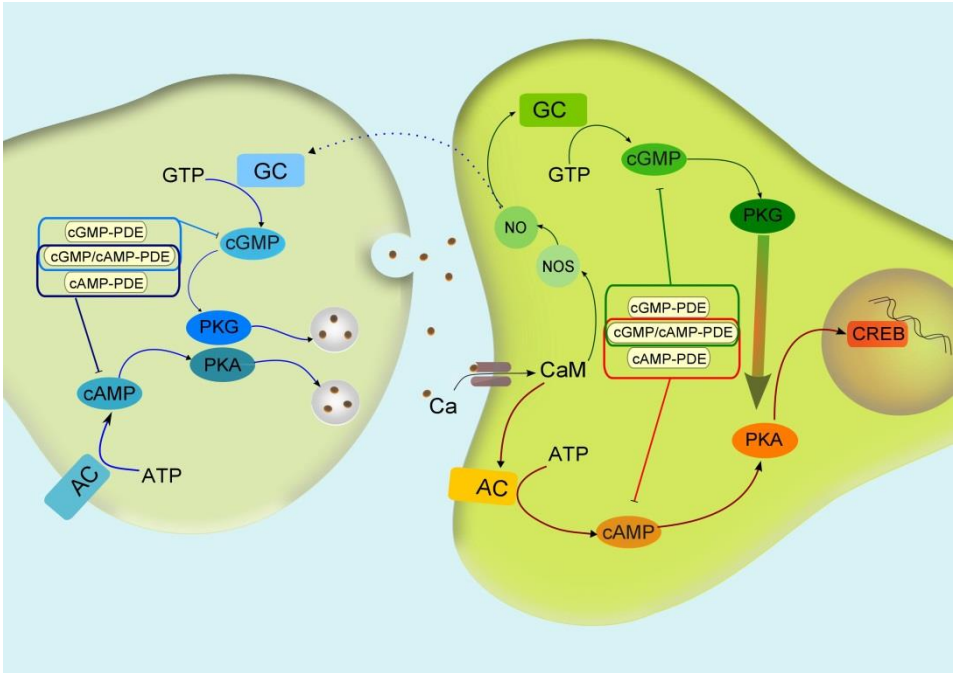

Fig. 1 Schematic diagram of pre- and postsynaptic cellular processes related to the second messengers cAMP and cGMP involved in LTP-related signal transduction.

1.4 Localization

Table 2 provides a short overview of the localization of the different PDEs in the brain of rodents and humans based on mRNA expression and situ histochemistry (Lakics et al, 2010; Pérez-Torres et al, 2010).

Table 2. Localization of the different phosphodiesterases (PDEs) in the brain of rodents and humans in adulthood (adapted from Prickaerts, 2010; based on Lakics et al, 2010; Pérez-Torres et al, 2010).

PDE	**Localization in the Brain**
PDE1A-C	Hippocampus, cortex, olfactory bulb, striatum (highest expression levels), thalamus, amygdala, cerebellum; Expression levels are in general highest for 1A and lowest for 1C
PDE2A	Hippocampus, cortex, striatum, hypothalamus, amygdala, midbrain
PDE3	Throughout the brain low expression levels
PDE4A-D	Hippocampus, cortex, olfactory bulb, striatum, thalamus, hypothalamus, amygdala, midbrain, cerebellum; Expression levels are in general highest for 4A-4D (differs per brain structure) and lowest for 4C
PDE5A	Hippocampus, cortex, cerebellum
PDE6	Pineal gland
PDE7A-B	Hippocampus, cortex, olfactory bulb, striatum, thalamus, hypothalamus, midbrain; Expression levels are in general highest for 7B
PDE8A-B	Hippocampus, cortex, olfactory bulb, striatum, thalamus, hypothalamus, midbrain; Expression levels are in general highest for 8B
PDE9A	Hippocampus, cortex, olfactory bulb, striatum, thalamus, hypothalamus, amygdala, midbrain, cerebellum
PDE10A	Hippocampus, cortex, striatum (highest expression levels), midbrain, cerebellum
PDE11A	Throughout the brain low expression levels

Only clear expression levels are taken into consideration. Note that this table does not provide information with respect to the level of expression (protein or mRNA) of the different PDEs.

1.5 Beyond the memory domain

Recently, PDE-Is are receiving increased attention as possible therapeutic tools for treatment of cognitive decline. The effects on memory processes have received most attention when it comes to the application of PDE-Is as therapeutic agents (for an overview see Blokland et al., 2012; Reneerkens et al., 2009), since impaired memory functioning is linked to several disorders, including depression, schizophrenia, Alzheimer´s disease (Blaney, 1986; Folstein and Whitehouse, 1983; Frith, 1996). This review will, however, focus on the possible role of PDEs in cognitive decline beyond the memory domain. Here we will discuss the involvement of PDEs on three related domains: attention, information filtering (sensory and sensorimotor gating) and response inhibition (drug-induced hyperlocomotion). Currently, these are emerging cognitive domains in the field of PDE research. Here we discuss experimental studies and the potential beneficial effects of PDE-I drugs on these cognitive domains.

2. Attention

2.1 Attentional concepts and biology

Posner and Petersen (Fan and Posner, 2004; Petersen and Posner, 2012; Posner and Petersen, 1990) describe the concept that is referred to as ´attention´ in terms of three basic concepts. The first concept encompasses that “the attention system is anatomically separate from processing systems, which handle incoming stimuli, make decisions, and produce outputs”. The second concept states that “attention utilizes a network of anatomical areas”. The third concept comes down to these anatomical areas carrying out different attentional processes that can be specified in cognitive terms.

The most unique aspect is the anatomical basis of the attention system: divided into three networks, each representing a different set of attentional processes. The first component contains mainly subcortically located arousal and alerting networks, mainly the ascending reticular activating system, and project to the whole brainstem and thalamus and, through the striatum, up to the limbic system to form cortical projections. The main function of this component is the activation and synchronization of the cortex during behavior and motivation, and has affinity for distinctive stimuli. The second component is the mixed cortical-subcortical orienting network which detects novel stimuli (superior colliculi), filters out irrelevant stimuli (pulvinar) or disengages attentional focus (posterior parietal cortex). The third attentional component is the executive attentional network.

This involves frontal brain structures for generation of voluntary saccades (frontal eye fields), induces motor intention (premotor cortex), is linked to working memory (dorsolateral prefrontal cortex (dlPFC) or medial prefrontal cortex (mPFC) in rodents (Uylings et al., 2003)) and is modulated by the anterior cingulate cortex (ACC) (Fan and Posner, 2004; Petersen and Posner, 2012; Posner and Petersen, 1990).

2.2 PDE inhibitors and attention

Abundant PDEs can be found in the many human brain area´s involved in the attention network including the thalamus, striatum and prefrontal cortex (PFC) (Lakics et al., 2010). However, so far only a few studies have been conducted investigating the effects of PDE inhibition on attention in both animals and humans (see Table 3). Of note, most animal tests for attention tap into the executive attention network but studies generally do not specify any clear distinction between the different components of attention.

Table 3. Overview studies testing the effects of PDE inhibition on attention-related functions.

Task	Model (species)	Treatment	Results	Reference
Latent inhibition	MK-801-induced deficit model (0.5 mg/kg, i.p.)(mice)	PDE4-I: Rolipram (0.05 and 0.075 mg/kg, i.p.)	Rolipram 0.075 mg/kg restored levels of latent inhibition	Davis & Gould, 2005
Latent inhibition	Bupropion-induced deficit model (1.0 and 4.0 mg/kg, i.p.), amphetamine-induced deficit model (0.5, 1.0, 2.5 mg/kg, i.p.)and GBR12783-induced deficit model (5.0, 15.0 mg/kg, n.p.)(mice)	PDE4-I: Rolipram (0.5 mg/kg, i.p.)	Rolipram restored levels of latent inhibition in all three deficit models	Lipina & Roder, 2009
Latent inhibition	Amphetamine-induced deficit model (2.5 mg/kg, i.p.) and disruption induced by increased # of conditioning trials (mice)	PDE7-I: VP1.15 (3.0 mg/kg, i.p.)	In both deficit models VP1.15 enhanced disrupted latent inhibition	Lipina et al., 2013
Latent inhibition	PDE10A knockout	Not applicable	Latent inhibition	Piccart et al.,

	(mice)		was impaired in KO mice compared to wild type	2014
Spatial auditory attention task	Unimpaired young (human)	PDE5-I: Sildenafil (100 mg, oral)	No effects on behavioral measures, amplitude enhancement Nd-effect + P3 effect	Schultheiss et al., 2001
Periphery vigilance test combined with a tracking task	Unimpaired (human)	PDE5-I: Sildenafil (100 mg, oral)	Average output of tracking was lower in sildenafil group	Grass et al., 2001
Conditioned avoidance attention task	Scopolamine-induced deficit model (0.13 mg/kg, s.c.)(rats)	PDE9-I: PF-04447943 (0.1-3.0 mg/kg, i.p.)	Only 1 mg/kg PF-04447943 reversed scopolamine deficit on 1 and 3 sec trials	Vardigan et al., 2011
Attentional set-shifting	PCP-induced deficit model (5.0 mg/kg, i.p.)(rats)	PDE10-I: Papaverine (3.0, 10.0 and 30.0 mg/kg, i.p.)	All doses of papaverine restored deficit in EDS performance	Rodefer, et al., 2005
Attentional set-shifting	PCP-induced deficit model (5.0 mg/kg, i.p.)(rats)	PDE2-I: BAY 60-7550 (0.3-3 mg/kg, p.o.)	3 mg/kg restored deficit in EDS performance	Rodefer et al., 2012
Attentional set-shifting	PCP-induced deficit model (5.0 mg/kg, i.p.)(rats)	PDE4-I: rolipram (0.003-0.03 mg/kg, i.p.)	0.1 mg/kg restored deficit in EDS performance	Rodefer et al., 2012
Attentional set-shifting	PCP-induced deficit model (5.0 mg/kg, i.p.)(rats)	PDE5-I: sildenafil (0.3-3 mg/kg, i.p.)	3 mg/kg restored deficit in EDS performance	Rodefer et al., 2012
Attentional set-shifting	PCP-induced deficit model (5.0 mg/kg, i.p.)(rats)	PDE10-I: papaverine (1-10 mg/kg, i.p.)	10 mg/kg restored deficit in EDS performance	Rodefer et al., 2012

i.p. = intra peritoneal; i.m. = intra muscular; s.c. = subcutaneous; n.p. = not provided

2.2.1. PDE2 inhibitors

Only one study examined the effects of a PDE2-I in an attention model that is assumed to measure attentional deficits in schizophrenia (Rodefer et al., 2012). In this test model phencyclidine (PCP) treatment resulted in an increased number of trials when the rats had to learn an extra-dimensional shift. In this study BAY 60-7550 attenuated the PCP-induced deficit.

2.2.2 PDE 4 inhibitors

In 2005 the first study was conducted by (Davis and Gould, 2005). The latent inhibition (LI) paradigm was used to examine attention and study cognitive deficits associated with schizophrenia. LI is the phenomenon by which prior exposure to a non-rewarding conditioned stimulus (CS) decreases the salience of the CS when it is later paired with an unconditioned stimulus (US) (Weiner, 2003). This test requires attentional abilities. A deficit in selective attention results in better learning in this task. In their study, the cAMP-specific PDE4-I rolipram attenuated the disruptive effect of the N-methyl- D -aspartic acid (NMDA) antagonist MK-801, a commonly used deficit model in attention research, on LI in mice, thus linking PDE-Is for the first time to attention research. In another study using the same paradigm, rolipram reversed the LI deficits induced by bupropion (DA and norepinephrine (NE) reuptake inhibitor and noncompetitive nicotinic antagonist) treatment in mice (Lipina and Roder, 2009). Rolipram was also found to reverse the PCP-induced deficits in extra dimensional attentional set-shift learning (a test model for schizophrenia) (Rodefer et al., 2012). Taken together, these data show that a variety of test models show an improved attentional performance after PDE4-I treatment.

2.2.3 PDE 5 inhibitors

Two human studies examined the effects of PDE5 inhibition on attention. In 2001 both these studies were conducted using the cGMP-specific PDE5-I sildenafil. In the first study behavioral patterns and event-related potentials (ERPs) were recorded in a spatial auditory attention task (Schultheiss et al., 2001). While behavioral patterns did not reveal any overt effects of sildenafil, auditory ERPs were indicative of an enhanced ability to focus attention and to select relevant target stimuli in the sildenafil condition. The other study encompassed a pilot study (n=6) (Grass et al., 2001). These data could suggest that the attention performance was enhanced in the sildenafil condition, but it should be noted that the reported effects did not reach statistical significance. One animal study showed that sildenafil improved set-shifting learning in rats (Rodefer et al., 2012).

2.2.4 PDE 7 inhibitors

The dual inhibitor of cAMP-specific PDE7 and glycogen synthase kinase 3 (GSK-3), VP1.15, was able to ameliorate the disrupted LI induced by the increased number of conditioning trials and reversed an amphetamine-induced LI deficit in mice (Lipina et al., 2013). These data indicate that VP1.15 has an effect in models of early attention processing. However, further studies are needed to be shown to what extend these effects can be attributed to inhibition of PDE7 or GSK-3.

2.2.5 PDE 9 inhibitors

Vardigan and coworkers (Vardigan et al., 2011) showed that the selective cGMP-specific PDE9-I PF-04447943 attenuated a scopolamine-induced deficit in the conditioned avoidance attention task (CAAT) in rats. In this experiment animals were selected on basis of their performance in this task. After this titration this study demonstrated efficacy of PDE9-I treatment in this avoidance task (Vardigan et al., 2011). These findings suggest that PDE9-I may reverse a cholinergic deficit in attention.

2.2.6 PDE 10 inhibitors

Rodefer et al (2005) showed that acute treatment with the PDE10A-I papaverine reversed phencyclidine (PCP)-induced deficits in extra dimensional attentional set-shift learning (replicated in Rodefer et al., 2012). Thus, papaverine improved attentional functions in this schizophrenia model.
A more recent study by Piccart et al. (2014) measured LI via conditioned taste aversion in PDE10A knockout mice. PDE10A -/- deficient animals showed impaired LI as indicated by the aversion index. The data revealed that wild type animals showed decreased conditioning to saccharin that had been tasted before conditioning, as expected in LI. PDE10A -/- deficient animals however did not show differences in conditioning to saccharine, when pre-exposed, indicative of impaired LI. Taken together these data suggest a role of PDE10 in attentional functions in preclinical schizophrenia models.

2.2.7 Miscellaneous

Although not directly related to PDE inhibition, a study by Lesch *et al.* revealed an interesting relation between the PDE4D6 gene and attention-deficit/hyperactivity disorder (ADHD) (Lesch et al., 2010). In this study they performed a genome-wide copy number variation analysis in ADHD in an extended pedigree. They found that the PDE4D6 gene was potentially influencing ADHD-related psychopathology and was involved in aberrations inherited from affected parents. Furthermore, Paine and colleagues (Paine et al., 2009) showed that inhibition of PKA within the mPFC of rats, a region comparable to the dlPFC in humans (Uylings et al., 2003), produces inattention and hyperactivity. Since the level of the second messenger cAMP is directly related to PKA activity, a direct link between PDE4 inhibition and increased attention appears likely. Or more in general, cAMP specific PDEs that are located in frontal cerebral regions, i.e. PDE1, PDE2, PDE4 and PDE8, could be considered as interesting targets for PDE-Is in enhancing attention.

2.3 Conclusion PDE inhibitors and Attention

In conclusion, although highly variable in type of PDE-I, task type, read-out parameters and (deficit) models used, all animal studies into PDE inhibition and attention show a congruent pattern.

Inhibitors of PDE4, 5, 7, 9 and 10 all show positive effects on the measures of attention investigated. The most used model of attention in PDE animal research is LI. Within this model different deficit models are used to induce an LI impairment, i.e. MK-801, PCP, bupropion, scopolamine or amphetamine. Some human studies with PDE5-Is appear to support these findings in animal research (but see Reneerkens *et al.*, 2013).

The most interesting PDE-Is for improving attention based on their presence in the abundant attention network and the scarce data available, appear PDE4-Is and PDE5-Is as rolipram and sildenafil showed best results, respectively (see Table 3 for an overview). The field of attention research is hampered by inconsistencies in methods used and lack of overall consensus of the attention network itself. This led to the fact that studies into attention and PDE inhibition do not select target brain areas to be stimulated, but use existing or new attention tasks and generally try to stimulate cholinergic, dopaminergic and glutamatergic neurotransmission to find an effect. Most attention tasks are stimulus-driven and therefore use bottom-up processing. Bottom-up processing involves mostly orienting and alerting networks. However, if a response requires executive attention top-down processing plays a role as well. It is therefore very hard to distinguish between different brain areas involved in the attentional component of a task and consequently which brain area should specifically be stimulated. The advantage of PDE-Is is that they do not focus on one neurotransmitter system but can enhance intracellular signaling in all neuronal systems containing the particular PDE subtype being targeted, either glutamatergic, cholinergic or otherwise. Likewise, taking into consideration the localization of the different PDE subfamilies, knowledge of the target area is required to select the appropriate PDE-I. The latter is a challenge for attention tasks. Each behavioral task may activate (partly) different brain areas so that several distinct types of PDE-Is would be suited for each behavioral attention task. Nevertheless, based on human PDE expression patterns in the overall attentional network, PDE2-Is, PDE4-Is and PDE8-Is are most likely to show attention enhancing effects (Lakics et al., 2010). PDE5, though, is generally low expressed in the brain, yet results from human studies using the PDE5-I sildenafil appear positive. However, the PDE5-I vardenafil showed no results in humans after acute treatment. Consequently, one should be reserved when arguing that PDE5-Is are attention enhancers. PDE4-Is seem to be more promising but, yet again more studies are needed to substantiate this claim.

Several issues have to be considered in the field of attention and PDE-I research. Firstly, the number of studies that examined the effects of PDE-Is on attention are relatively scarce. Moreover, there are different types of attention and the test paradigms are quite heterogeneous which presently makes it difficult drawing firm conclusions with respect to PDE-Is and attention. A second main issue relates

to the underlying neurobiology of attention. In attention research there is the lack of overall consensus of what systems and brain areas make up the attention system. Next to Posner's theory there are other models of attention available (e.g. Knudsen, 2007). In theory, stimulation of an area within any of these extensive networks could lead to enhanced attentional performance. Therefore, it is not clear which brain area is effectively mediating the positive effects of a PDE-I on cognition. In most studies, attention tasks lack specification of which brain areas are involved in the task. Instead, the focus is mainly on boosting a particular neurotransmitter system. Especially the glutamatergic system, including the NMDA receptor, receives most attention in relation to disturbed attentional functioning in schizophrenia (e.g. Davis and Gould, 2005; Rodefer et al., 2005). Also the cholinergic and dopaminergic neurotransmitter systems are investigated within this context (e.g. Lipina and Roder, 2009; Vardigan et al., 2011).

Of note, another approach is provided by pharmacological treatments of ADHD, an attention-related disorder. Stimulant drugs such as methylphenidate (Ritalin) and amphetamine (Adderall) improve the attention of individuals with ADHD and can enhance attention in healthy people as well (Lakhan and Kirchgessner, 2012; Mehta et al., 2000). Methylphenidate increases activity in the PFC and attention-related areas of the parietal cortex during challenging mental tasks (Berridge et al., 2006; Mehta et al., 2000). These are the same areas that have been demonstrated to have shrunken in volume in ADHD brains (Castellanos et al., 2002). These studies indicate the PFC and parietal cortex as relevant brain areas for attentional functioning. Several PDEs can be found within these areas including PDE1, PDE2, PDE4 and PDE8. Therefore, these PDEs are interesting targets for attention enhancement, of which only PDE4 has received attention in this respect.

3 Gating

3.1 Gating concepts and biology

In this section of our review we will focus on another cognitive domain termed 'gating' including both sensory gating and sensorimotor gating. 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, Mears, Wan, and Boutros, 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 and 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.

Sensory gating can be observed throughout the brain in areas in all four lobes in both hemispheres, as well as, subcortical structures capable of eliciting a P50 response. Almost every sensory signal, except olfaction, entering the brain must pass through a thalamic "gate" before it is relayed to the other parts of the brain, including the hippocampus and the cortex contributing to gating of irrelevant stimuli. Furthermore, frontal inhibitory output, as well as, locally released inhibitory neurotransmitter, both as a response to the first stimulus, contribute to inhibition of the response to the second stimulus (Freedman et al., 1991). Extensive research has shown that the process of sensory gating is disrupted in patients suffering from clinical disorders including schizophrenia and AD (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 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 (de Bruin et al., 2001). 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, Klein, and Cromwell, 2006; Zhou, Ma, Liu, Chen, He, and Miao, 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; Witten et al., 2014).

Sensorimotor gating can be measured via one of the most prominent behaviors studied in vertebrates called the 'startle response'. The startle response is a fast contraction of body muscles caused by a sudden acoustic, tactile, or visual stimulus mediated by simple neuronal circuitry (Koch, 1999). It is interpreted to be an evolutionarily conserved form of protection against potential danger and predators. Increase of the acoustically generated startle (commonly called the acoustic startle response; ASR) is known to have different causes such as sensitization, fear-potentiation or pharmacological intervention, whereas decrease of the ASR can be caused by habituation, PPI or again pharmacological intervention (Koch, 1999). Compounds affecting the glutamatergic NMDA pathways or dopaminergic pathways are commonly used deficit models in gating research (Kehne et al., 1991; Klamer et al., 2004).

3.2 PDE inhibitors and sensory gating

Table 4. Overview studies sensory gating and PDE inhibition.

Task	Model (species)	Treatment	Results	Recording site	Reference
Sensory gating	Healthy (rats)	PDE2-I: BAY 60-7550 (1.0 mg/kg, p.o.) and PDE5-I: vardenafil (1.0 mg/kg, p.o.) and PDE10-I: PQ-10 (1.0 mg/kg, p.o.)	Administration of PDE-Is did not affect sensory gating	Striatum, dorsal hippocampus and vertex	Reneerkens et al., 2013B
Sensory gating	Impaired (DBA2 mice)	PDE2-I: Lu AF64280 (5.0 and 20.0 mg/kg, s.c.)	Lu AF64280 20.0 mg/kg reduced the T/C ratio	CA3 region hippocampus	Redrobe et al., 2014
Sensory gating	Healthy (mice) and amphetamine-induced deficit (5.0 mg/kg, i.p.)(mice)	PDE4-I: Rolipram (0.1, 1.0 and 2.0 mg/kg, i.p.)	Rolipram alone dose dependently enhances the amplitude of the first stimulus on the P20, N40 and P20N40 components. Additionally, rolipram normalizes the amphetamine-induced amplitude decrease on the first stimulus on each component	Hippocampus	Maxwell et al., 2004
Sensory gating	Healthy (mice) and amphetamine-induced deficit model (0.5 mg/kg, n.p.)	PDE4-I: RO-20-1724 (0.1, 0.25, 0.5, 1.0, and 2.5 mg/kg, s.c.)	RO-20-1724 0.25, 0.5, and 1.0 mg/kg increased the amplitude of the P20 component of	CA3 region hippocampus	Halene and Siegel, 2008

			the first click and increased the N40 amplitude. RO-20-1724 0.25 mg/kg restored gating and increased S1 amplitude compared to amphetamine alone		
Sensory gating	Healthy (rats) and healthy (human)	Rat: PDE5-I: vardenafil (0.3, 1.0 and 3.0 mg/kg, p.o.), human: vardenafil (10.0 and 20.0 mg, oral	Administration of vardenafil did neither have an effect on sensory gating in rats nor in humans	Rat: vertex, hippocampus and striatum, human: cortex (Fz, Fcz and Cz electrodes)	Reneerkens et al., 2013A
Sensory gating	Amphetamine-induced deficit model (1 mg/kg, i.v.)(mice)	PDE9-I: PF-4447943 (0.1, 0.32 and 1.0 mg/kg, s.c.) and PF-4449613 (1.0 mg/kg, s.c.)	PF-4447943 0.1 and 0.32 mg/kg and PF-4449613 1.0 mg/kg reversed amphetamine-induced deficit	CA3 region left hippocampus	Kleiman et al., 2012
Sensory gating	Amphetamine-induced deficit model (1 mg/kg, i.v.)(rats)	PDE10-I: TP-10 (3.0 mg/kg, i.v.)	TP-10 reversed gating deficit	CA3 region left hippocampus	Schmidt et al., 2008

i.p. = intra peritoneal; i.v. = intra venous; s.c. = subcutaneous; p.o. = per os; n.p. = not provided

3.2.1 PDE2 inhibitors

In a recent study the novel cAMP/cGMP-specific PDE2A-I Lu AF64280 significantly reduced the deficit in sensory gating of DBA2 mice (Redrobe, Jorgensen, Christoffersen, Montezinho, Bastlund, Carnerup, Bundgaard, Lerdrup, and Plath, 2014). Furthermore, Reneerkens and colleagues showed that the cAMP/cGMP-specific PDE2A-I BAY 60-7550 did not affect sensory gating in rats measured unilaterally in vertex or unilaterally in striatum or dorsal hippocampus (Reneerkens et al., 2013b). However, PDE2 inhibition increased the P1 peak after presentation of S1 at the vertex. PDE2 inhibition may thus affect auditory processing in general, yet not sensory gating. These two studies do not provide a clear picture as to the role of PDE2 in auditory gating. The different models applied in both studies may underlie these different findings.

3.2.2 PDE4 inhibitors

Maxwell et al. (Maxwell et al., 2004) showed that the cAMP-specific PDE4-I studied the effects of rolipram in a model for impaired auditory processing in schizophrenia. They recorded local field potentials in rats with and without amphetamine. They found that rolipram dose dependently enhanced the amplitude of the first stimulus on the P20, N40 and P20-N40 components. In addition, rolipram was able to normalize the amphetamine-induced amplitude decrease on the first stimulus on each component. In another study applying the same model for schizophrenia, the PDE4-I RO-20-1724 increased the amplitude of the P20 and N40 ERP components at the cortex (Halene and Siegel, 2008). Like rolipram, this PDE4-I was also able to reverse the amphetamine-induced decrease in amplitudes of these ERP components. These data support the notion that PDE4-Is may be relevant for disturbances of sensory processing as observed in schizophrenia.

3.2.3 PDE5 inhibitors

Reneerkens and colleagues (Reneerkens et al., 2013b) demonstrated that the cGMP-specific PDE5-I vardenafil did not affect sensory gating (double-click paradigm) in the striatum, dorsal hippocampus and vertex of rats. Another study by the same group (Reneerkens, Sambeth, Ramaekers, Steinbusch, Blokland, and Prickaerts, 2013a) showed that the cGMP-specific PDE5-I vardenafil had no effect on auditory sensory gating (similar paradigm was used as in rats) in the vertex of humans. These data indicate that PDE5 is not involved in the early stages of auditory information processing.

3.2.4 PDE9 inhibitors

Recordings of field potentials from the hippocampal CA3 region of anesthetized rats were used to monitor auditory evoked potentials (AEPs) that demonstrated auditory gating. Rats were anesthetized with chloral hydrate. Anesthetized rats were placed in a stereotaxic frame and amphetamine was administered to disrupt sensory gating. Acute administration of the cGMP/cAMP-specific PDE9A-I PF-4447943 or PDE9A-I PF-4449613 reversed the amphetamine-induced deficit in auditory gating in anesthetized rats (Kleiman et al., 2012). Although this study was conducted in anesthetized animals, these data suggest that PDE9 could be regarded as a relevant target for improving auditory processing deficits in schizophrenia.

3.2.5 PDE 10 inhibitors

In 2008, field potentials were recorded from the CA3 region of the left hippocampus of Sprague-Dawley rats after administration of the cGMP/cAMP-specific PDE10A-I TP-10. TP-10 significantly reversed the gating deficit induced by amphetamine pre-treatment (Schmidt et al., 2008). Furthermore, Reneerkens and colleagues (Reneerkens et al., 2013b) showed that the PDE10-I PQ-10 did not affect sensory gating in rats as measured in the vertex and unilaterally in striatum and dorsal

hippocampus. However, PQ-10 increased the N1 peak after presentation of S1 as compared to vehicle treatment at the hippocampus. PDE10 inhibition may thus affect auditory processing in general but not sensory gating (see Table 4).

Inspection of the sensory gating data reveals a remarkable observation: PDE-Is exerting an effect in deficit models only. Experiments in healthy rats or humans show no effects of any type of PDE-I (see Table 4). Standard deficit model in sensory gating research is the amphetamine-induced deficit model. Amphetamine is known to enhance, among others, the levels of catecholamines in the brain. Catecholamines are thought to play an important role in sensory gating. Studies with unimpaired participants showed that decreasing levels of catecholamines decrease sensory gating (Braff and Huey, 1988). Studies with animals showed the same effects of catecholamines on sensory gating, both at the behavioral level and the neuronal level (Swerdlow et al., 1986). Other deficit models are the MK-801-induced, apomorphine- and quinpirole-induced deficit models.

3.3 PDE inhibitors and sensorimotor gating

As mentioned earlier, sensorimotor gating is measured via PPI. PPI is a neurological phenomenon in which a weaker prestimulus (prepulse) inhibits the reaction of the animal to a subsequent strong startling stimulus (pulse). The stimuli are usually acoustic, but tactile stimuli (e.g. via air puffs onto the skin) and light stimuli are also used. The reduction of the amplitude of the startle reflects the ability of the nervous system to temporarily adapt to a strong sensory stimulus when a preceding weaker signal is given to warn the organism. PPI is detected in numerous species ranging from mice to humans.

Although the extent of the adaptation affects numerous systems, the most comfortable to measure are the muscular reactions, which are normally diminished as a result of the nervous inhibition. Effects of PDE-Is on sensorimotor gating are most likely induced via enhanced signaling in the startle response circuitry (i.e. cochlear nuclei, caudal pontine reticular nucleus (PnC) and motor neurons)(Koch, 1999). The brain mechanisms underlying the mediation of PPI are still not fully understood, though, the attenuating effect on the ASR of acoustic prepulses probably affects the primary ASR pathway at the level of the PnC (Carlson and Willott, 1998; Lingenhohl and Friauf, 1994; Willott et al., 1994; Wu et al., 1988) most likely by activation of an inhibitory cholinergic (muscarinic) projection from the pedunculopontine tegmental nucleus (PPTg) to the PnC (Koch et al., 1993; Swerdlow and Geyer, 1993). Lesions of the inferior colliculi disrupt PPI by auditory prepulses (Leitner and Cohen, 1985; Li et al., 1998) suggesting that the ascending auditory pathway activates the PPI circuit at the level of the midbrain.

Table 5. Overview studies sensorimotor gating and PDE inhibition.

Task	Model (species)	Treatment	Results	Reference
Prepulse inhibition	Unknown	PDE1-I: name unknown	Compound did not alter prepulse inhibition	Zhang et al., 2010
Prepulse inhibition	DARPP-32 knockout and wildtype (mice)	PDE4-I: Rolipram (3.0 and 10.0 mg/kg, i.p.)	Both doses increased prepulse inhibition in wildtype mice but failed to do so in DARPP-32 knockout mice	Kuroiwa et al., 2012
Prepulse inhibition	Unimpaired (rats)	PDE4-I: Rolipram (0.05 – 10.0 mg/kg, i.p.)	Rolipram produced a rapid and dose-related increase in the amplitude of the acoustic startle response.	Kehne, Boulis and Davis, 1991
Prepulse inhibition	Impaired (C57BL/6J mice) and amphetamine-induced deficit model (10.0 mg/kg, i.p.)(mice)	PDE4-I: Rolipram (0.1, 0.66, 1.0 and 10.0 mg/kg, i.p.)	0.66, 1.0 and 10.0 mg/kg rolipram increased PPI and 0.66 mg/kg rolipram blocked disruptive effects of amphetamine	Kanes et al., 2007
Prepulse inhibition	Two independently derived ENU-induced mutations in Exon 2 of *Disc1* (mice)	PDE4-I: Rolipram (0.5 mg/kg, i.p.)	Rolipram increased the PPI of 100P/100P and 100P/+ mice to the level of +/+ mice but did not reverse the milder PPI deficit of 31L/31L mice	Clapcote et al., 2007
Prepulse inhibition	Amphetamine-induced deficit model (5.0 mg/kg, n.p.)(mice)	PDE4-I: RO-20-1724 (0.25, 2.5, and 4.0 mg/kg, s.c.)	No effects of any dose on deficit in prepulse inhibition	Halene and Siegel, 2008
Prepulse inhibition	PDE4B knockout (mice)	No treatment	Knockout mice showed a reduction in prepulse inhibition	Siuciak et al., 2008
Prepulse inhibition	Amphetamine-induced deficit model (5 mg/kg, i.p.) and MK-801-	PDE7-I: VP1.15 (3.0 mg/kg, i.p.)	VP1.15 reversed only amphetamine-induced deficit	Lipina et al., 2013

	induced deficit model (0.3 mg/kg, i.p.)(mice)		(not MK-801-induced deficit)	
Prepulse inhibition	Impaired (C57BL/6J mice) and MK-801-induced deficit model (0.178 mg/kg, s.c.)(mice)	PDE9-I: PF-4447943 (1.0, 3.2, 10.0 and 32.0 mg/kg, s.c.)	No effect in both models	Kleiman et al., 2012
Prepulse inhibition	Impaired (C57BL/6J mice) and MK-801-induced deficit model (0.178 mg/kg, s.c.)(CD-1 mice)	PDE10-I: TP-10 (0.32, 1.0, 3.2 and 10 mg/kg, s.c.) and TP-10 (1.0, 3.2 and 10 mg/kg, s.c.)	No effects of any dose in both models	Schmidt et al., 2008
Prepulse inhibition	MK-801-induced deficit model (0.085 mg/kg, s.c.)(rat) and impaired (C57BL/6J mice)	PDE10-I: Papaverine (Rat) (3.0, 10.0 and 30.0 mg/kg, i.p.) and MP-10 (1.0, 3.0 and 10.0 mg/kg, i.p.) (mice) (1.0, 4.5, 10.0, 30.0 and 54.0 mg/kg, i.p.) and MP-10 (1.0, 3.0, 10.0, 30.0 and 54.0 mg/kg, i.p.)	Rat: 10.0 and 30.0 mg/kg papaverine and 3.0 and 10.0 mg/kg MP-10 reversed deficit in prepulse inhibition Mice: only 54.0 mg/kg papaverine and 54.0 mg/kg MP-10 showed greater prepulse inhibition	Grauer et al., 2009
Prepulse inhibition	Apomorphine-induced deficit model (0.1 and 0.5 mg/kg, s.c.) and amphetamine-induced deficit model (4 mg/kg, s.c.)(rats)	PDE10-I: Papaverine (3.0, 10.0 and 30.0 mg/kg, i.p.)	Papaverine failed to reverse apomorphine- and amphetamine-induced PPI deficits at all doses, strains, pretreatment times, and prepulse intervals	Weber et al., 2009
Prepulse inhibition	Apomorphine-induced deficit model (0.5 mg/kg, s.c.) and quinpirole-induced deficit model (0.5 mg/kg, s.c.)(rats)	PDE10-I: TP-10 (0.32, 1.0, 3.2 and 10.0 mg/kg, s.c.)	Only 3.2 mg/kg TP-10 combined with SCH23390 (0.005 mg/kg, s.c.) increased PPI in apomorphine-induced deficit model	Gresack et al., 2013
Acoustic startle response	Unimpaired (larval zebrafish)	PDE4-I: Rolipram (3.0, 10.0 and	All doses increased the	Best et al., 2008

		30.0 μM)	acoustic startle response	

i.p. = intra peritoneal, s.c. = subcutaneous, n.p. = not provided

3.3.1 PDE1 inhibitors

A novel and highly selective cGMP/cAMP-specific PDE1B-I has been developed by Intra-Cellular Therapies Inc (Zhang, 2010). Although the drug showed some anti-cataleptic potential in preclinical studies showed, this compound did not alter PPI or startle magnitude. It was suggested that this drug may work via DA D1 receptors, but at present no effects have been reported on sensorimotor gating.

3.3.2 PDE4 inhibitors

Systemic administration of the cAMP-specific PDE4-I rolipram produced a rapid and dose-related increase in the amplitude of the ASR in rats. The (-) isomer was more potent than the (+) isomer in enhancing startle amplitude. Rolipram increased startle responses that were elicited by brief electrical stimulation of the ventral cochlear nucleus or nucleus reticularis pontis caudalis, two brainstem relay nuclei of the startle neural circuit (Kehne et al., 1991). Rolipram also significantly increased PPI at doses that did not alter the acoustic startle response (lowest effective dose 0.66 mg/kg). In addition, rolipram blocked the disruptive effects of amphetamine on PPI (Kanes et al., 2007). Furthermore, rolipram increased the PPI of homozygous and heterozygous 100P mice (a mouse model for schizophrenia) to the level of wildtype mice but did not reverse the milder PPI deficit of heterozygous 31L mice (a mouse model for depression) (Clapcote et al., 2007). Additionally, rolipram did not affect startle responses to acoustic pulses at 120 dB in DARPP-32 knockout mice. DARPP-32 knockout mice naturally show a higher level of PPI compared to control mice. Rolipram did, however, decrease PPI in the control mice (Of note, DA D1 receptors signal through the cAMP/PKA/DARP-32 second messenger cascade, which is modulated by PDE4)(Kuroiwa et al., 2012). The study by Halene and Siegel (Halene and Siegel, 2008) used another PDE4-I: RO-20-1724. This compound did not significantly increase PPI in trials at any prepulse intensity for any tested dose in an amphetamine-induced deficit model. Further evidence is provided by PDE4B knockout mice that showed a significant reduction in PPI compared to wildtype mice (Siuciak et al., 2008). Finally, Best and colleagues (2008) demonstrated that zebrafish larvae (7 days post fertilization) exhibit iterative reduction in a startle response to a series of acoustic stimuli. Administration of rolipram increased the acoustic startle response in the zebrafish. Different models and drugs have been used to examine the effects of PDE4 inhibition on sensorimotor gating. In summary, these studies do not support a clear role of PDE4-I in sensorimotor gating paradigms (e.g., increase after rolipram, decrease in PDE4B knockout mice). The discrepancy in the effects between studies could be explained by the

different test models that were used. Nevertheless, it seems that PDE4 is involved in sensorimotor gating processes.

3.3.3 PDE7 inhibitors

The dual inhibitor of PDE7 and GSK-3, named VP 1.15, facilitated PPI and reversed the amphetamine-induced PPI deficits. In contrast, VP 1.15 did not reverse the MK-801-induced PPI deficits (Lipina et al., 2013). This single study suggest that this PDE7-I may be gating processes but further studies need to show the specificity of these effects with respect to the underlying mechanism. Thus, it needs to be studied to what extend is this related to PDE7 or GSK-3, and how does this relates to a dopamine and glutamate.

3.3.4 PDE9 inhibitors

Administration of the cGMP-specific PDE9-I PF-4447943 had no effect on PPI in the poor-gating C57BL/6J mice. However, the drug interacted with a sub threshold dose of risperidone (0.32 mg/kg) to significantly increase PPI at multiple doses. PF-4447943, however, was without effect on the disruption of PPI produced by the NMDA receptor antagonist MK-801 (Kleiman et al., 2012). These effects suggest a synergism between the effects of PF-4447943 and dopamine/serotonin, but not glutamate.

3.3.5 PDE 10 inhibitors

The cGMP/cAMP-specific PDE10A-I TP-10 was tested in two models by Schmidt and colleagues (Schmidt et al., 2008). In the first model (C57BL/6J mice), TP-10 had no effect at any dose at any of the prepulse intensities, nor did the compound reduce startle amplitude. In a second model, CD-1 mice were administered MK-801 before assessment of PPI. MK-801 significantly reduced PPI. Coadministration of TP-10 had no effect on the MK- 801-induced deficit. Another study tested TP-10 in different PPI experiments in combination with other dopaminergic drugs (Gresack et al., 2013). In line with the previous study, TP-10 had no significant effect on PPI alone, nor did it prevent the apomorphine-induced disruption of PPI. However, in a second experiment TP-10 treatment reversed the PPI impairment induced by quinpirole. In addition, the apomorphine-induced decrease in PPI was depended on the combined pretreatment of TP-10 with SCH23390. These effects cannot be attributed to TP-10 alone, as the TP-10/SCH23390 combination treated group also exhibited significantly higher PPI than the TP-10 alone group. Taken together these data suggest that PDE10-I by itself may not be very active in gating processes, but that there is a potential interaction with dopaminergic mechanisms.

Two other cGMP/cAMP-specific PDE10A-Is, papaverine and MP-10, both blocked NMDA antagonist-induced deficits in PPI of acoustic startle response in rats, while improving baseline sensory gating in mice (Grauer et al., 2009). Furthermore, Weber and colleagues (Weber et al., 2009) showed that the cAMP/cGMP-selective PDE10A-I papaverine failed to reverse apomorphine- and amphetamine-induced PPI deficits at all doses, strains, pretreatment times, and prepulse intervals (see Table 5). In addition, a study using PDE10A knockout mice failed to report any effects on PPI (Piccart et al., 2014).

3.4 Conclusion PDE inhibitors and gating

Compared to the other two cognitive domains being discussed in this review, the cognitive concept of gating received most attention as a possible target for PDE-I treatment, mainly due to disruption of gating in several patients suffering from clinical disorders including schizophrenia and AD (e.g. Adler et al., 1982; Ally et al., 2006; Javitt, 2009; Jessen et al., 2001). All but one study were conducted in animals. Because it has been shown that the ERPs of humans and rats show a significant amount of similarities (e.g. Sambeth et al., 2003), we expect that the effects of drugs, like PDE-Is, on ERPs are translational between humans and animals (Maxwell et al., 2004).

The overall picture regarding PDE inhibition and gating is most promising for PDE4-Is, PDE10-Is appear interesting, while PDE1-Is, PDE2-Is and PDE5-Is may be least promising, although it has to be noted that research is scarce with the latter types of PDE-Is. Results are either negative (PDE1-Is and PDE2-Is) or mixed as in the case of PDE9-Is (Kleiman et al., 2012). PDE5-Is were tested in two studies, of which one study involving human participants. No effects are found at all for the potent inhibitor vardenafil, indicating that PDE5 is probably not involved in auditory gating in both rodents and humans.

Abundant evidence is provided for a role of the PDE4 subfamily in gating, although these data are almost exclusively based on the PDE4-I rolipram. In rats, mice and zebrafish positive effects are found of rolipram on gating. All studies, though, use different (deficit) models. ASRs were enhanced at a normal level, in an amphetamine-induced deficit model and in genetically modified animals. The latter model was used to investigate whether DARPP-32 is involved in the machinery by which rolipram might improve sensorimotor gating (Kuroiwa et al., 2012). DARPP-32 knockout mice showed a significantly higher level of PPI compared to control mice. Siuciak *et al.* (Siuciak et al., 2008) found that PDE4B knockout mice showed a significant reduction in PPI compared to wildtype mice indicating a role for PDE4B in auditory gating. Though, negative results were also found. Rolipram increased the PPI of 100P mice to the level of wild type mice but did not reverse the milder PPI deficit of 31L mice (Clapcote et al., 2007). The only true negative results came from a study using the PDE4-I

RO-20-1724. RO-20-1724 failed to reverse the amphetamine-induced deficit in PPI (Halene and Siegel, 2008). Though, RO-20-1724 did reverse the effects of d-amphetamine on the N40 component. The failure to reverse the amphetamine-induced deficit in PPI can most likely be attributed to the potency of the compound since rolipram did reverse PPI in an amphetamine-induced deficit model. In summary, it appears that PDE4-Is are most promising to improve sensory gating as well as sensorimotor gating.

PDE10-Is were investigated in five studies involving rats and mice. Schmidt *et al.* (Schmidt et al., 2008) showed that the PDE10-I TP-10 reversed sensory gating deficits in the hippocampus induced by d-amphetamine but found no effects in an MK-801 deficit model of PPI. Findings from Gresack et al. (Gresack et al., 2013) are in line with the previous study showing, again, no effects of TP-10 on PPI or only an effect when combined with a D1 antagonist. Thirdly, Piccart et al. (2014) also found no effects on PPI in PDE10A -/- deficient mice. In contrast, Grauer et al. (Grauer et al., 2009) significantly reduced the MK-801 deficit in PPI with both papaverine and MP-10. Though, the former study was performed in anesthetized rats while the latter used freely moving rats in Plexiglas cylinders as testing chambers. Additionally, the routes of administration differed between studies (i.p. versus s.c.), which might be of influence. In contrast to Grauer et al., a study by Weber et al. (2009) found no effects of papaverine on PPI in both an apomorphine- and amphetamine-induced deficit model. In conclusion, no definitive judgments can be made about PDE10A-Is as results within both sensory gating and sensorimotor gating are contradictory. The only tentative conclusion may be that the PDE10A-I TP-10 does not affect PPI per se since this is the only consistent finding.

PDE-Is improving in particular sensory gating act by targeting PDEs expressed in the neurons of the thalamic “gate”, in the frontal inhibitory output neurons or in the neurons that locally release inhibitory neurotransmitter. In addition, many modulatory neuronal connections could also play a role and PDEs in these modulatory brain areas and neurons can thus exert an effect on sensory gating. PDE4-Is, PDE9-Is and PDE10-Is all showed positive effects in animals and they are expressed relatively high in the thalamus. Yet, how expression patterns of PDEs look like in the other brain areas is unknown and making predictions about how all the modulatory mechanisms might lead to an overall effect is almost impossible without further fundamental knowledge of PDE expression patterns.

For sensorimotor gating the story seems even more complex. Further detailed knowledge about the expression patterns in the startle response circuitry is essential. Since the attenuating effects on the ASR of acoustic prepulses are most likely induced at the level of the PnC, this might be an

appropriate starting point for future studies, though other areas of the circuitry may be equally suited. Since detailed knowledge on expression patterns is currently lacking, no predictions from a neurobiological standpoint can yet be made. Derived from experimental results, only PDE4-Is seem to provide constant positive outcomes and can currently be viewed as the most promising PDE-I subtype for enhancement in the cognitive domain of sensorimotor gating.

4. Drug-induced hyper locomotion

4.1 (Hyper) locomotion and biology

Locomotion is a complex behavior that results from neural activation in the cerebral cortex, basal ganglia, cerebellum, brainstem locomotor regions and central pattern generators in the spinal cord. While different neurochemical mechanisms are involved in this neural activation, DA appears to play an essential role (Le Moal and Simon, 1991; Salamone et al., 2005). Pharmacological blockade of DA transmission inhibits spontaneous locomotion and greatly attenuates behavioral activation, independent of its triggering mechanisms (Kiyatkin, 2002). On the other hand, various environmental challenges that induce locomotor activation also increase DA transmission and vice versa as hyperlocomotion and stereotypy also occur during pharmacological increase in DA transmission induced by both direct (i.e. bromocriptine, apomorphine) and indirect (i.e. amphetamine, methamphetamine, cocaine) DA agonists.

Interestingly, all substances inducing hyperlocomotion also induce increased motor impulsivity (Bickel et al., 2012; Dalley et al., 2011; de Wit, 2009). Motor impulsivity in general, or response inhibition in particular, i.e. the ability to inhibit a prepotent response, is assumed to find its neurobiological origin in frontostriatal circuitry, as does (hyper)locomotion (Albrecht et al., 2014; Dalley et al., 2008; Feil et al., 2010; Trifilieff and Martinez, 2014). Disorders involving deficits in motor impulsivity may therefore benefit from PDE-Is in a manner similar to hyperlocomotion. Disturbances in motor impulsivity are characteristic of many neurodegenerative disorders such as AD and Parkinson's disease (PD), psychiatric illness including schizophrenia, depression and obsessive compulsive disorder (OCD), as well as pervasive developmental disorders such as ADHD (Chudasama and Robbins, 2006) indicating a high medical need for these disorders.

In the current section we will first discuss the relation between PDE-Is and drug-induced hyperlocomotion. In the next section we will further illustrate response inhibition and motor impulsivity.

4.2 PDE inhibitors and drug-induced hyperlocomotion

Table 6. Overview studies hyperlocomotion and PDE inhibition.

Task	Model (species)	Treatment	Results	Reference
Open field test	Ethanol-induced hyperlocomotion (4 times 5 g/kg, i.p.)(mice)	PDE1: Vinpocetine (10.0 and 20.0 mg/kg, i.p.)	Vinpocetine 20.0 mg/kg ameliorated hyperactivity	Nunes et al., 2011
Open field test	Methamphetamine-induced hyperlocomotion (5 days 4.0 mg/kg followed by 2.0 mg/kg challenge after 1-week withdrawal, i.p.)(rats)	PDE4: Rolipram (5 days 4 mg/kg, i.p.)	Rolipram prevented methamphetamine-induced behavioral sensitization	Iyo et al., 1996
Open field test	Methamphetamine-induced hyperlocomotion (1.0 and 2.0 mg/kg, s.c.), PCP-induced hyperlocomotion (2.5 and 5.0 mg/kg, s.c.) and morphine-induced hyperlocomotion (20.0 mg/kg, s.c.)(mice)	PDE4: Rolipram (1.0, 3.2 and 10.0 mg/kg, i.p.)	Rolipram suppressed methamphetamine-induced hyperlocomotion at all doses, morphine-induced hyperlocomotion at 3.2 and 10.0 mg/kg and had no effect on PCP-induced hyperlocomotion	Mori et al., 2000
Open field test	Amphetamine-induced hyperlocomotion (0.5 mg/kg, s.c.)(rats)	PDE10: MP-10 (0.08 – 20.0 mg/kg, p.o.)	MP-10 5.0 and 20.0 mg/kg inhibited amphetamine-induced locomotor hyperactivity	Sotty et al., 2009
Open field test	PCP-induced hyperlocomotion (dose n.p., i.p)(mice)	PDE10: Compound 56 (20 mg/kg, i.p.)	Compound 56 reduced hyperactivity	Gage et al., 2011
Open field test	MK-801-induced hyperlocomotion (0.1 mg/kg, i.p.)(rats)	PDE10: Compound 66 (0.1 mg/kg, p.o.)	Compound 66 reversed MK-801-induced hyperactivity	Malamas et al., 2012
Open field test	PCP-induced hyperlocomotion (no further information provided)(mice)	PDE10: Compound 55 (5 mg/kg, p.o.) and compound 61 (4 mg/kg, p.o.)	Both compounds reduced PCP-induced hyperlocomotion	Cutshall et al., 2012
Open field test	MK-801-induced hyperlocomotion (no further information provided)(rats)	PDE10: THPP-1 (1.0, 3.0 and 10.0 mg/kg, p.o.)	THPP-1 3.0 and 10.0 mg/kg displayed full attenuation of MK-801-induced	Raheem et al., 2012

			hyperlocomotion	
Activity monitoring in home cage	Cocaine-induced hyperactivity (15 mg/kg, i.p.)(rats)	Non-specific: Isobutylmethylxanthine (IBMX) (0.1, 1.0 and 2.0 µg/µL, i.c.v.)	IBMX did not attenuate increase in activity	Schroeder et al., 2012
Locomotor activity assays	Novelty-induced locomotion, amphetamine-induced hyperlocomotion (1.25 mg/kg, s.c.), scopolamine-induced hyperlocomotion (0.31 mg/kg, i.v.)and PCP-induced hyperlocomotion (1.25 mg/kg, i.v.) (rats)	PDE10: JNJ-42314415, TP-10, PQ-10 and MP-10 (all compounds tested in many dosages, s.c.)	JNJ-42314415 (1.54, 6.2, 1.18, 1.54 mg/kg were minimal effective dosages in the four mentioned deficit models in the left column, respectively), PQ-10 (4.7, 9.4, 4.8, 4.7 mg/kg, respectively), TP-10 (0.77, 4.1, 0.78, 0.51 mg/kg, respectively) and MP-10 (0.44, 3.1, 0.44, 0.44 mg/kg, respectively)	Megens et al., 2014

i.m. = intra muscular; i.p. = intra peritoneal; i.v. = intra venous; i.c.v. = intra cerebrovascular; s.c. = subcutaneous; p.o. = per os

4.2.1 PDE 1 inhibitors

Early alcohol exposure significantly increased locomotor activity in the open field test, which has been linked to attention-deficit/hyperactivity disorder that can be observed in the fetal alcohol spectrum disorder (Nunes et al., 2011). In these animals a reduced cAMP level can be found in the hippocampus. The acute treatment of ethanol-exposed animals with the cAMP/cGMP-specific PDE1-I vinpocetine restored both their hyperlocomotor activity and cAMP levels to control levels (Nunes et al., 2011). These data suggest that hippocampal cAMP may underlie the hyperactivity and that this could be restored with a PDE1-I.

4.2.2 PDE 4 inhibitors

The administration of methamphetamine once a day for 5 days significantly enhanced hyperlocomotion and rearing induced by a 2-mg/kg methamphetamine challenge after a 1-week withdrawal period, compared with controls or coadministration with the cAMP-specific PDE4 inhibitor rolipram (Iyo et al., 1996). Furthermore, the effects of rolipram on the hyperlocomotion induced by several abused drugs (methamphetamine, morphine and PCP) and a DA D1-receptor agonist (SKF81297) in mice were investigated. Methamphetamine, morphine, PCP and SKF81297 each induced dose-dependent hyperlocomotion. Rolipram suppressed methamphetamine and

morphine-induced hyperlocomotion, but not PCP-induced hyperlocomotion. These results suggest that cAMP in the brain is involved in methamphetamine- and morphine-induced hyperlocomotion, while the underlying mechanism(s) of PCP-induced hyperlocomotion may be different from those of methamphetamine- and morphine-induced hyperlocomotion. It is well known that methamphetamine- and morphine-induced hyperlocomotion are mediated by the dopaminergic system and that interaction between postsynaptic D1- and D2-receptors may play an important role in the expression of various DA-mediated behaviors. SKF81297-induced hyperlocomotion was significantly but not completely suppressed by the highest dose of rolipram. Therefore, it is unlikely that postsynaptic D1-receptor-mediated functions are involved in the suppressive effects of rolipram on methamphetamine- and morphine-induced hyperlocomotion. These results suggest that rolipram may inhibit methamphetamine- and morphine-induced hyperlocomotion via increased cAMP levels at D2-receptors (Mori et al., 2000).

4.2.3 PDE10 inhibitors

The cGMP/cAMP-specific PDE10A inhibitor MP-10 blocked amphetamine-induced hyperlocomotion as well as amphetamine-induced DA efflux in the NAc in a dose-dependent manner (Sotty et al., 2009). Furthermore, the hydrazone-based cGMP/cAMP-specific PDE10A inhibitor compound 56 reduced PCP-induced hyperlocomotion in the search for novel treatment options in schizophrenia (Gage et al., 2011). Another hydrazone-based drug, called compound 55, also reduced PCP-induced hyperlocomotion in a separate study (Cutshall et al., 2012). The triazine-based PDE10A inhibitor compound 66 reversed MK-801 induced hyperlocomotion (Malamas et al., 2012). A fifth tetrahydropyridopyrimidine-based PDE10A inhibitor, THPP-1, displayed full attenuation of MK-801-induced hyperlocomotion (Raheem et al., 2012). Lastly, the PDE10A-I JNJ-42314415 was tested for its effects on locomotion induced by novelty (normal locomotion), amphetamine, scopolamine and PCP (hyper-locomotion) (Megens et al., 2014). The compound was compared to other known PDE10A-Is (MP-10, PQ-10, TP-10) and DA D2 receptor blockers. All four PDE10A-Is used in the study reduced (hyper)locomotion induced by either novelty, amphetamine, scopolamine or PCP.

4.2.4 Non-selective PDE inhibitors

The non-selective PDE-I isobutylmethylxanthine (IBMX) did not affect the acute hyperlocomotor response to cocaine, but when coadministered with cocaine for 7 consecutive days, attenuated development of behavioral sensitization (Schroeder et al., 2012)(see Table 6).

4.3 Conclusion PDE inhibitors and drug-induced hyperlocomotion

It is well known that psychostimulants, i.e. amphetamine, methamphetamine, cocaine, as well as other drugs of abuse, i.e. alcohol, heroin, induce hyperlocomotion in rodents, which is believed to be mediated by dopaminergic, and especially the mesolimbic dopaminergic, system (Koob and Bloom, 1988; Nestler et al., 1996). There are two superfamilies of dopaminergic receptors, designated D1-like and D2-like receptors (Sibley et al., 1993). D1-receptor agonists and D2-receptor agonists work synergistically in the expression of hyperlocomotion, stereotypies and rewarding effects. This may be indicative that the interaction between D1 and D2 receptors plays an important role in DA-mediated behaviors (Mori et al., 2000).

Administration of either drug discussed in the drug-induced hyperlocomotion section results in an increase in DA release in the mesolimbic and nigrostriatal DA pathways (Lobo and Nestler, 2011). Drug-induced increases in extracellular DA result in an up or down regulation of cAMP signaling in frontostriatal circuitry, depending on binding to D1 or D2 receptors in the direct or indirect pathway, respectively (Nishi et al., 2008; Nishi et al., 2011). Drugs of abuse thereby facilitate activity in the direct pathway and inhibit activity in the indirect pathway, both inducing excitation of the cortex and thus (extra) facilitation of movement (hyperlocomotion) (Lobo and Nestler, 2011). PDE10, to a less extent, PDE4 and, to an even lesser extent, PDE1, is expressed in both direct and indirect pathways with a preference for the latter (Nishi et al., 2008). PDE-Is of these subtypes therefore function mainly as DA D2 receptor antagonists (causing activation of the indirect pathway leading to less activation of the cortex and thus decreased movement) and function to a lesser extent as DA D1 receptor agonists (causing enhanced activation of the direct pathway leading to more activation of the cortex and increased movement). Because of the dominant expression of these PDEs in the indirect pathway, the activation of the indirect pathway offsets the activation of the direct pathway, inducing a net effect of less activation of the cortex, less activation of movement and thus counteraction of hyperlocomotion.

Though, cells are likely to be more sensitive to PDE inhibition at higher AC activity as the cAMP hydrolysis capacity is strained. Therefore, we propose that, for instance, PDE10A inhibition would exert a stronger effect on D1-expressing versus D2-expressing neurons in condition of increased dopaminergic transmission, since in this scenario, cAMP hydrolysis capacity is strained in D1-expressing neurons. The effect on D2-expressing neurons would predominate in basal conditions, in line with findings of a study conducted in neostriatal slices (Nishi et al., 2008). In the end, this may sound like a competition of what gets there first. If effects of drugs of abuse are already present and of sufficient strength to strain cAMP hydrolysis, subsequent PDE inhibition will mainly affect cAMP in the direct pathway. Alternatively, if PDE-Is have started their effect before cAMP hydrolysis is

strained via elevated DA levels through drugs of abuse, PDE inhibition will mainly affect cAMP in the indirect pathway. Though, PDE inhibition will also affect the release of DA presynaptically, either from substantia nigra (SN) or VTA. So even when DA is already being released after administration of drugs of abuse, the presynaptic effect of PDE inhibition can still shift the main postsynaptic effect from indirect to direct pathway. This could lead to an aggravation of drug-induced hyperlocomotion or shift the main postsynaptic effect from direct to indirect pathway leading to a reduction of drug-induced hyperlocomotion.

5. Miscellaneous

Three studies could be added to the previous section, though, the output parameters of these tasks do not provide a clear indication of response inhibition, thus allowing ambiguity (see Table 7).

Table 7. Overview studies response inhibition, attention and PDE inhibition.

Task	Model (species)	Treatment	Results	Reference
Object retrieval task	Unimpaired adult (cynomolgus macaques)	PDE4: Rolipram (0.003-0.03 mg/kg, i.m.) or sildenafil (0.3-3 mg/kg, i.m.)	Rolipram (0.01 mg/kg) and sildenafil (1 mg/kg) increased correct first reaches	Rutten et al., 2008
Object retrieval task	Unimpaired adult (cynomolgus macaques)	PDE4D: Rolipram (0.003-0.03 mg/kg, i.m.) and D159687 (0.05-5 mg/kg, p.o.) and D159797 (0.05-1 mg/kg, p.o.)(negative allosteric modulators)	Rolipram (0.03), D159687 (0.05-5 mg/kg) and D159797 (0.5 and 1 mg/kg) increased correct first reaches	Sutcliffe et al. 2014
Object retrieval detour task	Ketamine-induced deficit model (individual dosing, i.m.)(rhesus monkeys)	PDE10: THPP-1 (3.0 and 10.0 mg/kg, day 1-4 p.o. and day 5 oral)	THPP-1 10.0 mg/kg attenuated impairment in correct first reaches	Smith et al., 2013
Stroop task	Unimpaired adults (human)	PDE5: Vardenafil (10.0 and 20.0 mg, oral)	No effects on behavioral measures, main treatment effect for P300 on ERPs	Reneerkens et al., 2013

i.m. = intra muscular; p.o. = per os

5.1 PDE 4 inhibitors

In 2008 Rutten et al. (Rutten et al., 2008) showed that the cAMP-specific PDE4-I rolipram enhanced the percentage of correct first reaches (indicative of response inhibition and attention) in the object retrieval detour task (ORDT) in adult cynomolgus macaques. This improved performance was also found with a PDE4D negative allosteric modulator (Sutcliffe et al., 2014). Although these data strongly support a role of PDE4 in response inhibition, it should be noted that this task not only involves response inhibition but also involves cognitive functions.

5.2 PDE 5 inhibitors

In the same study by Rutten and colleagues (Rutten et al., 2008) the cGMP-specific PDE5-I sildenafil either enhanced the percentage of correct first reaches in the ORDT in adult cynomolgus macaques. So far, only one study involving human participants and including an impulsivity measure is known to us. (Reneerkens et al., 2013a) found no effect of the cGMP-specific PDE5 inhibitor vardenafil in the Stroop task, which is well known for its ability to induce interference, and assesses response inhibition and focused attention. These data do not suggest a role for PDE5 in response inhibition.

5.3 PDE 10 inhibitors

The cGMP/cAMP-specific PDE10A inhibitor THPP-1 has also been tested in the ORDT. However, the effects were tested in a ketamine deficit model, as a model of schizophrenia. THPP attenuated a ketamine-induced deficit in the ORDT in rhesus monkeys (Smith et al., 2013). The effects of THPP-1 alone were not tested in this study. These data may suggest a role for PDE10 in response inhibition, but may be limited to a ketamine-induced deficit.

5.4 Conclusion miscellaneous

The three studies discussed above are miscellaneous in the sense that the task used allows ambiguity as to what it actually measures. The main output parameter of the tasks measures both attention and response inhibition. With regard to the former subject they could be added to the attention section, with regard to the latter subject a new field of PDE research is addressed, though ambiguity allows neither. Response inhibition is part of the impulsivity taxonomy and can be assigned to the subdivision ‘motor impulsivity’ (Evenden, 1999).

As discussed previously, both PDE4 and PDE10A inhibition are expected to increase cAMP in both D1- and D2-expressing striatal MSNs and thus in effect, potentiate the effect of D1 receptor activation and counteract the effect of D2 receptor activation as a result of their inverse coupling to AC. In this respect, PDE4 and PDE10 inhibition may, by facilitating D1 receptor-dependent excitatory effect and/or counteracting D2 receptor-dependent inhibitory effect of striatal MSNs activity, indirectly

modulate feedback regulation of DA neuron activity thereby providing a reduction in drug-induced hyperlocomotion, with or without additional presynaptic effects (Nishi et al., 2008). Due to a larger expression of PDE10 in MSNs, compared to PDE4 (or PDE1), PDE10-Is are expected to be the most suitable tool for reducing drug-induced hyperlocomotion, which is in line with findings of previously discussed studies (Nishi et al., 2011).

Response inhibition is assumed to find its neurobiological origin in frontostriatal circuitry, as does locomotion (Albrecht et al., 2014; Dalley et al., 2008; Feil et al., 2010; Trifilieff and Martinez, 2014). Disorders involving deficits in motor impulsivity may therefore benefit from PDE-Is in the same way as does hyperlocomotion. This notion is reinforced by the fact that all substances in the drug-induced hyperlocomotion section also induce increased motor impulsivity (Bickel et al., 2012; Dalley et al., 2011; de Wit, 2009).

6. General Discussion

Besides the already known traditional PDE-Is such as sildenafil, more PDE-Is are now entering the stage of clinical research. However, in contrast to the large number of preclinical investigations, the number of clinical studies is limited since the PDE research field is still a relatively young field that really took a start in 1998 when Viagra (sildenafil) was approved by the American Food and Drug Administration (FDA) for treatment of erectile dysfunction. In the present review we focused on PDE-Is as putative drugs for cognition enhancement beyond the memory domain focusing on the cognitive domains of attention, information filtering (sensory and sensorimotor gating) and response inhibition (drug-induced hyperlocomotion).

In summary, although the total number of studies is rather limited, most of the studies into the effects of PDE inhibition on attention, show improved performance on one of the many aspects of attention after PDE-I treatment. Because of the enormous extend of the attention network, stimulating intracellular signaling in a diverse range of brain structures could, theoretically, lead to enhanced performance on a behavioral task measuring one of the many aspects of attention. Several of these aspects of attention were measured by the studies discussed in this review, while investigating different types of PDE-Is. Therefore, only early conclusions can be drawn as to which (sub)type of PDE is the most promising target for enhancing attention performance. Most support for PDE-Is as possible attention enhancers is provided for PDE4 and PDE5 (see Table 8). Both subtypes are highly expressed in areas associated with Posner's attention network, especially PDE4 (Lakics et al., 2010). This may suggest that future studies should focus on these two PDEs using a wide variety of attention tasks to examine which aspects of attention are enhanced. Concluding, PDEs seem to be

a promising target for enhancing attention and PDE4-Is and PDE5-Is seem to be the most promising tools, though subtype specificity should be further investigated.

Gating received more attention as a possible target for PDE-I treatment compared to the other cognitive domains. This is mainly because gating is disrupted in several patients suffering from clinical disorders including schizophrenia and AD (e.g. Adler et al., 1982; Ally et al., 2006; Javitt, 2009; Jessen et al., 2001). In the search for new and improved antipsychotics PDE-Is have been researched elaborately in sensory and sensorimotor gating paradigms. In both paradigms PDE4-Is have been most extensively investigated with rolipram exceeding all other PDE-Is. In conclusion, experiments in both paradigms all indicate PDEs, especially PDE4, to be considered as a promising target for improving gating (see Table 8). Rolipram showed positive results on gating in rats, mice and zebrafish; though no human trials have been conducted so far.

For drug-induced hyperlocomotion both PDE4-Is and PDE10-Is show best results (see Table 8) due to an expected potentiation of the effect of D1 receptor activation and counteraction of the effect of D2 receptor activation as a result of inverse coupling to AC. By facilitating the D1 receptor-dependent excitatory effect and/or counteracting the D2 receptor-dependent inhibitory effect of striatal MSNs, PDE-Is indirectly modulate feedback regulation of DA neuron activity thereby providing a reduction in drug-induced hyperlocomotion (Nishi et al., 2008). In conclusion, PDEs seem to be a promising target for counteracting drug-induced hyperlocomotion. Due to a larger expression of PDE10 in MSNs, compared to PDE4 (or PDE1), PDE10-Is are expected to be the most suitable tool for reducing drug-induced hyperlocomotion.

Another cognitive domain in early information processing, that we have briefly touched upon in this review, is response inhibition (motor impulsivity). Since response inhibition and locomotion are both believed to find their origin in frontostriatal circuitry, disorders involving response inhibition, like ADHD and possibly addiction, might benefit from PDE-I treatment as does (drug-induced hyper)locomotion. Especially, since the drugs used to induce hyperlocomotion, all induce motor impulsivity as well (Bickel et al., 2012; Dalley et al., 2011; de Wit, 2009), likely via frontostriatal circuitry. Furthermore, the OR, measuring response inhibition in animals, also showed positive effects of PDE inhibition. These findings could not be replicated in humans using the Stroop task, although it should be mentioned that the tasks cannot be compared directly. Motor impulsivity might thus be a next promising cognitive domain benefitting from PDE-I treatment.

Table 8. Overview of potential PDE-specific targets for improving cognitive functions: attention, information filtering (sensory and sensorimotor gating) and response inhibition (drug-induced hyperlocomotion).

Cognitive domain	PDE type
Attention	PDE4 and PDE5
Sensory gating	PDE4, PDE9 and PDE10
Sensorimotor gating	PDE4
Response inhibition (motor impulsivity)	PDE4 and PDE10

Though, several issues should be kept in mind when interpreting results thus far regarding all cognitive domains. First, it is important to know the exact localization and the level of expression of specific PDE enzymes in the normal brain. Secondly, it must be taken into account that the constitution of the brain changes with age and brain diseases and that this may affect the distribution and expression of PDEs in the brain. 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 is a field of great interest. Most PDE-Is currently available are selective for one particular PDE family. However, this implies that all PDE enzyme isoforms of that family will be inhibited. In case of PDE4 this implicates about 25 isoforms (Gurney et al., 2011), of which some can be associated with adverse side effects. This relates to a fourth point of consideration. It is clear that more selective PDE-Is are needed to have more specific biological activity without unwanted side effects. An example of this is the recent development of selective PDE4-Is for only one of the four PDE4 gene products. In particular nausea and emesis (vomiting) are linked to PDE4 inhibition. There are now PDE4D-Is which are devoid of emetic effects (Burgin et al., 2010) or have at least greatly reduced emetic effects (Bruno et al., 2011). Development of subtype specific PDE-Is has to be the next step. Increasing the selectivity of PDE-Is poses a major challenge which has to be achieved by influencing compound-enzyme interactions most likely outside the catalytic domain of the PDE enzymes (Gurney et al., 2011).

To summarize, PDEs seem to be a promising target in the field of cognition enhancement even beyond the memory domain. Although these new fields in PDE research are just emerging, clear positive effects have already been found in animals and, like in the memory domain, we are now awaiting translational confirmation by human data. Thus, providing further clinical proof of concept for cognition enhancing effects of PDE-Is and the generation of isoform selective PDE-Is are the final hurdles to overcome in developing safe and efficacious novel PDE-Is for the treatment of cognitive decline (also) beyond the memory domain.

Conflict of Interest

There is no conflict of interest for the work being reported.

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Chapter 3

Phosphodiesterase inhibition and regulation of dopaminergic frontal and striatal functioning: clinical implications

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Abstract

Background: The fronto-striatal circuits are the common neurobiological basis for neuropsychiatric disorders including schizophrenia, Parkinson's disease, Huntington's disease, attention deficit hyperactivity disorder (ADHD), obsessive-compulsive disorder and Tourette's syndrome. Fronto-striatal circuits consist of motor circuits, associative circuits and limbic circuits. All circuits share two common features. Firstly, all fronto-striatal circuits consist of hyper direct, direct and indirect pathways. Secondly, all fronto-striatal circuits are modulated by dopamine. Intracellularly, the effect of dopamine is largely mediated through the cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) signaling cascade with an additional role for the cyclic guanosine monophosphate (cGMP)/ protein kinase G (PKG) pathway, both of which can be regulated by phosphodiesterases (PDEs). PDEs are thus a potential target for pharmacological intervention in neuropsychiatric disorders related to dopaminergic regulation of fronto-striatal circuits.

Methods: Clinical studies of the effects of different phosphodiesterase inhibitors (PDE-Is) on cognition, affect and motor function in relation to the fronto-striatal circuits are reviewed.

Results: Several selective PDE-Is have positive effects on cognition, affect and motor function in relation to the fronto-striatal circuits.

Conclusion: Increased understanding of the subcellular localization and unraveling of the signalosome concept of PDEs including its function and dysfunction in the fronto-striatal circuits will contribute to the design of new specific inhibitors and enhance the potential of PDE-Is as therapeutics in fronto-striatal circuits.

Keywords: fronto-striatal circuits, dopamine, phosphodiesterase, phosphodiesterase inhibitors, cyclic adenosine monophosphate

Introduction

Several neuropsychiatric disorders, including Parkinson's disease, Huntington's disease, attention-deficit hyperactivity disorder (ADHD), Tourette's syndrome, schizophrenia and obsessive-compulsive disorder, share the fronto-striatal circuits, also known as cortico-striatal-thalamic circuits, as their neurobiological basis. The fronto-striatal circuits comprise motor, cognitive and limbic circuits (Alexander et al., 1986; Alexander and Crutcher, 1990; Alexander et al., 1990). These circuits operate in a very complex manner which is extensively described elsewhere (Surmeier et al., 2007; Haber and Rauch, 2010; Gerfen and Surmeier, 2011; Surmeier et al., 2011; Calabresi et al., 2014). Dysfunction of these circuits produces the wide range of motor, cognitive and affective symptoms observed in related neuropsychiatric disorders. One prominent feature of the complex functioning of the fronto-striatal circuits is their modulation by dopamine, both at the level of the frontal cortex as well as the striatum. As a result, dopaminergic receptors are strongly expressed throughout all fronto-striatal circuits (Gerfen and Surmeier, 2011; Nishi et al., 2011; Kuroiwa et al., 2012). Unsurprisingly, dopaminergic medication has been the first-line therapy for several disorders related to dysfunctional fronto-striatal circuits, however efficacy is often moderate at best and accompanied by severe side effects (e.g. ADHD, schizophrenia and Parkinson's disease).

DA originating from substantia nigra pars compacta (SNc) and/or ventral tegmental area (VTA) (nigrostriatal and mesolimbic pathways) binds to both DA type1 (D1) receptors and DA type2 (D2) receptors on medium spiny neurons (MSNs) in the striatum (Gerfen and Surmeier, 2011). D1 receptors are mainly found on MSNs of the direct pathway and D2 receptors are mainly found on MSNs of the indirect pathway where they establish antagonistic interactions with adenosine A_{2a} receptors (Gerfen et al., 1990; Ferre et al., 2011). Additionally, DA released from VTA (mesocortical pathway) also binds to D1 receptors in the frontal cortex (Kuroiwa et al., 2012). D1 receptors activate the $G\alpha_{s/olf}$ family of G proteins to stimulate cyclic adenosine monophosphate (cAMP) production and thereby striatonigral and frontal signaling (Sibley et al., 1993; Beaulieu and Gainetdinov, 2011). In contrast, the D2 receptors couple to the $G\alpha_{i/o}$ family of G proteins and thus induce inhibition of cAMP production, thereby inhibiting striatopallidal signaling which eventually leads to disinhibition of the frontal cortex (see figure 1). Actions of the DA receptors in both pathways can be viewed as synergistically or complementary.

Intracellularly, the effect of DA on striatonigral, striatopallidal and frontal neurons, is largely mediated through the cAMP-activated cascade (Nishi et al., 2008; Nishi and Snyder, 2010; Nishi et al., 2011; Kuroiwa et al., 2012). cAMP is synthesized from adenosine triphosphate (ATP) by adenylyl cyclase (AC) which is activated directly by activated G-protein coupled receptors, or by calmodulin-

dependent protein kinase II (CaMKII) after Ca^{2+} influx. cAMP affects synaptic plasticity through both presynaptic neurotransmitter release and postsynaptic intracellular pathways (see figure 1). The former might be mediated via a presynaptic calcium (Ca^{2+})/calmodulin-dependent protein kinase (CaMK)/cAMP/cAMP-dependent protein kinase (PKA) cascade and elevation of cAMP has been found to result in the synthesis and/or release of several neurotransmitters including two main players in the fronto-striatal circuits: glutamate and DA (Schoffelmeer et al., 1985; Imanishi et al., 1997; Rodriguez-Moreno and Sihra, 2013).

The influence on postsynaptic intracellular pathways occurs through activation of postsynaptic PKA by cAMP produced by AC stimulated by either glutamatergic-induced Ca^{2+} influx or DA signaling-stimulated G_s. PKA exerts several effects related to neuroplasticity and neuroprotection. The fastest postsynaptic response in relation to neuroplasticity mediated by cyclic nucleotides is the activation and insertion of stored receptors by PKA through phosphorylation of GluR1 subunits promoting α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor trafficking into the postsynaptic membrane for potentiation of glutamatergic transmission (Song et al., 2013). In addition to the mobilization and membrane insertion of stored receptors, the process of protein synthesis (e.g. AMPA receptors) further increases neuroplasticity (Carew and Sutton, 2001; Izquierdo et al., 2006).

PKA also phosphorylates cAMP response element- binding protein (CREB) (Mayr and Montminy, 2001) and Dopamine- and cAMP-Regulated PhosphoProtein MR 32 kDa (DARPP-32) (Greengard, 2001; Svenningsson et al., 2004). Phosphorylated CREB (pCREB) is also involved in neuroplasticity (e.g. synthesis of other proteins)(Impey et al., 1996; Lu et al., 1999; Sakamoto et al., 2011) and neuroprotection (e.g. neuronal arborization, synaptogenesis and neurogenesis) (Mantamadiotis et al., 2002; Bruel-Jungerman et al., 2006; Sakamoto et al., 2011). One of the genes transcribed by pCREB is *bdnf* (Scott Bitner, 2012). After release, the protein BDNF binds to the tropomyosin-related kinase B (TrkB) receptor, which is the receptor with the highest affinity for BDNF. BDNF is involved in the proliferation, survival and differentiation of new neurons (i.e., neurogenesis in the brain)(Minichiello, 2009).

In addition, the activity-dependent release of BDNF and subsequent TrkB-mediated activation of CREB is also an important mechanism of enhancing neuronal communication, specifically in active neurons of the brain. For instance, BDNF increases synaptic strength with adjacent neurons by processes like long-term potentiation (LTP), thus ameliorating their connectivity (Lu et al., 2008;

Minichiello, 2009). Interestingly, LTP itself has been linked to both synaptogenesis and neurogenesis (Bruel-Jungerman et al., 2006).

DARPP-32 is phosphorylated at Thr34 in both striatal and frontal neurons. DARPP-32 converts thereby into a potent inhibitor of protein phosphatase-1 (PP-1). DARPP-32 is also phosphorylated at Thr75 by Cdk5 and this converts DARPP-32 into an inhibitor of PKA. Thus, DARPP-32 has the unique property of being a dual-function protein, acting either as an inhibitor of PP-1 or of PKA influencing neuroplasticity (Svenningsson et al., 2004). The inhibition of PP-1 controls the phosphorylation state and activity of many downstream physiological effectors, including various neurotransmitter receptors (e.g. AMPA receptor GluR1 subunit, N-methyl-D-aspartate (NMDA) receptor NR1 subunit), ion channels and pumps (e.g. N/P-type Ca^{2+} channels, Na^{+} channel, Na^{+}, K^{+}-ATPase), and transcription factors (e.g. CREB, c-Fos, ΔFosB)(Greengard et al., 1999). Striatal LTP and long-term depression (LTD) are dependent on cAMP and DARPP-32 phosphorylation (Calabresi et al., 2000).

The cAMP/PKA cascade is thus a potential target for pharmacological intervention in neuropsychiatric disorders related to dopaminergic frontal and striatal dysfunction. cAMP is degraded by cAMP-specific phosphodiesterases (PDEs) and dual substrate PDEs. Eleven PDE families have been described, 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 central nervous system (CNS) (Lakics et al., 2010; see Table 1). Selective phosphodiesterase inhibitors (PDE-Is) prevent the degradation of cyclic nucleotides leading to increased concentrations of cAMP. Due to the differential expression of PDE subtypes in one or more of the frontal and striatal pathways or dopaminergic terminals, different subtype-specific PDE-Is enable stimulation of dopamine synthesis, inhibtion of D2 receptor signaling or stimulation of D1 receptor signaling (Nishi et al., 2011). However, the level of expression of different PDE family members in these fronto-striatal circuits in both physiological and pathological conditions is incompletely understood and a subject of intense investigation. In the fronto-striatal circuits, the main therapeutic mechanism of PDE inhibition is enhanced neuroplasticity and neuroprotection through previously discussed CREB and DARPP-32 signaling cascades (see figure 1). However, known effects of PDE-Is on neuroinflammation and cytokine-mediated responses may play additional roles (Hebb and Robertson, 2008; Wilson and Brandon, 2015).

Table 1. Localization of the different phosphodiesterases (PDEs) in the brain of rodents and humans in adulthood (adapted from Prickaerts, 2015) based on Lakics et al, 2010; Pérez-Torres et al, 2010).

PDE	Localization in the Body	Localization in the Brain
PDE1A-C	Heart, smooth muscles, lungs	Hippocampus, cortex, olfactory bulb, striatum (highest expression levels), thalamus, amygdala, cerebellum; Expression levels are in general highest for 1A and lowest for 1C
PDE2A	Heart, adrenal cortex, platelets	Hippocampus, cortex, striatum, hypothalamus, amygdala, midbrain
PDE3	Heart, smooth muscles, kidneys, platelets	Throughout the brain low expression levels
PDE4A-D	Wide variety of tissues: e.g., smooth muscles, lungs, kidneys, testes	Hippocampus, cortex, olfactory bulb, striatum, thalamus, hypothalamus, amygdala, midbrain, cerebellum; Expression levels are in general highest for 4A-4D (differs per brain structure) and lowest for 4C
PDE5A	Smooth muscles, skeletal muscles, lungs, kidneys, platelets	Hippocampus, cortex, cerebellum
PDE6	Rod and cone cells in retina	Pineal gland
PDE7A-B	Heart, skeletal muscles, liver, kidneys, testes, pancreas	Hippocampus, cortex, olfactory bulb, striatum, thalamus, hypothalamus, midbrain; Expression levels are in general highest for 7B
PDE8A-B	Heart, liver, kidneys, lungs, testes, thyroid	Hippocampus, cortex, olfactory bulb, striatum, thalamus, hypothalamus, midbrain; Expression levels are in general highest for 8B
PDE9A	Kidneys, spleen, prostate, various gastrointestinal tissues	Hippocampus, cortex, olfactory bulb, striatum, thalamus, hypothalamus, amygdala, midbrain, cerebellum
PDE10A	Heart, skeletal muscles, lungs, liver, kidneys, testes, pancreas, thyroid	Hippocampus, cortex, striatum (highest expression levels), midbrain, cerebellum
PDE11A	Skeletal muscles, liver, kidneys, testes, prostate, thyroid	Throughout the brain low expression levels

Note that this table does not provide information with respect to the level of expression (protein or mRNA) of the different PDEs.

The integration of individual PDEs into specific signalosomes within different functional compartments has revealed the functional roles of these PDEs and linked the large number of PDE isoforms to the compartmentalized regulation of specific cyclic nucleotide signaling pathways and biological responses. This compartmentalization contributes therefore to both the fine-tuning and specificity of cyclic nucleotide signaling (Jurevicius and Fischmeister, 1996; Zaccolo et al., 2000; Mongillo et al., 2006; Maurice, 2011; Stangherlin et al., 2011; Stangherlin and Zaccolo, 2012). More in detail, compartmentalization provides spatially distinct pools of PKA and PKG to be activated in different ways. This idea was confirmed by the observation of accumulation of cAMP in localized pools (Houslay, 1995). These pools are created by physical interactions between different components of signaling cascades and structural elements of the cell. Localization and activation of both cyclases and PDEs are important determinants in the process of cyclic nucleotide homeostasis by modulating fluctuations in the compartments. For PDEs sequestration and anchoring is the principal mechanism to create cyclic nucleotide gradients (Houslay and Milligan, 1997; Houslay and Adams, 2003). Subsequently, different PKA isoforms are anchored at specific intracellular sites by AKAPs (A-kinase anchoring proteins) (Rubin, 1994). AKAPs control the gradients of cAMP in the cell and modify localized target proteins thereby causing sequestration of PKA into distinct cellular compartments. This also applies to the fronto-striatal circuits and is considered the main determinant of the target PDE isoform within a specific neuropsychiatric disorder. In addition, different PDE isoforms can integrate multiple distinct cellular inputs and allow crosstalk between cyclic nucleotides and other signaling networks and systems (Dodge-Kafka et al., 2005; Mongillo et al., 2006; Houslay et al., 2007; Stangherlin et al., 2011; Wilson et al., 2011; Kritzer et al., 2012).

Since the focus of this review is on frontal and striatal dopaminergic regulation, in particular PDE1B, PDE2A, PDE4, PDE7B, PDE9A and PDE10A are of special interest (Lakics et al., 2010). PDE1B, PDE7B and PDE10A are highly enriched in striatum and/or frontal cortex. PDE2A, PDE4 (A, B, D) and PDE9A are more widely distributed but are also expressed in striatum and/or frontal cortex. There is only very limited preclinical data on PDE2A, PDE7A and PDE9A inhibition (e.g. Duinen et al., 2015). To date, most research has been devoted to the potential of PDE1B, PDE4 and PDE10A for regulation of dopaminergic frontal and striatal signaling and therefore these subtypes will be discussed below.

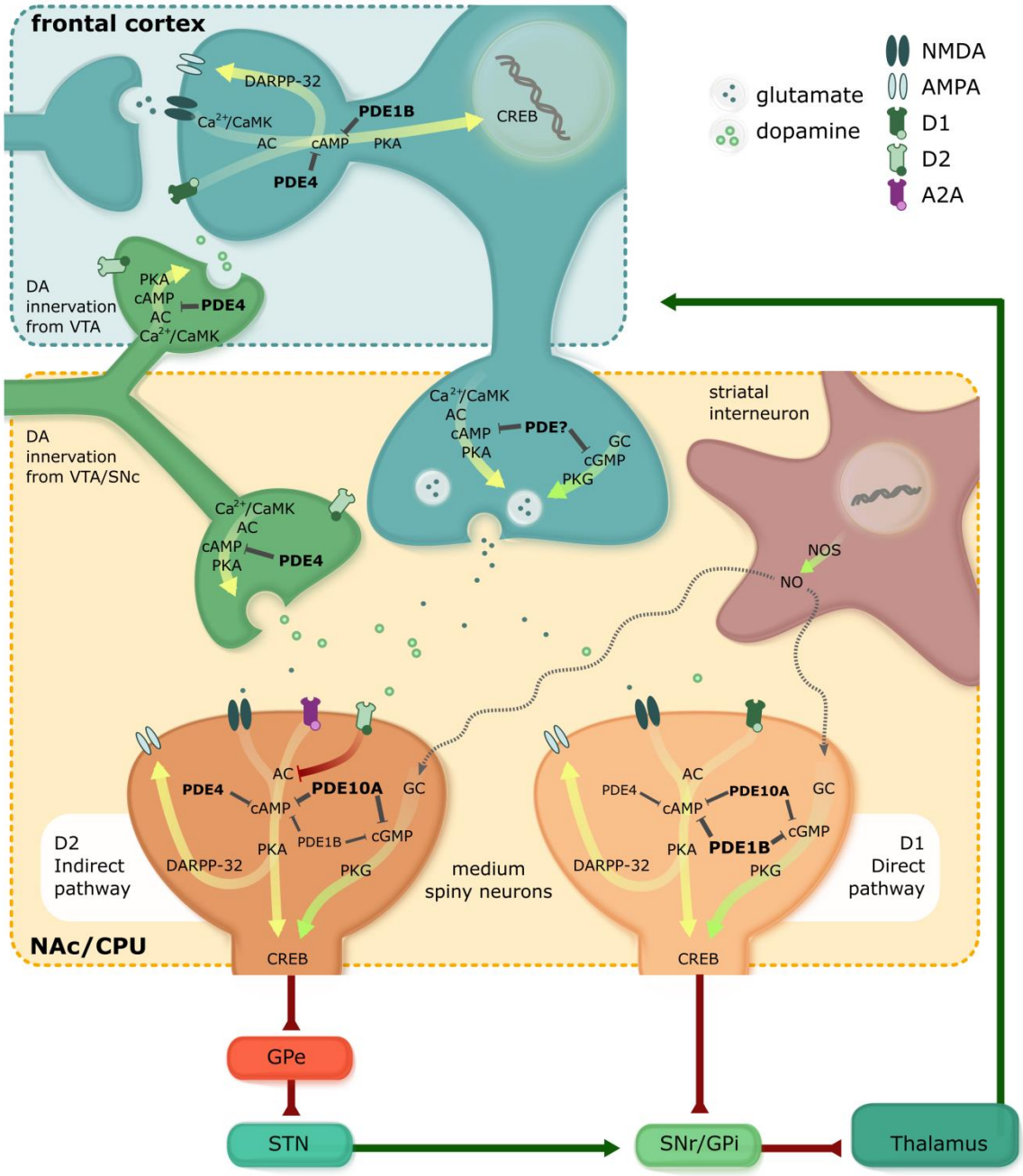

Figure 1. Fronto-striatal circuits originate in the frontal cortex, pass through the basal ganglia, which project via the thalamus back to frontal brain areas. Output neurons in the striatum are medium spiny neurons (MSNs), which consist of direct pathway and indirect pathway neurons. The direct pathway neurons inhibit tonically active neurons in globus pallidus interna (GPi)/substantia nigra pars reticulata (SNr). The indirect pathway neurons activate neurons in GPi/SNr via inhibition of the globus pallidus externa (GPe) and activation of the subthalamic nucleus (STN). Direct and indirect pathway neurons induce opposing effects on the output neurons in GPi/SNr, resulting in dis-inhibition and pro-inhibition of output, respectively. Within the basal ganglia all projections are GABAergic except those from the STN. Main phosphodiesterases (PDEs) expressed in fronto-striatal circuits are PDE1B, PDE4 and PDE10A.

PDE1B is generally co-localized with dopamine (DA) D1 receptors in the brain and thought to represent a major inactivation mechanism of D1 receptors. By acting like a DA D1 agonist PDE1B-Is can enhance phosphorylation of cAMP response element binding protein (CREB) as well as Dopamine- and cAMP-Regulated PhosphoProtein MR 32 kDa (DARPP-32) enhancing synaptic transmission (e.g. AMPA receptors), neuron excitability, and synapto- and neurogenesis resulting in neuroplasticity and neuroprotective effects at glutamatergic frontal and fronto-striatal synapses.

Regarding fronto-striatal signaling, the effect of PDE4 inhibition on cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) signaling is linked to indirect pathway adenosine A2a receptor signaling and has no major role in D1 receptor direct pathway signaling. An opposite situation is observed at frontal dopaminergic signaling. In the frontal cortex PDE4 is –just as PDE1B- localized at DARPP-32 expressing neurons. In contrast to the striatum, PDE4 inhibition enhances DA D1 receptor-induced phosphorylation of DARPP-32 in the frontal cortex, indicating a prominent role of PDE4 in frontal DA receptor signaling. Finally, DA release from DAergic midbrain terminals can be influenced with a PDE4 inhibitor as DA is expressed at DAergic terminals in neurons of the substantia nigra pars compacta (SNc) in which cAMP has been reported to be a strong inducer of tyrosine hydroxylase gene transcription rate and mRNA affecting DA synthesis and release.

In direct pathway neurons, PDE10A inhibition activates cAMP/PKA signaling related to D1 receptor signaling whereas in indirect pathway neurons PDE10A inhibition activates cAMP/PKA signaling by simultaneous potentiation of adenosine A2A receptor signaling and inhibition of D2 receptor signaling. Effects of PDE10A inhibition are suggested to predominate the indirect pathway. In contrast to PDE4 inhibition, PDE10A inhibition does not increase tyrosine hydroxylase phosphorylation and therefore has no effects on DA synthesis and release. Nevertheless, it cannot be ruled out that selective PDE inhibitors might influence both the direct and indirect pathway via enhancing the release of DA from frontal DAergic projections depending on the –to be determined- presence of PDEs in these terminals.

In striatal interneurons containing nitric oxide synthase (NOS), nitric oxide (NO) is produced and diffuses into dendrites of MSNs which contain high levels of guanylate cyclase (GC), which, when activated, lead to the synthesis of cyclic guanosine monophosphate (cGMP). In the striatum, transient elevations in intracellular cGMP –next to cAMP- primarily act to increase neuronal excitability and to facilitate glutamatergic fronto-striatal transmission. Thus, inhibition of selective PDE subtypes can also target the cGMP/protein kinase G (PKG) pathway and have an effect on fronto-striatal functioning.

Phosphodiesterase 1

Phosphodiesterase 1 and dopamine signaling

PDE1 hydrolyzes both cAMP and cGMP. PDE1, unlike any other class of PDE, is uniquely activated by the binding of a complex of Ca^{2+} and calmodulin (CaM). PDE1 is encoded by three separate genes, *PDE1A*, *PDE1B* and *PDE1C*. PDE1B is highly co-localized with D1 receptors in the brain, and is particularly rich in the striatum, hippocampus and prefrontal cortex (Lakics et al., 2010). PDE1 activity

has first been described as cytosolic, however it now appears that PDE1A is not restricted to the cytosol but is also present in the nucleus where it contributes to the regulation of transcription factors (Nagel et al., 2006). This opens a new field of research in transcriptional regulation. Changes in PDE1 location associated with cell differentiation might contribute to compartmental signaling (Nagel et al., 2006).

Since PDE1B is strongly and selectively expressed in the striatum and frontal cortex it could be a relevant target for modulating fronto-striatal behaviors. However, only a few studies have been published with PDE1-Is (Medina, 2011; Nunes et al., 2011). Indeed, as noted by the authors, in these studies no potent and selective inhibitors of PDE1 isoforms were available for research. Vinpocetine, often referred to as a PDE1-I, has substantial other activities including inhibition of Na^{2+} channels and IκB kinase (IKK). Vinpocetine therefore should not be considered as a selective PDE1-I. Since PDE1B is activated by Ca^{2+} and CaM, it provides a mechanism for crosstalk between Ca^{2+} and cyclic nucleotide signaling (Nishi et al., 2008; Nishi and Snyder, 2010; Nishi et al., 2011). PDE1B was localized to all DARPP-32-positive MSNs indicating expression in both striatal pathways (Nishi et al., 2011). Behavioral profiles of PDE1B knockout (KO) mice showed rather mild behavioral effects. The authors show an increased spontaneous locomotor activity in the presence of methamphetamine administration. Cognitive aspects are reported as similar to wild type. Overall the data suggest predominant effects of PDE1B-Is would be seen in the striatonigral direct pathway (Reed et al., 2002; Ehrman et al., 2006; Siuciak et al., 2007; Zhang, 2010). However, regarding effects on frontal and striatal DA release or effects on cAMP/PKA signaling in the frontal cortex, these areas have so far been understudied.

Implications and clinical overview of PDE1B-Is

PDE1B is generally co-localized with DA D1 receptors in the brain and thought to represent a major inactivation mechanism of D1 receptors. PDE1B is not membrane bound but contained mainly in a soluble intracellular compartment (Fusco and Giampa, 2015). Targeting this subfamily of PDEs is therefore considered a promising therapeutic strategy in disorders characterized by frontal cognitive dysfunction, like schizophrenia and ADHD. Negative and cognitive symptoms of schizophrenia, are associated with reduced DA (D1) function in the prefrontal cortex, also referred to as hypofrontality (Liemburg et al., 2012; Arnsten, 2013). The reduced prefrontal DA function disturbs the balance of excitatory to inhibitory synaptic interactions in this area (Winterer, 2006). Thus, the decreased ratio of D1/D2 signaling in schizophrenia would favor unstable cortical representation of internal and external stimuli (Winterer and Weinberger, 2004) and as such, affect cognition. Likewise, dopaminergic hypofrontality is observed in ADHD patients (Pliszka, 2005; Sagvolden et al., 2005;

Arnsten and Pliszka, 2011) and linked to inattentiveness, hyperactivity and impulsivity. By acting supposedly like a DA D1 agonist, a PDE1B-I can enhance phosphorylation of GluR1 subunits, to potentiate glutamatergic fronto-striatal signaling. In addition, potentiation of DA receptors will increase phosphorylation of DARPP-32 and CREB, subsequently inducing gene expression in the prefrontal cortex, and benefiting clinical symptoms by activation of neuroplasticity at prefrontal synapses.

Regarding striatal disorders like movement disorders and positive symptoms in schizophrenia, not much data is available. Assuming effects of PDE1B are indeed preferentially induced in the DA D1 direct pathway, subsequent DARPP-32 and CREB phosphorylation will enhance synaptic transmission, neuron excitability, and synapto-neurogenesis inducing neuroplasticity and neuroprotection at glutamatergic fronto-striatal synapses. In theory, neuropsychiatric disorders related to striatal hypofunction (like hypokinetic movement disorders such as Parkinson's disease), would benefit from stimulated plasticity in striatonigral neurons (Nishino et al., 1993; Heckman et al., 2015). This, in contrast to desired mechanism of action of treatment for hyperkinetic movement disorders (like Huntington's disease) and anti-psychotic treatment, as both preferably target the D2 receptor striatopallidal pathway (Strange, 1998; Walker, 2007). Support for this hypothesis comes from studies that found impaired cyclic nucleotide signaling mechanisms to occur in human Parkinson's disease as well as in experimental animals (Belmaker et al., 1978; Volicer et al., 1986; Nishino et al., 1993; Sancesario et al., 2004). Additional upregulation in PDE1B activity has also been observed following 6-hydroxydopamine (6-OHDA) lesions (as a Parkinson's model) in rats (Sancesario et al., 2004). The opposite has been observed in Huntington's disease (Luthi-Carter et al., 2000; Nucifora et al., 2001). These studies found decreased cyclic nucleotide (cAMP) levels in the deafferented striatum accompanied by decreased PDE1B activity. The decreased PDE1B activity may be compensatory to the decrease in cyclic nucleotide levels. CREB-mediated transcriptional dysregulation has also been reported to occur during Parkinson's disease pathology. PDE1-Is have been tested in an animal model of haloperidol-induced catalepsy. As haloperidol is a potent D2 DA receptor antagonist, interference with D2 DA receptors causes significant motor disturbances seen frequently with schizophrenics treated with antipsychotic medicines. The haloperidol-induced catalepsy model is capable of testing agents for exacerbation or lessening of these motoric effects. The potent and selective PDE1-I, ITI-214 (see below) reversed the haloperidol-induced catalepsy, indicating the potential use of this mechanism to reverse such motoric effects (Wennogle et al., 2010).

BDNF has been demonstrated to exert protective actions on nigral dopaminergic neurons in *in vivo* and *in vitro* models of Parkinson's disease (Hyman et al., 1991; Levivier et al., 1995; Shults et al., 1995; Hung and Lee, 1996; Feng et al., 1999; Mohapel et al., 2005; Sun et al., 2005), whereas inhibition of nigral BDNF expression has been reported to cause dopaminergic neuronal loss (Porritt et al., 2005). Postmortem studies have demonstrated reduced levels of BDNF within the SNc in Parkinson's disease patients (Mogi et al., 1999; Parain et al., 1999; Howells et al., 2000; Chauhan et al., 2001). Furthermore, during *in vitro* experiments, BDNF has been demonstrated to promote the survival and differentiation of mesencephalic dopaminergic neurons (Hyman et al., 1991; Feng et al., 1999). The enhancement of cerebral cyclic nucleotide levels by PDE1B inhibition would improve CREB mediated signaling mechanisms providing therapeutic effects in Parkinson's disease.

Recently, a set of four clinical studies were performed with a truly selective and potent PDE1-I, ITI-214 (Li et al., 2016a). With the exception of work performed with vinpocetine, a non-selective agent as noted above, ITI-214 is the first selective PDE1-I studied in humans. Clinical evaluations included a series of Phase I single and multiple ascending dose studies performed in the United States (US) and Japan. ITI-214 was given orally to healthy volunteers and to patients using once-a-day dosing and was shown to be safe and well-tolerated, with a linear pharmacokinetic profile. This study has been reported in a press release (http://www.intracellulartherapies.com/products-technology/pde-inhibitor-platform.html), where the company concludes that "these studies represent a significant milestone as the first demonstration of the safety of a potent and highly specific PDE1-I in humans".

Phosphodiesterase 4

Phosphodiesterase 4 and dopamine signaling

PDE4, which is cAMP-specific, is encoded by four distinct genes in mammals, *PDE4A*, *PDE4B*, *PDE4C* and *PDE4D*, and is expressed as at least 25 splice variants. Each of these variants has a modular structure consisting of a variant-specific N-terminal domain, regulatory domains (upstream conserved region 1 and 2 (UCR1 and UCR2)), a conserved catalytic domain and an isoform-specific C-terminal domain (McCahill et al., 2008; Gurney et al., 2011; Richter et al., 2013). Transcription of a number of PDE4 genes is activated by the cAMP/PKA/CREB cascade (D'Sa et al., 2002; Le Jeune et al., 2002), and PKA induction of PDE4 genes serves as a long-term feedback mechanism. The N-terminal domain and UCR1/2 interact with variant-specific binding proteins, to direct the subcellular targeting of PDE4 variants (McCahill et al., 2008). Various targeting proteins have been identified, including arrestin, A-kinase anchoring protein (AKAPS), receptor for activated C kinase 1 (RACK1), disrupted in schizophrenia 1 (DISC1), Src, and extracellular receptor kinase (ERK) (see Nishi and Snyder, 2010).

Nishi and colleagues (Nishi et al., 2008) showed that the inhibition of PDE4 by rolipram weakly enhanced cAMP/PKA signaling both in neostriatal slices and *in vivo* (Nishi et al., 2008). Rolipram increased the phosphorylation of DARPP-32 but only at high concentrations. Rolipram treatment enhanced adenosine A_{2a} receptor-mediated phosphorylation of DARPP-32, but had no effect on D1 receptor/cAMP/PKA-mediated phosphorylation at the level of DARPP-32. Enhanced adenosine A_{2a} receptor-mediated signaling is expected to oppose actions of the DA D2-receptor in striatopallidal neurons. These findings may suggest that PDE4 is exclusively expressed in indirect pathway neurons. However, immunohistochemical analysis of previously mentioned neostriatal slices revealed that PDE4B expression can be found in both pathways but with a higher expression in indirect pathway neurons. Regarding striatal dopaminergic signaling it seems that the effect of PDE4 inhibition on cAMP/PKA signaling is linked to adenosine A_{2a} receptor signaling and has no major role in striatal DA signaling. An opposite situation is observed at frontal dopaminergic signaling. In the frontal cortex several PDE isoforms are expressed in cortical neurons (Cherry and Davis, 1999; Pérez-Torres et al., 2000). For the mouse frontal cortex it has been described that PDE4B is localized at DARPP-32 expressing neurons (Nishi and Snyder, 2010). In contrast to the striatum, rolipram enhanced DA D1 receptor-induced phosphorylation of DARPP-32 in the frontal cortex, indicating prominent role of PDE4 in frontal DA receptor signaling. Finally, DA is known to be expressed at dopaminergic terminals in neurons of the SNc (Cherry and Davis, 1999), where cAMP has been reported to be a strong inducer of tyrosine hydroxylase (TH) gene transcription rate and mRNA affecting DA synthesis (Kumer and Vrana, 1996; Chen et al., 2008). Rolipram enhanced haloperidol-induced phosphorylation of TH at Ser40 in presynaptic DA terminals with a proportional increase in DA synthesis, though failed to do so in the absence of haloperidol. Also, rolipram enhanced levels of 3,4-Dihydroxyphenylacetic acid (DOPAC) and DOPAC/DA ratio indicating an increased DA metabolism. However, no increase in the level of DA itself was found indicating the absence of a direct effect on DA release (Nishi et al., 2008).

Implications and clinical overview of PDE4-Is

Compared to PDE1 much more is known regarding the role of PDE4 in frontal and striatal dopaminergic functioning. However, by no means have all the effects been unraveled and all the questions been resolved for PDE4. PDE4 inhibition has been shown to increase dopaminergic tone in striatal neurons by increasing both synthesis and metabolism, though lacking a direct effect on release. It is known that basal ganglia functioning depends on specific amounts of DA in order to function at peak performance. Low levels of DA cause movement difficulties, while excessive DA causes involuntary movements. Even if PDE4 inhibition itself may not be involved in the process of releasing DA, the enhanced production of the DA precursor levodopa by enhanced cAMP-stimulated

TH gene transcription, may result in enhanced stimulus-driven DA release. PDE4-Is may therefore constitute an interesting treatment for neuropsychiatric disorders involving hypofunctioning striatal DA systems, like Parkinson's disease. Indeed, rolipram has been reported to attenuate MPTP-induced DA depletion in the striatum and reduce the loss of nigral TH-positive neurons *in vitro* (Hulley et al., 1995a; Yamashita et al., 1997a; Yamashita et al., 1997b) and *in vivo* (Hulley et al., 1995b; Yang et al., 2008). Next, it remains to be seen if these effects on synthesis and metabolism also apply to frontal dopaminergic terminals arriving from the VTA. If so, PDE4 would be an interesting target for disorders characterized by frontal dopaminergic dysfunction, like ADHD or schizophrenia (cognitive and negative symptoms). Frontal dopaminergic hypofunctioning can not only be opposed by PDE4 inhibition via augmented release of neurotransmitters at dopaminergic terminals, but also, like previously discussed for PDE1B, by means of increased DA D1 receptor/cAMP/PKA signaling inducing DARPP-32 and CREB phosphorylation leading to concomitant gene transcription related to neuronal plasticity. Because PDE4 inhibition affects both these mechanisms, PDE4-Is are particularly interesting as a potential treatment for ADHD and schizophrenia. Finally, in the striatum, PDE4 inhibition regulates adenosine A_{2a} signaling and is therefore often viewed as exerting DA D2 antagonistic effects, although it may also be viewed as mimicking the effect of an adenosine A_{2a} agonist. Both would indicate anti-psychotic potential of PDE4-Is by counteracting hyperdopaminergia, which has been confirmed by several studies over the years including early clinical trials (Casacchia et al., 1983; Parkes et al., 1984; Siuciak, 2008). Based on results of rolipram, this latter mechanism is also applicable to Huntington's disease. Rolipram exerted neuroprotective effects in two rodent Huntington's disease models via increased CREB phosphorylation and subsequent targets like BDNF (DeMarch et al., 2007; DeMarch et al., 2008; Fusco and Giampa, 2015). Neuroprotective effects of rolipram were induced by sparing of striatal neurons, prevention of intranuclear inclusion formation and attenuation of microglial reactivity (DeMarch et al., 2008). Furthermore, rolipram was effective in preventing CREB binding protein sequestration into striatal neuronal intranuclear inclusions, sparing interneurons of R6/2 mice and rescuing motor coordination and activity deficits (Giampa et al., 2009b; but see Hannan, 2009). However, there are a range of molecular and cellular mechanisms implicated in the pathogenesis of Huntington's disease (Gil and Rego, 2008).

When examining actual clinical data, the first clinical trials into PDE4 were done in the field of depression research (Esposito et al., 2009). First clinical studies showed a good antidepressant response to rolipram treatment (Zeller et al., 1984; Fleischhacker et al., 1992). However, rolipram produces severe dose-limiting side effects including emesis, headache, gastric hyper secretion, nausea and vomiting. This has put a serious hold on the further development of rolipram and other

related PDE4-Is. It also prevented rolipram from reaching the market. Yet, a clinical Phase II trial started in 2006 to reevaluate the antidepressant properties of rolipram (estimated study completion date: December 2013). No details are yet available to the scientific community. Another PDE4-I, ND1251, was reported to improve memory in a group of 8 depressed subjects (http://www.outsourcing-pharma.com/Preclinical-Research/PDE4-re-emerges-as-depression-therapy).

Although rolipram was primarily developed for treating depression (Zeller et al., 1984; Fleischhacker et al., 1992), rolipram has also been investigated in early clinical trials as a treatment for Parkinson's disease (Casacchia et al., 1983; Parkes et al., 1984). Some positive effects of rolipram were observed, however not exceeding efficacy of levodopa or other dopaminergic drugs. At the moment, ''second generation'' PDE4-Is are being developed, which are supposed to have less-emetic side effects, and are being studied for other disorders besides that of depression. As a result, roflumilast has been approved by the Food and Drug Administration (FDA) in 2011 as an anti-inflammatory drug for the treatment of Chronic Obstructive Pulmonary Disease (COPD) exacerbations (Izquierdo and Aparicio, 2010; Puhan, 2011).

PDE4-Is were also tested in clinical trials as treatment for schizophrenia. Takeda has recently finished a proof of mechanism Phase I clinical study with the PDE4-I roflumilast in combination with second generation antipsychotics in schizophrenia patients (ClinicalTrials.gov Identifier: NCT02079844).

PDE4-Is were also examined as a treatment for Huntington's disease. The new experimental PDE4-I GSK356278 was tested by GlaxoSmithKline as a new treatment for Huntington's disease in 2 subsequent Phase I studies. In 2012, the first Phase I study was completed investigating the safety, tolerability, pharmacokinetics and pharmacodynamics of GSK356278 (ClinicalTrials.gov Identifier: NCT01573819). GSK356278 was well tolerated when it was given as a single dose to healthy people and in this study GlaxoSmithKline the objective was to observe effects of GSK356278 after daily intake. Subsequently, a second Phase I positron emission tomography (PET) brain occupancy study of GSK356278 was conducted in male healthy volunteers (ClinicalTrials.gov Identifier: NCT01602900; no results are disclosed). It is currently unclear whether this PDE4-I treatment is aimed at the motor or cognitive symptoms observed in Huntington's disease.

Of note, Ibudilast (or AV-411) is another PDE4-I in development as an anti-inflammatory drug to treat for instance Amyotrophic Lateral Sclerosis (ALS) (ClinicalTrials.gov Identifier: NCT02238626). However, this compound not only inhibits PDE4 but also serves as a glial activator. CNS applications

of AV-411 are being explored in clinical Phase II studies, i.e. pain and drug abuse (ClinicalTrials.gov Identifier: NCT00723177, NCT01217970, NCT02025998, NCT01860807).

Additionally, different genetic studies have shown a positive relationship between PDE4B polymorphisms and schizophrenia, which likely results in significantly decreased PDE4B levels as detected in postmortem brain tissue (Fatemi et al., 2008b; Guan et al., 2012). Low PDE4B levels, which might be considered as compensatory mechanism, do not necessarily result in increased cAMP levels as several mechanisms can also be activated that counteract the decreased degradation of cAMP by PDE4B. Another genetic link is related to the gene Disrupted-in Schizophrenia-1 (DISC1; Harrison and Weinberger, 2005). A chromosomal translocation of this gene increases susceptibility for schizophrenia (Millar et al., 2000; Sachs et al., 2005) and interestingly binding of DISC1 to PDE4B is disrupted which might result in an overactivity of the latter (Millar et al., 2005; Murdoch et al., 2007).

Phosphodiesterase 10

Phosphodiesterase 10 and dopamine signaling

PDE10, which is encoded by *PDE10A*, is a dual substrate PDE, hydrolyzing both cAMP and cGMP. PDE10A is present both in striatonigral direct and striatopallidal indirect pathway MSNs (Xie et al., 2006; Nishi et al., 2008). Additionally, PDE10A regulates cAMP/PKA signaling (Nishi et al., 2008) and gene expression (Strick et al., 2010) in the MSNs of both pathways. Interestingly, PDE10A hydrolyzes both cAMP and cGMP, but it has an approximate 20-fold higher affinity for cAMP (Bender and Beavo, 2006) making it an interesting target for disorders involving the fronto-striatal circuits. In the striatum, PDE10A is expressed in both direct and indirect pathway MSNs, but not in interneurons (Xie et al., 2006; Nishi et al., 2008; Sano et al., 2008). Of the three splice variants, PDE10A2 is associated with the membrane, whereas PDE10A1 and PDE10A3 are found in the cytosol (Kotera et al., 2004). In the striatum, mainly PDE10A2 is expressed and it is found at membranes in dendrites and spines of medium spiny neurons (Xie et al., 2006). PDE10A2 is phosphorylated by PKA at Thr16 within the N-terminal region (Kotera et al., 2004). Nishi and colleagues argue that this seems to induce the translocation of PDE10A2 from membrane to cytosol, thereby controlling cAMP/PKA signaling within the spines (Nishi and Snyder, 2010; see also Wilson and Brandon, 2015).

Through this effect on cAMP/PKA signaling, PDE10A inhibition by papaverine showed enhanced phosphorylation of CREB and ERK (Rodefer et al., 2005; Siuciak et al., 2006; Becker and Grecksch, 2008) and of their downstream targets DARPP-32 and GluR1 (Nishi et al., 2008) at PKA sites in striatal

MSNs both *in vitro* and *in vivo*. More specifically, in both direct and indirect pathway neurons, PDE10A shows equal expression patterns (Xie et al., 2006; Nishi et al., 2008; Sano et al., 2008), regulation of cAMP/PKA signaling (Nishi et al., 2008) and gene expression (Strick et al., 2010). However, distinguishing between both pathways, in direct pathway neurons, PDE10A inhibition activates cAMP/PKA signaling related to D1 receptor signaling (Nishi et al., 2008), whereas in indirect pathway neurons PDE10A inhibition activates cAMP/PKA signaling by simultaneous potentiation of adenosine A_{2A} receptor signaling and inhibition of D2 receptor signaling. A study of neuronal type-specific regulation of DARPP-32 phosphorylation at Thr34 using neostriatal slices showed that papaverine increased DARPP-32 phosphorylation by six-fold in indirect pathway neurons, whereas it increased DARPP-32 phosphorylation only by two-fold in direct pathway neurons indicating that effects of PDE10A inhibition predominate the indirect pathway (Bateup et al., 2008; Nishi et al., 2008). Recent electrophysiological results support this conclusion (Threlfell et al., 2009). More support is provided by recent behavioral studies published by different groups, however, these latter studies also show substantial D1 direct pathway effects of PDE10A-Is (Megens et al., 2014b; Megens et al., 2014a; Gentzel et al., 2015; Suzuki et al., 2016).

In vivo, PDE10A-Is are studied mostly for effects on spontaneous or stimulated behaviors providing evidence for predominant indirect pathway effects (similar to effects of D2 receptor blockers). This would include inhibition of spontaneous or stimulant-induced behavior, inhibition of conditioned avoidance behavior, reversal of stimulant-induced sensory gating deficits and preferential activity against apomorphine-induced climbing (Schmidt et al., 2008; Grauer et al., 2009; Kehler and Nielsen, 2011; Gresack et al., 2013; Megens et al., 2014b). However, concomitant D1 receptor stimulation causes reduced efficiency against behavioral stimulants, via direct pathway activation (Menniti et al., 2007; Sotty et al., 2009; Gresack et al., 2013; Megens et al., 2014b). D1 receptor stimulation is also responsible for the cognition-enhancing effects (Rodefer et al., 2005; Grauer et al., 2009) and socializing effects (Grauer et al., 2009) of PDE10A-Is. So indeed, there is compelling evidence for substantial direct pathway activation of PDE10A-Is. This latter notion is supported by recent work from Megens and coworkers in suppressed behavior via D1 receptor blockade, D2 receptor blockade or DA depletion (Megens et al., 2014a). Their results indicate that PDE10A-Is reverse behavioral suppression after D1 receptor blockade (hypolocomotion) via direct pathway activation (next to suppressing stimulant behavior via indirect pathway activation). These effects are indicative of substantial D1 agonistic effects of PDE10A-Is (next to their D2 antagonistic effects). Still, the main effects of PDE10A-Is are suggested to be exerted trough the indirect pathway. By this route PDE10A-Is can cause extrapyramidal side-effects, resembling D2 receptor blockers. The latter may explain why PDE10A-Is have not yet reached the market as antipsychotic treatment. This notion is supported by

the recent failure of the Pfizer PDE10A-I MP-10 (or PF-02545920) in a Phase II clinical trial as antipsychotic treatment, where it showed no efficacy on positive and negative symptoms and produced motor side-effects (akathisia and dystonia) in patients with schizophrenia (DeMartinis et al., 2012).

Finally, in contrast to PDE4 inhibition by rolipram, PDE10A inhibition by papaverine showed no increases on TH phosphorylation at Ser40 (PKA site) suggesting no effects of PDE10A inhibitors on DA synthesis. Of note, only at high concentration papaverine did show an effect on TH phosphorylation. Also, results for papaverine should be confirmed by using the more potent PDE10A-Is TP-10 and MP-10. Additionally, PDE10A inhibition showed no effects on DA metabolism (Nishi et al., 2008). Therefore, in contrast to PDE4, it is assumed that PDE10A does not play a major role at dopaminergic terminals.

Implications and clinical overview of PDE10A-Is

PDE10A is even more extensively studied in relation to the fronto-striatal circuits than the previously discussed PDE4 and PDE1B subtypes. Due to the hypothesis of a higher expression in indirect pathway neurons PDE10A-Is have received much attention as potential DA D2 antagonists and as such for their antipsychotic properties. Historically, positive symptoms in schizophrenia have been linked to overstimulation of DA receptors in the striatum (Baumeister and Francis, 2002), which is attenuated by (PDE10A inhibition-induced) DA D2 receptor antagonism. Because of the expected predominant effects in the indirect pathway PDE10A is also hypothesized as therapeutic target in Huntington's disease. Increases in cAMP are expected to drive CREB dependent signaling pathways, known to be dysregulated in Huntington's disease mouse models (Choi et al., 2009). In line, like rolipram, TP-10 was shown to be neuroprotective in the quinolinic acid model of Huntington's disease through CREB-mediated neuroprotection (Giampa et al., 2009a). In a follow-up study, PDE10A-I treatment of R6/2 mice showed significant delays in development of the motor deficits measured in this model accompanied by reduced striatal and cortical cell loss (Giampa et al., 2010). This was accompanied by increased CREB phosphorylation, suggesting that increased cAMP signaling in these brain regions could slow progression of neurodegeneration. Additionally, gene expression studies have implicated PDE10A and cAMP signaling as a therapeutic strategy for Huntington's disease (Hebb et al., 2004). Chronic treatment of wildtype mice with TP-10 resulted in an increase in gene expression of members of the, for Huntington's disease neuroprotective, ERK and PKA signaling pathways and an increase in ERK and MSK phosphorylation (Roze et al., 2008; Kleiman et al., 2011; Martin et al., 2011). Hypothetically, in Parkinson's disease PDE10A-Is could be used in the same way to treat DA agonist- or levodopa-induced dyskinesias. Chronic treatment with both classes of drugs

leads to improvement in symptoms but causes unwanted side-effects. These unwanted symptoms are thought to be due to D1 receptor functional supersensitivity, abnormal cAMP signaling and enhanced ERK signaling (Bezard et al., 2001; Aubert et al., 2005; Santini et al., 2007). Cyclic nucleotide levels were found to be decreased in the brains of rats treated with a combination of levodopa and 6-OHDA (Giorgi et al., 2008). Consistent with this finding, treatment of levodopa-induced dyskinesias with TP-10 reduced the severity of dyskinesias observed in 6-OHDA rats. In this way PDE10A-Is rescue decreases in cyclic nucleotide levels and prolong the use of levodopa (Wilson and Brandon, 2015).

Preclinical antipsychotic effects of PDE10A-Is may have initiated fronto-striatal disorder-related research, though lack of clinical efficacy and possible extrapyramidal side effects are hampering PDE10A-Is in reaching the market as antipsychotic treatment. An example of the latter is provided by the failure of the Phase II clinical trial of the Pfizer PDE10A-I MP-10 (or PF-02545920). MP-10 showed no efficacy and produced motor side-effects. Despite the serious challenges, there remains interest in PDE10A-Is as antipsychotic treatment. For instance, Takeda is currently recruiting participants for a clinical Phase II study to evaluate the efficacy, safety and tolerability of TAK-063 compared with placebo in treatment of acutely exacerbated schizophrenia. Efficacy was explained as determining whether cognitive impairment associated with schizophrenia would be attenuated (ClinicalTrials.gov Identifier: NCT02477020). Also, a Phase I study by Hoffmann-La Roche has just been completed in which the safety, tolerability and pharmacokinetics of RO5545965 in patients with schizophrenia on risperidone was tested (no results have been posted; ClinicalTrials.gov Identifier: NCT02019329). Of note, in 2012 Amgen started and terminated a Phase I study to assess the safety and tolerability of their PDE10-I AMG 579 following a single oral dose administration in healthy subjects and in patients with schizophrenia or stable schizoaffective disorder (ClinicalTrials.gov Identifier: NCT01568203).

A recent study found no difference in PDE10A mRNA expression between schizophrenia patients and comparison subjects in any of the brain regions studied (thalamus, caudate, putamen, nucleus accumbens, globus pallidus, and substantia nigra). This is the first *in vivo* assessment of PDE10A expression in patients with schizophrenia. However, this should not be interpreted as a case against developing PDE10A drugs in schizophrenia. The study of intracellular signaling pathways makes a persuasive case for how PDE10A inhibitors could influence the overall signaling in a therapeutic direction, regardless of whether there is an intrinsic change in PDE10A in schizophrenia (Marques et al., 2016).

Pharmaceutical companies have also started to redesignate their PDE10-Is to Huntington's disease. A Phase II proof-of-concept trial is now being initiated in which Pfizer's PDE10A-I MP-10 will be tested for safety and efficacy in subjects with Huntington's disease (ClinicalTrials.gov Identifier: NCT02197130). Omeros initiated a Phase II clinical trial in Huntington's disease patients with OMS824 after an earlier Phase II trial in schizophrenia patients (no results disclosed; ClinicalTrials.gov Identifier: NCT01952132). The Huntington's disease trial is a sequential-cohort dose-escalation study that evaluates the safety and tolerability of OMS824 over four weeks (ClinicalTrials.gov Identifier: NCT02074410). In parallel with the clinical OMS824 trial, Omeros is conducting preclinical rat studies to support clinical trials of longer duration. However, based on that data, there might be a safety issue and based on follow-up communications with the FDA, Omeros has suspended the ongoing Huntington's disease trial. The FDA has requested that Omeros further evaluates the preclinical data in order to characterize the compound more fully prior to reinitiating the clinical trial (http://investor.omeros.com/phoenix.zhtml?c=219263&p=irol-newsArticle_Print&ID=1979683).
Additional support for the use of PDE10-Is in Huntington's disease comes from a recent study which shows that PDE10 levels are lowered early before symptom onset in Huntington's disease (Niccolini et al., 2015b). Whether this is cause or consequence remains to be determined, however it most likely resembles a consequence of the degeneration of striatal cells and therefore the PDE10 enzymes within. These results were recently confirmed by a study with the radioligand [18F] MNI-659A (Russell et al., 2016). A comparable large scale Phase 0 study is currently recruiting new participants. The aim of this study is to measure the availability of the PDE10A enzyme in Huntington's disease gene expansion carriers (HDGECs) using the recently developed radioligand [18F] MNI-659. The study will be cross-sectional, examining HDGECs at different stages of the disease (pre-manifest, stage 1 and stage 2), in comparison with healthy controls (ClinicalTrials.gov Identifier: NCT02061722).

Of note, Niccolini et al. also demonstrated striatal and pallidal loss of PDE10A expression in Parkinson's disease patients, which is associated with Parkinson's disease duration and severity of motor symptoms and complications (Niccolini et al., 2015a). These results suggest that dopaminergic nigrostriatal degeneration affects the expression of PDE10A in striatum and pallidum. Hypothesizing, it most likely resembles a compensatory mechanism. Less dopaminergic input from the SNc equals less cAMP activation in striatal and pallidal areas decreasing the required levels of PDE10. In another, more far-fetched scenario the decrease is causative. The decrease in PDE10 levels reflects the overall expression of PDE10 in these brain areas, not specified for the direct and indirect pathway. Because of the stronger expression of PDE10 in the indirect pathway compared to the direct pathway, PDE10 degeneration will affect the indirect pathway more strongly. Subsequently, reduced PDE10

expression results in enhanced activation of the indirect pathway resulting in increased inhibition of movement. In both Parkinson's disease and Huntington's disease altered PDE10 levels are likely compensatory/consequential instead of causative. In hyperkinetic movement disorders like Huntington's disease, PDE10 may thus be a promising target for pharmacological agents (PDE10-Is enhance the little cAMP signaling that is left in the indirect pathway).

Conclusion

Clinical trials investigating the effects of PDE-Is in neuropsychiatric disorders are overall very sparse and the wealth of positive preclinical data could not yet be translated into clinical efficacy. As a result, no definitive conclusions can be drawn merely based on clinical trial outcomes. Therefore, the current review provides a discussion of the role of PDEs in dopaminergic frontal and striatal signaling and the potential of their associated inhibitors in specific disorders of the fronto-striatal circuits. Subsequently, an overview is provided of the current clinical status.

The fronto-striatal circuits compose the neurobiological basis for several neuropsychiatric disorders, including Parkinson's disease, Huntington's disease, ADHD, Tourette's syndrome, schizophrenia and obsessive-compulsive disorder. The fronto-striatal circuits constitute a plurality of parallel segregated circuits, which can be clustered together in motor circuits, associative/cognitive circuits and limbic circuits (Krack et al., 2010). Together, dysfunctions in these circuits produce the wide range of symptoms observed in related neuropsychiatric disorders.

Intracellularly, direct and indirect pathway signaling in the striatum is largely mediated through the cAMP/PKA cascade (Nishi et al., 2008; Nishi and Snyder, 2010; Nishi et al., 2011). Cyclic nucleotide cascades are involved in synaptic transmission, neuron excitability, neuroplasticity and neuroprotection in all types of fronto-striatal circuits (see figure 1). Additionally, all fronto-striatal circuits are modulated by dopamine. Next to the effects of cAMP/PKA pathways on glutamatergic and GABAergic signaling in the fronto-striatal circuits, these cyclic nucleotide pathways also play a major role in the dopaminergic modulation of the circuits. The intracellular effect of dopamine is mediated through dopamine receptor-regulated activation of cAMP/PKA and subsequent DARPP-32 and CREB phosphorylation in both striatal and frontal neurons.

In the last decades PDEs have therefore received increased attention for their possible role in disorders involving the fronto-striatal circuits. Based on overall expression patterns in frontal and striatal dopaminergic terminals, indirect pathway neurons and direct pathway neurons, PDE1B, PDE2A, PDE4, PDE7B, PDE9A and PDE10A seem to be the most interesting targets (Lakics et al., 2010),

although most attention and resources have thus far been devoted to the potential of PDE1B, PDE4 and PDE10A due to their role in dopaminergic signaling. The main site of action and expression of PDE1B, PDE4 and PDE10A as discussed in this clinical review is inferred from biochemical analyses of striatal cAMP/PKA effectors, behavioral phenotypes of KO mice and the observation of effects of subtype-specific PDE-Is on dopamine-related behavior. The different PDE subtypes, and more specifically their splice-variants, can be related to different disorders due to their differential expression in one or more of the frontal and striatal pathways or dopaminergic terminals inducing stimulation of dopamine synthesis, the inhibtion of D2 receptor signaling or the stimulation of D1 receptor signaling. The different PDE isoforms contain a multiplicity of structural and biochemical properties and are located in specific subcellular compartments, with specific transcriptional and posttranscriptional regulation (Keravis and Lugnier, 2012). Therefore, expression of a PDE subtype in a brain area does not make it an interesting target per se. Their particular involvement in dopaminergic modulation of fronto-striatal signaling is what makes them an interesting target for related disorders. Preferably, the targeted cyclic nucleotide signaling cascade is involved in the pathology of the disorder or contributes to the reduction of the pathology. However, even if this is not the case, PDE inhibition could still influence the overall signaling in a therapeutic direction. Currently, researchers are just beginning to unravel the precise subcellular localization and the role of functional compartmentalization in physiological and pathological conditions of the fronto-striatal circuits (e.g. PDE10A: Russwurm et al., 2015; Li et al., 2016b; MacMullen et al., 2016). Another important consideration is that, in general, PDE-I research involving fronto-striatal disorders is based on the classical view of basal ganglia direct and indirect pathway functioning. Considerable evidence is congregating challenging this classical view (Cui et al., 2013; Calabresi et al., 2014; Keeler et al., 2014).

From a therapeutic perspective, inhibition of PDEs with increased expression appears most promising. This way, cognition and plasticity deficits resulting from impaired cAMP/PKA signaling might be improved by inhibiting specific PDE isoforms. However, PDE inhibition might have negative effects on cognition and plasticity when PDEs are already downregulated and cAMP levels and PKA activity are high. In this scenario, elevated cAMP levels might go over a physiological level and disrupt signaling. Along this line, high doses of rolipram impaired prefrontal cognitive function in aged, but not young monkeys, likely due to overstimulation of the already disinhibited cAMP/PKA signaling pathway in the aged prefrontal cortex (Ramos et al., 2003; Arnsten et al., 2005). This argues to specifically target PDEs that are overexpressed (see Table 2).

Table 2. Changes in human phosphodiesterase mRNA levels in several fronto-striatal disorders

	Schizophrenia	Parkinson's disease		Huntington's disease		
	cerebellum	striatum	pallidum	striatum	pallidum	thalamus
PDE1	+ (1C)	NS	NS	NS	NS	NS
PDE2	NS	NS	NS	NS	NS	NS
PDE3	NS	NS	NS	NS	NS	NS
PDE4	= (4A1, 4A5, 4A8) - (4B1, 4B2, 4B3, 4B4)	NS	NS	NS	NS	NS
PDE5	NS	NS	NS	NS	NS	NS
PDE6						
PDE7	NS	NS	NS	NS	NS	NS
PDE8	+ (8B)	NS	NS	NS	NS	NS
PDE9	NS	NS	NS	NS	NS	NS
PDE10	= (10A)	- (10A)	- (10A)	- (10A)	- (10A)	+ (10A)
PDE11	NS	NS	NS	NS	NS	NS

+ increased, - decreased, = no change; NS not studied; (Fatemi et al., 2008a; Fatemi et al., 2008b; Fatemi et al., 2010; Marques et al., 2016; Niccolini et al., 2015a; Niccolini et al., 2015b; Russell et al., 2016)

In addition to the backbone formed by frontal neurons, MSNs and their dopaminergic modulation, the importance of interneurons in physiological and pathological fronto-striatal functioning is becoming increasingly apparent. Several types of interneurons can be found in the striatum, like cholinergic and different GABAergic interneurons (Gerfen and Surmeier, 2011). In particular, nitric oxide synthase (NOS) containing GABAergic interneurons we would like to highlight. These nitric oxide (NO)-producing interneurons play an important role in fronto-striatal functioning (West and Tseng, 2011). NO diffuses from these interneurons into dendrites of MSNs which contain high levels of guanylate cyclase (GC), which, when activated, lead to the synthesis of cGMP (see figure 1). In the intact striatum, transient elevations in intracellular cGMP primarily act to increase neuronal

excitability and to facilitate glutamatergic fronto-striatal transmission (West and Tseng, 2011; Threlfell and West, 2013). Although the main focus in the fronto-striatal system has been on cAMP signaling, several PDE-Is (also) target cGMP (e.g. PDE1-Is and PDE10-Is) and may exert their effects (additionally) on the cGMP signaling cascade (Padovan-Neto et al., 2015).

Summarizing, increased understanding of the subcellular localization and unraveling of the signalosome concept of PDEs including its function and dysfunction in the fronto-striatal circuits will contribute to the design of new specific inhibitors and enhance the potential of PDE-Is as therapeutics in fronto-striatal circuits.

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Statement of interest

LW is an employee of Intra-Cellular Therapies, which has a financial interest in the PDE1 inhibitor ITI-214. MAvD, AB and JP have a proprietary interest in the PDE4 inhibitor roflumilast.

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Chapter 4

Acute administration of roflumilast enhances sensory gating in healthy young humans in a randomized trial

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Abstract

Research has shown that the process of sensory gating is disrupted in patients suffering from clinical disorders including attention deficit hyperactivity disorder (ADHD), schizophrenia and Alzheimer's disease. Phosphodiesterase inhibitors (PDE-Is) have received an increased interest as a tool to improve cognitive performance in both animals and man. One of the cognitive areas investigated is sensory gating. Therefore, we investigated the effects of the PDE4-I roflumilast in a sensory gating paradigm in 20 healthy young human volunteers (age range 18 – 30 years). We applied a placebo-controlled randomized cross-over design and tested 3 doses (100, 300, 1000 µg). The current study (ClinicalTrials.gov Identifier: NCT01433666) showed that roflumilast improved sensory gating in healthy young human volunteers only at the 100 µg dose. This means roflumilast shows a beneficial effect on gating at a dose that had no adverse effects reported following single-dose administration. This indicates that roflumilast 100 µg has a favorable side-effect profile. Roflumilast and PDE4 inhibition in general could therefore be seen as a promising treatment in disorders affected by disrupted sensory gating.

Introduction

Sensory gating is a process involved in early information processing which prevents overstimulation of higher cortical areas by filtering sensory information (Adler et al., 1998; Cromwell, Mears, Wan, & Boutros, 2008; Freedman, Adler, Waldo, Pachtman, & Franks, 1983). The typical sensory gating paradigm consists of two identical auditory stimuli that are presented with an interstimulus interval (ISI) between 0.5 and 2 seconds (s) and an intertrial interval (ITI) of at least 6 s. The main principle is that the response to the second stimulus (S2) will be smaller than the response to the first stimulus (S1). The duration of the ISI is crucial and should not be shorter than 0.5 s or longer than 2 s; else sensory gating will not be elicited. In humans the P50 (or P1; i.e. the response evoked 50 ms after stimulus onset) of the event-related potential (ERP) is believed to be the main component in the sensory gating paradigm. Although the P50 reflects information processing at early stages it has also been associated with different cognitive functions (Yadon, Bugg, Kisley, & Davalos, 2009).

Human research has shown that the process of sensory gating is disrupted in patients suffering from clinical disorders including attention deficit hyperactivity disorder (ADHD), schizophrenia and Alzheimer's disease (Adler et al., 1982; Ally, Jones, Cole, & Budson, 2006; Boutros, Belger, Campbell, D'Souza, & Krystal, 1999; Cancelli et al., 2006; Green et al., 2015; Javitt, 2009; Jessen et al., 2001; Micoulaud-Franchi et al., 2015). The P50 has been suggested as a biomarker for the evaluation of drugs that may potentially have a beneficial effect on cognitive functions in schizophrenia (Javitt, Spencer, Thaker, Winterer, & Hajos), but despite the prominent role that P50 abnormalities have played in our understanding of schizophrenia, more data is needed to fully incorporate P50 as clinical correlate (Potter, Summerfelt, Gold, & Buchanan, 2006). For example, it has been shown that a nicotinic alpha-7 agonist improved cognition in schizophrenic patients and that this was associated with normalization in P50 deficits (Olincy et al., 2006; Zhang et al., 2012). One advantage of this EEG-related measure is that it can be used for translational purposes (Blokland, Prickaerts, van Duinen, & Sambeth, 2015; Drinkenburg, Ruigt, & Ahnaou, 2015; Smucny, Stevens, Olincy, & Tregellas, 2015).

The last decades, phosphodiesterase inhibitors (PDE-Is) have received an increasing interest as a tool to improve cognitive performance in both animals and man (Blokland, Menniti, & Prickaerts, 2012; Gomez & Breitenbucher, 2013; Maurice et al., 2014; Menniti, Faraci, & Schmidt, 2006; Reneerkens, Rutten, Steinbusch, Blokland, & Prickaerts, 2009; Richter, Menniti, Zhang, & Conti, 2013; Wang, Zhang, Zhang, & Li, 2015). As the initial focus of cognition enhancement was directed towards memory function, mainly in relation to Alzheimer's disease (for a review see Garcia-Osta, Cuadrado-

Tejedor, Garcia-Barroso, Oyarzabal, & Franco, 2012; Gurney, D'Amato, & Burgin, 2015; Heckman, Wouters, & Prickaerts, 2015), nowadays the relation between phosphodiesterases (PDEs) and cognitive processing is also investigated beyond the memory domain (e.g. Duinen et al., 2015; Heckman, Blokland, Ramaekers, & Prickaerts, 2015; Heckman et al., 2016; Shim, Shuman, & Duncan, 2016; Siuciak, 2008). One cognitive process in which PDE-Is might play a role is sensory gating (see Figure 1).

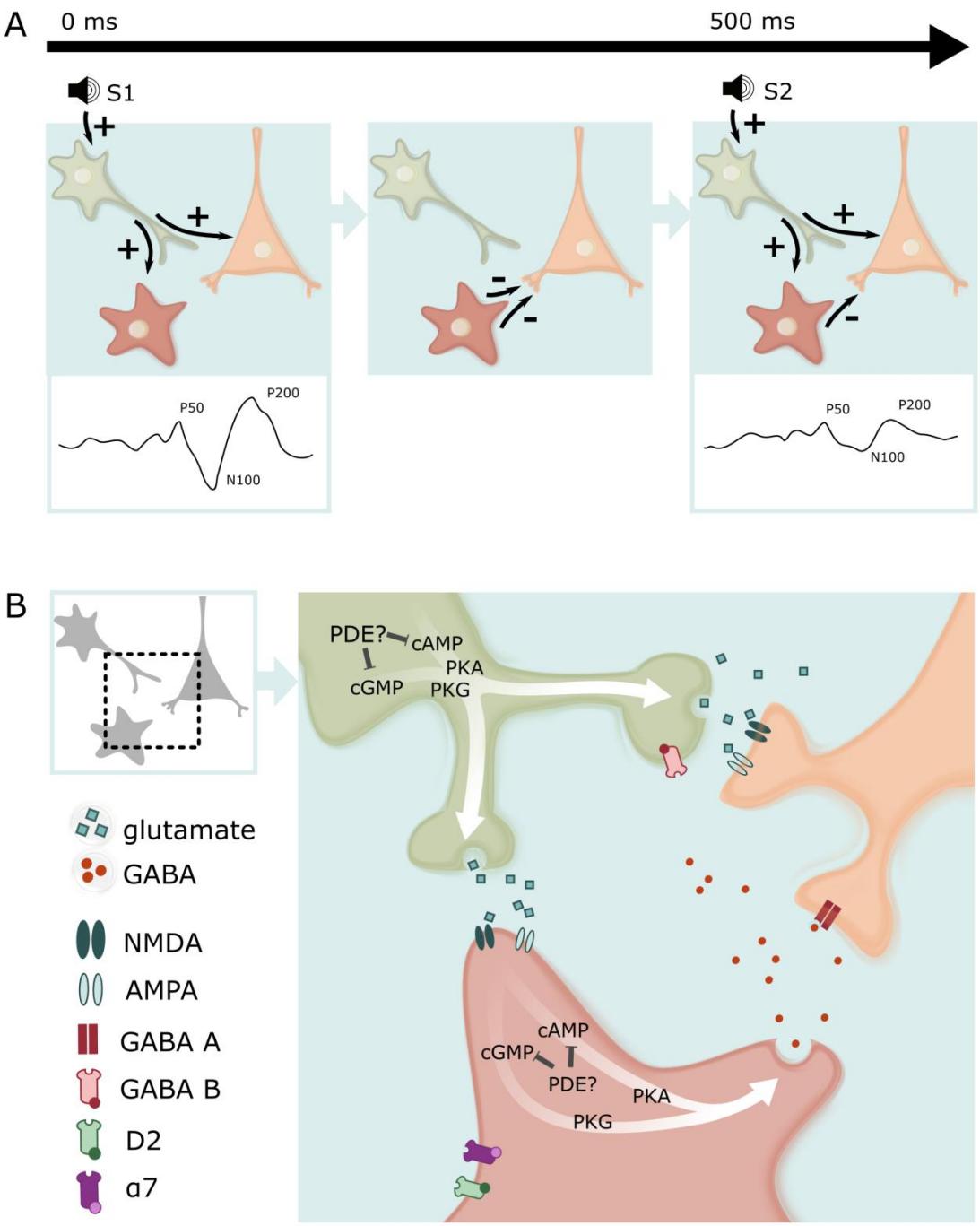

Fig. 1 Effects of PDE-Is on sensory gating are believed to be induced by targeting PDEs expressed in inhibitory interneurons of the auditory cortex and the thalamic 'gate', frontal inhibitory output neurons or in the interneurons that locally release inhibitory neurotransmitter in any other brain area capable of eliciting sensory gating. **A)** Auditory stimulus 1 (S1) excites an excitatory neuron, which in turn excites an inhibitory interneuron as well as an excitatory pyramidal neuron (left side figure). Activation of the inhibitory interneuron induces release of the inhibitory neurotransmitter GABA. GABA release causes fast inhibition of the pyramidal neuron

via postsynaptic GABA-A receptors (middle figure). Additionally, GABA released from the inhibitory interneurons induces slow, persistent inhibition of glutamate release from the first excitatory neuron onto the pyramidal neuron via presynaptic GABA-B receptors. This persistent inhibition reduces the activity of the pyramidal neuron for up to 8 s. Consequently, if S2 arrives the ERP amplitude will be reduced (right side figure).
B) More detailed depiction of the processes explained in A), showing the release site of glutamate and GABA as well as the location of their respective receptors. The postsynaptic GABA-A receptor inhibits the pyramidal neuron after activation by S1. The presynaptic GABA-B receptor induces the persistent inhibition of the first excitatory neuron thereby inducing gating.
Targeting any PDE subtype, e.g. PDE4, expressed in the inhibitory interneuron itself could enhance GABA release from the inhibitory interneuron when activated by S1. Additionally, any PDE subtype, e.g. PDE4, expressed in the first excitatory neuron's projections to the inhibitory interneuron could also enhance GABA release in the inhibitory interneuron. Consequently, both will result in an enhanced reduction of the S2-induced ERP amplitude. Note, however, that in the latter case, when the particular PDE subtype is expressed in the first excitatory neuron's projections to the inhibitory interneuron, this might also result in an enhanced response to S1. However, the latter is not observed in our study, indicating that the effect of roflumilast is more likely to occur in the inhibitory interneurons themselves.
Finally, the dopamine D2 receptor as well as the cholinergic α7 nicotinic receptor is depicted on the inhibitory interneuron. It is known from other studies that antipsychotic medication (D2 antagonists) enhances sensory gating. This possibly occurs via antagonism of the inhibitory effect D2 receptors exert on cAMP signaling and subsequent GABA release. In a similar but opposite manner, activating α7 nicotinic receptors on inhibitory interneurons enhances cAMP signaling in these neurons and increases associated GABA release.

Overall, only a limited number of human and animal studies have tested the effects of PDE-Is on sensory gating. Redrobe and colleagues (Redrobe et al., 2014), using the relatively new PDE2-I Lu AF64280, managed to induce an effect on sensory gating, i.e. reduction of an amphetamine-induced gating deficit in DBA2 mice. Both studies by Reneerkens et al. found no effects of either the PDE2-I BAY 60-7550 in rats, or the PDE5-I vardenafil in healthy rats as well as healthy young humans (in the absence of a deficit model)(Reneerkens, Sambeth, Blokland, & Prickaerts, 2013; Reneerkens, Sambeth, Van Duinen, et al., 2013). Another class of PDE-Is tested in the sensory gating paradigm are PDE10A-Is. PDE10A-Is are chosen with respect to the search for new antipsychotics in the field of schizophrenia research due to the high and exclusive expression of PDE10A in the striatum (Lakics, Karran, & Boess, 2010). Results for the PDE10A subclass of inhibitors are, however, mixed. On the one hand, no effects were found for the PDE10A-I PQ-10 in healthy rats (Reneerkens, Sambeth, Blokland, et al., 2013), an amphetamine-deficit model or a phencyclidine (PCP)-deficit model (Ahnaou, Biermans, & Drinkenburg, 2016). On the other hand, TP-10, another more potent PDE10A-I, did reverse impaired sensory gating in the hippocampus using the amphetamine-induced deficit model in rats (Schmidt et al., 2008).

The PDE9-Is PF-4447943 and PF-4449613 reversed an amphetamine-induced sensory gating deficit in mice (Kleiman et al., 2012). Additionally, PF-4447943 was tested in transgenic BACHD rats and Q175 mice (both transgenic animal models for Huntington's disease exhibiting impaired sensory gating) (Nagy, Tingley, Stoiljkovic, & Hajos, 2015). PF-4447943 dose-dependently improved the gating deficit in the primary auditory cortex and hippocampus of transgenic BACHD rats. Daily administration of PF-04447943 (1 mg/kg) over 7-days resulted in a complete recovery in their auditory gating in both brain structures. In Q175 mice, including wild-type, heterozygote and homozygote mice, PDE9 inhibition was without any effect.

PDE4-Is were tested in the sensory gating paradigm in two separate studies. The first study tested the first-generation PDE4-I rolipram (Maxwell, Kanes, Abel, & Siegel, 2004) and found that rolipram normalized the amphetamine-induced gating deficit in the hippocampus of mice. Another PDE4-I, RO-20-1724, (Halene & Siegel, 2008) restored gating in the hippocampus of mice using an amphetamine-induced deficit model.

Because it can be argued that the ERPs of humans and rats show a significant amount of similarities, although the basic components of sensory gating may still differ between animals and humans (e.g. de Bruin et al., 2001), we expect that the effects of PDE-Is on sensory gating in animals translate to humans (Maxwell et al., 2004). Based on the few studies that are available, it appears that PDE4 is a promising PDE subtype followed by PDE9 and PDE10 (see Heckman, Blokland, et al., 2015). The PDE4-I roflumilast was the first oral obtainable PDE4-I clinically approved at a daily dose of 500 µg, i.e. to treat chronic obstructive pulmonary disease (COPD). In the first clinical trials, roflumilast was able to improve lung function and to reduce the exacerbation of COPD, while the side effects consisted of very mild nausea, diarrhea and a light headache. Recently, we (Vanmierlo et al., 2016) and others (Jabaris et al., 2015) have shown that roflumilast is brain penetrant and improves short-term and long-term memory in rodents. Importantly, a PET study with the ligand [18F]B9302-107 for roflumilast confirmed that the currently marketed dose for COPD is also brain penetrant in humans (http://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/022522Orig1s000ClinPharmR.pdf, p. 150-151). On basis of these data, and the low emetic effects of roflumilast, this offered an excellent opportunity to investigate the cognitive effects of a PDE4-I in humans. Therefore, we investigated in the current study (ClinicalTrials.gov Identifier: NCT01433666) the acute effects of roflumilast in a sensory gating paradigm in healthy young human volunteers at three different doses, i.e. 100, 300 and 1000 µg. We hypothesized roflumilast to enhance sensory gating in healthy young humans

without exhibiting an effect on overall auditory processing as indicated by auditory evoked potentials (AEP).

Methods

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 (age range 18 – 35 years) were recruited through advertisements at Maastricht University between November 2011 and June 2012. Participants had to be willing to sign an informed consent form 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, hematological or psychiatric illness. Other exclusion criteria were excessive drinking (>20 glasses of alcohol-containing beverages a week), pregnancy or lactation, use of medication other than oral contraceptives, use of recreational drugs from two weeks before, and until the end of, the experiment, and any sensory or motor deficit which could reasonably be expected to affect test performance. In addition, participants who had a first-degree relative with a (history of) psychiatric disorder were excluded as well. The participants could leave the study at any given time without any consequences. In total 20 participants (mean age 20.9 ± 2.3 years; 4 male/16 female) were included in the study.

EEG recordings

An EEG cap was used to place a set of 32 EEG electrodes according to the international 10–20 system (Klem, Luders, Jasper, & Elger, 1999). 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 (Wan, Crawford, & Boutros, 2006). In addition, the Fz electrode has been demonstrated to show a similar amount of P200 gating and was therefore included as well (Wan, Crawford, & Boutros, 2007). A reference and a ground were placed at the left mastoid and at the forehead, respectively. Eye movements were detected by horizontal and vertical electro-oculogram (EOG) recordings. Before electrode attachment, the positions were 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 and 10 s. The participants were familiarized with the test during a training session.

Design and treatment

The study was conducted according to a double-blind, placebo-controlled, four-way cross-over design. The current study was part of a larger project with the same ClinicalTrials.gov Identifier (NCT01433666), investigating the cognition enhancing effects of roflumilast. The treatment order was balanced over the four test days and separated by a washout period of at least 10 days. The balancing of the treatment order was accomplished by counterbalancing. Roflumilast HCl (Daxas) 500 µg tablets were grinded, and the appropriate quantities (i.e., 100, 300, 1000 µg) were distributed over capsules with lactose monohydrate as the principle constituent. The placebo capsules only contained lactose monohydrate in an equivalent amount and the appearance was identical to the roflumilast capsules. The capsules were manufactured, blinded, and labelled by Basic Pharma Technologies BV (Geleen, the Netherlands) according to GMP regulations. Randomization personnel (not otherwise involved in the study) generated the randomization schedule, which was provided to the contract packaging facility prior to the start of the study. All randomization information was stored in a secured area, accessible only by authorized personnel. Treatment on each of the four test days consisted of a single capsule containing either placebo, 100, 300 or 1000 µg roflumilast. Previous studies have shown that peak plasma levels of roflumilast were reached 30–120 min (median, 60 min) after a single dose of 500 µg roflumilast; the terminal half-life was around 17 h for roflumilast and 30 h for its N-oxide metabolite (Bethke et al., 2007). Since this study was part of a larger project comprising multiple cognitive tasks investigating several cognitive domains, our sensory gating paradigm was tested 90 min after drug treatment. The drugs were ingested orally and combined with a low-fat breakfast, because fatty food might affect the absorption of roflumilast. The experimenter and participants were blind to the compound and doses tested. All testing was conducted at the department of Neuropsychology and Psychopharmacology at Maastricht University.

Questionnaire

After each session the subjects were asked to fill in a questionnaire. Physical complaints were measured by a general list consisting of 31 items with a 4 point scale ranging from 0: ‘not at all’ to 3: ‘strongly’.

Statistical analysis

All EEG data was analyzed with Vision Analyzer 2.0 (Brain Products GmbH, Gilching, Germany). After offline re-referencing the signal combining the left and right mastoids, the EEG signal was filtered with a high-pass filter of 10 Hz and a low-pass filter of 40 Hz. Next, eye movement artefacts were removed using the Gratton and Coles method (Gratton, Coles, & Donchin, 1983). Segments between 100 ms before until 500 ms after stimulus onset were constructed for each stimulus type (S1 and S2) separately, using the last 100 ms before S1 onset as baseline for both stimuli. The segments were visually checked for artefacts and removed from the dataset if an artefact occurred during the first 500 ms after stimulus presentation. The grand average over participants was used to determine the AEP components. P50 was defined as most positive value between 65 and 110 ms after stimulus onset, N100 as most negative value between 90 and 170 ms and P200 as most positive value between 170 and 260 ms. Due to violation of normality, data was analyzed using nonparametric tests for the amplitudes of the AEP component at the Fz, FCz and Cz locations (channels). First, outliers were removed from the raw data. Next, effects of roflumilast on basal information processing were evaluated by comparing treatment effects on auditory evoked potentials (S1). Subsequently, the responses to the S1 and S2 (S1-S2/S2) were compared by means of Wilcoxon Signed-ranks tests for the placebo condition to determine whether sensory gating occurred. To correct for variation in S2, relative gating scores were calculated (S1-S2/S2). Next, the responses to the roflumilast conditions were compared with the placebo condition for the scores by means of Wilcoxon Signed-ranks tests.

Results

Physical Complaints

After administration of 100 µg, the subjects did not report any physical complaint. At 300µg four subjects reported mild nausea, but no other complaints. After the highest dose mild nausea was reported by 3 subjects and 5 subjects reported a higher level of nausea. In addition, two subjects reported diarrhea at the highest dose.

Effects of roflumilast on auditory evoked potentials (S1)

No effects of PDE4 inhibition by roflumilast (100 µg, 300 µg and 1000 µg) compared to placebo treatment were found with the Wilcoxon Signed-ranks test on the S1 or S2 stimulus for the P50 peak for neither the Fz channel nor the FCz and Cz channels (data not shown). Also, no effects were found on the two other ERP components (N100 and P200; data not shown).

The sensory gating paradigm

Effects of placebo treatment on sensory gating for the Fz electrode are depicted in figure 2. Analysis by means of a Wilcoxon Signed-ranks test for the three channels Fz, FCz and Cz separately showed

that gating occurred in all three channels for the P50 peak (Z = -2.05, p < .05; Z = -3.10, p < .01; Z = -2.91, p < .01). Additionally, sensory gating occurred for the N100 peak for all three channels (Z = -3.54, p < .001; Z = -3.88, p < .001; Z = -3.85, p < .001) as well as for the P200 peak of all three channels (Z = -3.68, p < .001; Z = -3.92, p < .001; Z = -3.82, p < .001).

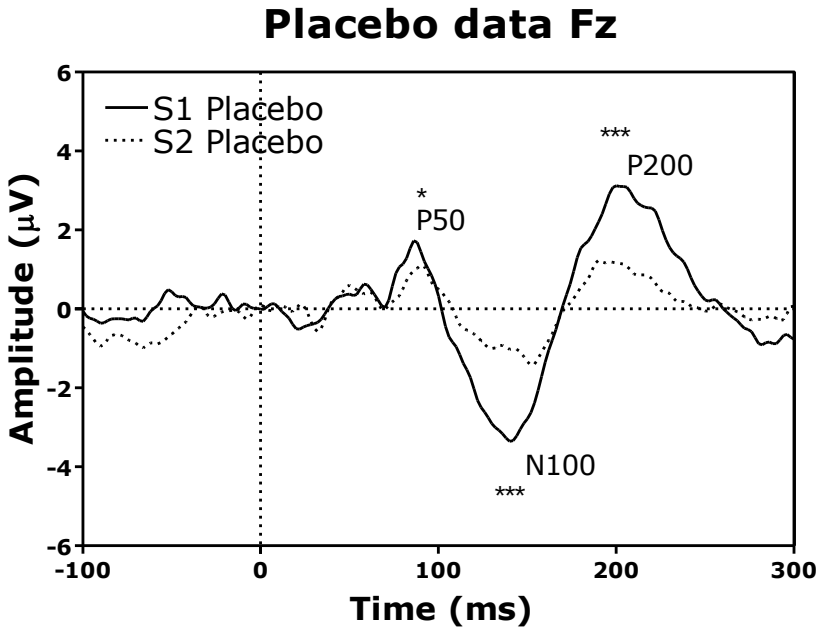

Fig. 2 Placebo ERPs (P50, N100 and P200 peaks) after presentation of S1 and S2. Sensory gating, i.e. a difference between S1 and S2, is depicted with *asterisks* (Wilcoxon Signed-ranks test: *p<.05; ***p<.001). Latencies are shown on the x-axis in milliseconds, amplitudes on the y-axis in microvolts.

Effects of roflumilast on sensory gating

The effect of roflumilast (100 μg, 300 μg and 1000 μg) on sensory gating is shown in figure 3. A Wilcoxon Signed-ranks test indicated that sensory gating significantly improved at the Fz electrode for the P50 component after treatment with 100 μg roflumilast compared to placebo, Z = -2.01, p < .05. No effects of roflumilast are found on the N100 or P200 ERP components of any of the channels.

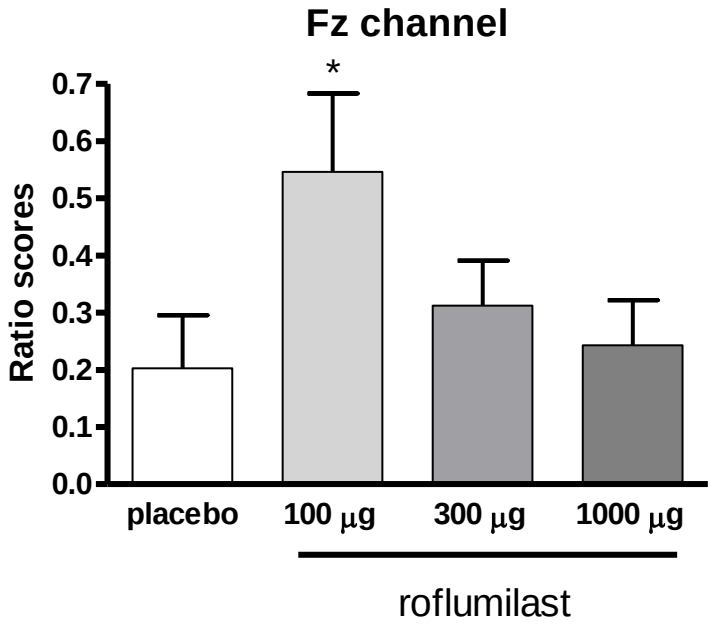

Fig. 3 Effects of treatment with the PDE4 inhibitor roflumilast on the mean relative gating score (±SEM) of the P50 peak of the Fz channel. An effect on sensory gating, i.e. different gating score compared to placebo, is depicted with an *asterisk* (Wilcoxon Signed-ranks test: *p<.05). Compounds/doses are depicted on the x-axis; ratio scores are depicted on the y-axis (higher ratio scores indicate better sensory gating)

Discussion

In the current study we investigated whether the PDE4-I roflumilast could enhance sensory gating in a dose-dependent manner in healthy young human volunteers without exhibiting an effect on overall auditory processing as indicated by AEP. Results showed that roflumilast significantly improved sensory gating in healthy young human volunteers. The effect was induced in a dose-dependent manner. The effective dose of 100 μg is 5 times lower than the clinically approved dose for the treatment of acute exacerbations in COPD. Notably, no emetic side effects were reported by the participants after administration of this low dose. This means roflumilast shows a beneficial effect on gating at a dose that had no adverse effects reported following single-dose administration in the present study. This indicates that roflumilast 100 μg has a favorable side-effect profile. In the current study, the three doses of roflumilast administered resulted in plasma levels at the time of testing which could be expected based on PK studies measuring roflumilast, 2.09, 6.27 and 8.19 ng/mL, respectively (Lahu, Nassr, & Hunnemeyer, 2011). Most importantly, based on the present EEG data, it is clear that a dose of 100 μg roflumilast is centrally active.

As shortly mentioned before, a clear distinction should be made between effects on AEPs (S1) and effects on sensory gating, even though both are considered 'early information processing'. Different PDE families and their inhibitors can distinctively affect AEPs and sensory gating. Furthermore, whether sensory gating is expressed as a ratio score (e.g. S2/S1), difference score (e.g. S1-S2), proportional score (e.g. S1-S2/S2) or percentage score (e.g. (S1-S2/S2)*100), it always explains S2 in terms of S1. An effect on AEPs after S1 will also change the ratio between S1 and S2 which has to be taken into consideration when interpreting an effect on sensory gating. An effect on S1 indicates an effect on basic information processing. To induce a true effect on sensory gating, S1 should not be affected by the drug. A significant S2 effect (decreasing amplitude) would support drug effects on sensory gating though is not necessary, as long as the relative gating score is showing significant drug effects, i.e. there is a difference on this score between drug conditions. We found that S1 did not differ between the placebo and the 100 µg roflumilast condition. We found that roflumilast did not affect S1 and that the S1-S2 ratio was enhanced after treatment with the 100 µg dose. This indicates that roflumilast enhances P50 gating in young healthy volunteers.

Another point of attention regards the fact that in preclinical studies an amphetamine-induced deficit was reversed by a PDE4 inhibitor (Halene & Siegel, 2008; Maxwell et al., 2004). This might be regulated by a very different mechanism compared to the mechanism involved in enhanced unimpaired sensory gating in healthy volunteers. In schizophrenia the dopamine hypothesis has been revised to postulate that positive symptoms, in particular, arise from hyperactivation of the dopaminergic D2 receptor subtype in mesolimbic brain regions (Brisch et al., 2014). Disruptive effects of amphetamine on sensory gating are suggested to be caused by hyperactive dopamine transmission resembling the dopamine hypothesis in schizophrenia (Smucny et al., 2015). The released dopamine will bind to the D2 receptors thereby inhibiting the inhibitory interneurons. Excess dopamine will thus lead to excessive throughput impairing normal gating. This hypothesis is supported by the fact that dopamine D2 receptor antagonists can prevent the amphetamine-induced deficits in sensory gating (During, Glenthoj, Andersen, & Oranje, 2014; Freedman et al., 1987; Light et al., 1999; Witten et al., 2016). Dopamine D2 receptor antagonism prevents inhibition of inhibitory interneurons. Note, however, that in the field of schizophrenia research dopaminergic drugs generally show no gating enhancing effects, outside of the amphetamine-deficit model, in schizophrenia patients, healthy humans and animals, and animal models of schizophrenia other than the amphetamine-deficit model (Adler et al., 1990; Arango, Summerfelt, & Buchanan, 2003; During et al., 2014; Freedman et al., 1983; Light et al., 1999; Oranje et al., 2004; Sanchez-Morla et al., 2009; Siegel et al., 2005; Simosky, Stevens, Adler, & Freedman, 2003). Thus, so far D2 antagonism has not convincingly proven to be effective on sensory gating in healthy subjects (either animal or man; e.g.

(Nagamoto et al., 1996). Therefore, reversal of the amphetamine-deficit model by PDE inhibition could be merely seen as proof of its additional antipsychotic potential resembling D2 antagonism.

Dopamine is not the only neurotransmitter that can affect sensory gating. In theory, any signaling system, combined or by itself, affecting downstream structures capable of exhibiting gating, can show effects in a sensory gating paradigm. Both noradrenaline (e.g. Adler et al., 1994; Witten et al., 2016) and acetylcholine (e.g. Adler, Hoffer, Wiser, & Freedman, 1993; Adler, Hoffer, Griffith, Waldo, & Freedman, 1992; Adler et al., 2001; Adler et al., 1998) have been shown to affect sensory gating. Especially, the cholinergic system is of interest as a treatment for gating deficits in schizophrenia as the inhibitory interneurons contain, next to the dopamine D2 receptors, α7 nicotinic acetylcholine receptors which upon activation stimulate GABA (γ-aminobutyric acid) release (Albuquerque, Pereira, Braga, Matsubayashi, & Alkondon, 1998; Frazier et al., 1998; Young & Geyer, 2013)(see Figure 1). In theory, PDEs expressed in any neuron modulating the inhibitory interneuron, like the dopaminergic and cholinergic projections, could modulate sensory gating.

Our results with roflumilast are thus not easily explained by the neurobiological mechanisms underlying sensory gating. In general, effects of PDE-Is on sensory gating are believed to be induced by targeting PDEs expressed in inhibitory interneurons of the auditory cortex and the thalamic 'gate', frontal inhibitory output neurons or in the interneurons that locally release inhibitory neurotransmitter in any other brain area capable of eliciting sensory gating. More simplified, in the above mentioned brain areas, S1 excites an excitatory neuron, which in turn excites an inhibitory interneuron as well as an excitatory pyramidal neuron (Smucny et al., 2015)(see figure 1). Activation of the inhibitory interneuron induces release of the inhibitory neurotransmitter GABA. GABA release causes fast inhibition of the pyramidal neuron via postsynaptic GABA-A receptors. Additionally, GABA released from the inhibitory interneurons induces slow, persistent inhibition of glutamate release from the first excitatory neuron onto the pyramidal neuron via presynaptic GABA-B receptors (Hershman, Freedman, & Bickford, 1995). This persistent inhibition reduces the activity of the pyramidal neuron for up to 8 s. Consequently, if S2 arrives the ERP amplitude will be reduced.

Hypothetically, targeting any PDE subtype, e.g. PDE4, expressed in the first excitatory neuron's projections to the inhibitory interneuron as well as PDEs expressed in the inhibitory interneuron itself could enhance output of both neurons when activated by S1. Consequently, the S2-induced ERP amplitude will be further reduced. Note, however, that PDEs expressed in the first excitatory neuron's projections directly to the pyramidal neuron must not be enhanced since this would increase the response to S1 and therefore positively affect general auditory information processing.

Roflumilast enhances sensory gating without exhibiting an effect on S1. Therefore, the effect of roflumilast is more likely to occur in the inhibitory interneurons themselves. PDE4 is indeed relatively highly expressed in brain areas associated with the neurobiology of sensory gating (Lakics et al., 2010). However, due to the extended neurobiology of sensory gating and the potential expression of PDE subfamilies within the brain areas involved, it is conceivable that inhibition of more than one subtype of PDE could enhance sensory gating (potentially accounting for the aforementioned positive effects of PDE9-Is and PDE10-Is).

Additionally, PDE4-Is may function like dopamine D2 receptor antagonists although it needs to be determined whether this is directly beneficial for sensory gating. Taken together, future preclinical studies will have to provide more insight into the mechanism by which PDE4 inhibition enhances sensory gating in healthy and pharmacologically-impaired volunteers, and eventually patients. Of note, when comparing results several translational considerations should be taken into account, like site of measurement (local in rodents vs superficial in humans), mental state (anesthetized in rodents vs awake in rodents or humans), treatment duration (acute vs chronic) and route of drug administration (mostly intraperitoneal/subcutaneous in rodents vs mostly oral in humans), but also differences in PDE4 expression and pharmacokinetic properties of the drug (cf. Blokland, van Goethem, Heckman, Schreiber, & Prickaerts, 2014).

Due to the positive effects of roflumilast on sensory gating observed in the current study the compound seems to be a promising treatment to test in disorders affected by disrupted sensory gating, like schizophrenia, Alzheimer's disease and ADHD. Additional support for roflumilast in these disorders is provided by recent studies that showed roflumilast to induce memory enhancing effects in rodents (Jabaris et al., 2015; Vanmierlo et al., 2016). In this study effects are exerted at the same low acute dose of roflumilast, thereby minimizing emetic adverse events. A low dose of roflumilast might therefore be seen as a promising treatment for cognitive symptoms in ADHD, schizophrenia and Alzheimer's disease.

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Statement of interest

TU is an employee of Takeda Development Center Americas. MAvD, AB, JP and AS have a proprietary interest in the PDE4 inhibitor roflumilast.

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Chapter 5

Effects of the phosphodiesterase type 4 inhibitor roflumilast on the tri-phasic response of the substantia nigra pars reticulata after infralimbic cortex stimulation

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ABSTRACT

Fronto-striatal circuits constitute the neurobiological basis of many neuropsychiatric disorders. Part of the intracellular signaling within these circuits, including its dopaminergic modulation, is regulated by the cAMP/PKA signaling cascade. Based on the overall expression in human fronto-striatal circuitry, we tested the effects of a cAMP selective PDE4 inhibitor on the tri-phasic response in the dorsomedial substantia nigra pars reticulata (SNr) upon stimulation of the infralimbic cortex in rats. Our results show for the first time that stimulation of the cognitive infralimbic cortex leads to a tri-phasic response in SNr neurons. In addition and in line with previous biochemical and behavioral studies, PDE4 inhibition by roflumilast affects both the direct pathway as well as the indirect pathway of which the latter appears more sensitive than the former.

Keywords: phosphodiesterase inhibitor, roflumilast, substantia nigra pars reticulata, fronto-striatal circuits

INTRODUCTION

The fronto-striatal circuits are parallel organized circuits running from the frontal cortex, through the basal ganglia structures, to the thalamus from where they project back to the frontal cortex (Alexander et al., 1986; Alexander and Crutcher, 1990). The fronto-striatal circuits can be divided into three groups based on biological function, i.e. motor, associative/cognitive and limbic (Alexander et al., 1986). All circuits are characterized by their modulation by dopamine at the level of the striatum (Greengard et al., 1999; Greengard, 2001; Svenningsson et al., 2004) and their division into three pathways within the basal ganglia, i.e. the hyperdirect pathway, the direct pathway and the indirect pathway. Altogether, the fronto-striatal circuits comprise a complex mechanism of action and functionality, which is abundantly described elsewhere (e.g. Surmeier et al., 2007; Haber and Rauch, 2010; Gerfen and Surmeier, 2011; Surmeier et al., 2011; Calabresi et al., 2014).

In the striatum, dopaminergic neurotransmission is regulated by the cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) signaling cascade targeting effectors like cAMP response element-binding protein (CREB) and Dopamine- and cAMP-Regulated PhosphoProtein MR 32 kDa (DARPP-32) (Mayr and Montminy, 2001; Svenningsson et al., 2004). Consequently, dopaminergic signaling is regulated by phosphodiesterases (PDEs), as cAMP is degraded by PDEs. Because of its substrate cAMP and based on the overall expression in human fronto-striatal circuitry (Lakics et al., 2010), the PDE4 family is of particular interest (for a review see Heckman et al., 2016). However, little is known regarding the distinct expression of PDE4 within the three individual basal ganglia pathways.

Therefore, in the current study we used an *in vivo* electrophysiology approach to investigate the three basal ganglia pathways. For this we measured extracellular neural activity in the substantia nigra pars reticulata (SNr) during frontal cortex stimulation (Kolomiets et al., 2003). In particular, we were interested in the circuits involving cognitive function rather than fronto-striatal circuits involving motor or limbic functions (Deniau et al., 1996). In rats, these encompass the fronto-striatal circuits originating in the medial prefrontal cortex (mPFC; prelimbic and infralimbic cortices) and the orbitofrontal cortex. We focused on the circuit originating in the infralimbic cortex instead of the prelimbic cortex or orbitofrontal cortex, because sparse evidence confirms the existence of the three pathways (hyper, direct and indirect pathways) in this circuit. Also, the infralimbic cortex projections leave the basal ganglia mainly via the ventral pallidum (e.g. Deniau et al., 1994; Groenewegen et al., 1999) with less projections via the SNr (Vertes, 2004). We hypothesize that stimulation of the infralimbic cortex may lead to a tri-phasic response in the SNr (Maurice et al., 1999), topographically and functionally associated with the ventral parts of the basal ganglia. The temporal and topographic

sensitivity of this electrophysiological response combined could directly address the distinctive effects of PDE4 inhibition in the three basal ganglia pathways, and thus determine PDE4 function and PDE4 inhibitor applicability to specifically influence fronto-striatal cognitive function.

EXPERIMENTAL PROCEDURES

Animals

A total of 24 male Wistar rats (age 3 months, 260–380 g, Charles River, Margate, Kent, UK) were used. Animals were housed collectively with *ad libitum* access to food and water. In the animal facility a normal 12-h light/dark cycle was maintained. *In vivo* electrophysiological experiments including drug administration were conducted during day time. All experiments were conducted at the University of Oxford at the University Department of Pharmacology in accordance with the Animals (Scientific Procedures) Act 1986 (UK) and were approved by a local Ethical Review Process at the University of Oxford.

Electrophysiological recordings

General anesthesia was induced with isoflurane (Isoflu, Abbott, Queenborough, Kent, UK) and maintained with urethane (1.3–1.5 mg/kg, ethyl carbamate, Sigma, Steinheim, Germany), supplemented with doses of ketamine (30 mg/kg, i.m.; Narketan, Vetoquinol, Buckingham, Buckinghamshire, UK) and xylazine (10 mg/kg, i.m.; Rompun, Bayer, Newbury, Berkshire, UK) whenever necessary. Subsequently, animals were placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). Surgery site was pretreated with bupivacaine for local anesthesia. Corneal dehydration was prevented by applications of Lacrilube eye gel (Allergan, Coolock, Dublin, Ireland). Body temperature was maintained at 37 ± 0.5 °C using a homeothermic heating blanket (Harvard Apparatus, Kent, UK).

An electrocorticogram (ECoG) was recorded over the left motor cortex (2.6 mm posterior, 2.0 mm lateral to bregma) to monitor the depth of anaesthesia (see Schweimer et al. 2011 for details). Craniotomies were performed above the infralimbic cortex (3.2 mm anterior, 0.6 mm lateral to bregma) for the stimulation electrode; and above the SNr (5.2 mm posterior, 2.0 mm lateral to bregma) for the recording electrode (Paxinos and Watson, 2009). Saline was applied to the exposed brain surface to prevent dehydration.

Extracellular neuronal activity was monitored with a 10–25 MΩ glass microelectrode filled with 1.5% Neurobiotin (Vectorlabs, Burlingame, CA, USA) in 0.5 M NaCl (tip diameter 1–1.5 µm). The

microelectrode was lowered into the SNr with a single-axis in vivo micromanipulator (IVM) controlled via LINLAB software (Scientifica, Uckfield, UK). Electrode signals were alternating current (AC)-coupled, amplified (1000x), and band-pass filtered (0.3–5 kHz) using a Neurolog system (Digitimer, Welwyn Garden City, Hertfordshire, UK) and acquired on-line through a Micro1401 interface and Spike2 software (Cambridge Electronic Design, Cambridge, Cambridgeshire, UK). Mains noise at 50 Hz was eliminated ('Humbug' filter, Brown et al., 2002) for single unit and ECoG recordings.

Electrical stimulation of the medial prefrontal cortex (infralimbic cortex)

Electrical stimulation of the infralimbic cortex (anterior: 3.2; lateral: 0.6 to bregma; ventral: -4.5 mm from the cortical surface), ipsilateral to the recording SNr site, was performed with a bipolar coaxial stainless steel electrode (200 µm tip diameter, 500 µm shaft diameter, 500 µm exposed inner and outer contact; NE-100 Harvard Apparatus, UK) positioned stereotaxically according to Paxinos and Watson (2009). Electrical stimuli were generated from a constant current isolated stimulator (WPI A360 stimulus isolator, Sarasota, Florida, USA) controlled via a Master-8 (A.M.P.I., Israel). Stimulation consisted of monopolar pulses of 0.6 ms width and 500 µA intensity delivered at a frequency of 1.4 Hz.

Drugs

All drugs were freshly prepared before each experiment. Roflumilast (kindly provided by Takeda, Konstanz, Germany) was administered i.v. in accumulating doses (0.0025, 0.005 and 0.01 mg/kg). This was done to establish a dose-response curve and maximizing the data per animal, and thereby reduce animal numbers. Roflumilast was dissolved in 10% Kolliphor HS 15 (Sigma-Aldrich, Schnelldorf, Germany) and 90% isotonic saline (0.9%, Scheller et al., 2014) to make it suitable for i.v. administration.

Extracellular single unit recordings of the SNr neurons, pharmacological treatment and experimental design

Initially, the recording electrode was lowered to: anterior: -5.2 mm, lateral: 2.0 mm lateral to bregma; ventral: -7.0 mm from the cortical surface according to Paxinos and Watson (2009). For each experiment the dorsal border of the SNr was located by identifying dopaminergic neurons of the SNc. Criteria for dopaminergic SNc neurons were broad action potentials (>1.1 ms), and low to moderate frequency discharge (2-10 Hz) (Bunney et al., 1973; Ungless and Grace, 2012). Ventral of the SNc layer of dopaminergic neurons, SNr neurons can be found. Electrophysiological characteristics of the SNr neuron were thin spikes (width < 0.55 ms), high-frequency discharge (>10 Hz) without a decrease in the spike amplitude (Bunney et al., 1973; Deniau et al., 1978; Kolomiets et al., 2003). Once an SNr

neuron was detected a stable baseline firing rate was established for 2 minutes, followed by a 2 min stimulation period to check for the typical tri-phasic response of the SNr (excitation-inhibition-excitation). Presence of a tri-phasic response was also used as electrophysiological characteristic of the SNr neuron. The recording pattern was repeated if the neuron exhibited a tri-phasic response to determine stable baseline spike train parameters as well as a stable tri-phasic response; followed by drug injections (i.v.) at 4 min intervals (2 min stimulation off and 2 min stimulation on). This allowed for testing of the effects of different doses of roflumilast on baseline spike train parameters as well as the SNr tri-phasic response (see Table 1).

Table 1. Schematic overview of the experimental stimulation and recordings

Time (min)	Stimulation infralimbic cortex (on/off)	Experimental step
T=0	off	Detection SNr neuron according to electrophysiological characteristics
T=2	off	Establishment of stable baseline firing
T=4	on	Verification of typical tri-phasic response of the SNr (excitation-inhibition-excitation) after stimulation infralimbic cortex
T=6	off	Verification stable baseline spike train parameters
T=8	on	Verification stable tri-phasic response
T=10	off	Injection vehicle (i.v.)
T=10	off	Measuring effects dosing on baseline spike train parameters
T=12	on	Measuring effects dosing on tri-phasic response
T=14	off	Injection 0.0025 mg/kg roflumilast (i.v.)
T=14	off	Measuring effects dosing on baseline spike train parameters
T=16	on	Measuring effects dosing on tri-phasic response
T=18	off	Injection 0.005 mg/kg roflumilast (i.v.)
T=18	off	Measuring effects dosing on baseline spike train parameters
T=20	on	Measuring effects dosing on tri-phasic response
T=22	off	Injection 0.01 mg/kg roflumilast (i.v.)
T=22	off	Measuring effects dosing on baseline spike train parameters
T=24	on	Measuring effects dosing on tri-phasic response
T=26	off	Juxtacellular labelling or iontophoresis
T=86	off	Leave to settle
T=131	off	Transcardial perfusion

SNr = substantia nigra pars reticulata; i.v. = intravenous injection

Histochemistry

Following pharmacological treatment, animals were overdosed with pentobarbitone, brains were removed and kept in 4% PFA overnight, before being transferred to a 30% sucrose solution for cryoprotection. Coronal sections (30 µm) were cut using a cryostat (Bright Instruments Ltd, Luton, Bedfordshire, UK). Posterior sections containing the complete SNr were checked using a light-microscope (Leica Dialux 20) to verify recording electrode location. Additionally, PFC sections were stained using a standard Nissl-staining protocol to verify stimulation electrode location. Prior to the Nissl-staining, sections were mounted and hydrated in decreasing concentrations of ethanol. Following the Nissl-staining, sections were first dehydrated in increasing concentrations of ethanol and subsequently treated with isopropanol and xylene. Sections were examined under a light-microscope (Leica Dialux 20). Brightness and contrast of the images were adjusted using ImageJ software (version 1.50, ImageJ, National Institute of Health, Bethesda, MD, USA).

Data and statistical analysis

For the baseline periods, the interspike interval, coefficient of variation of the interspike interval, firing rate, and spike waveform width (time taken from a 5% increase from baseline to the first trough) were calculated for all neurons (e.g. Schweimer et al., 2011; Brouard et al., 2015).

We calculated the peristimulus-time histograms (PSTH) and response magnitudes as previously described in Beyeler et al. (2010). PSTH were generated for every 2 min stimulation period (5-ms bins). The baseline was defined as the mean of the spikes of the 100 ms epoch in each, individual PSTH preceding the cortical stimulation. In addition, the standard deviation (S.D.) of the baseline was determined. A significant excitation was considered if the mean of all bins within the excitatory epoch exceeded the mean baseline activity by 2 S.D. An inhibition was defined as a period during which the mean of bins of the inhibitory epoch is below 70% of the baseline mean (Georges and Aston-Jones, 2002).

Excitatory response magnitudes (R_{mags}) were normalized for different levels of baseline activity, allowing for comparison of drug effects on evoked responses independent of effects on baseline activity or differences in firing rate (Georges and Aston-Jones, 2002). R_{mags} for excitation were calculated with the following equation: Excitation R_{mag} = (counts in excitatory epoch) – (mean counts per baseline bin x number of bins in excitatory epoch). In contrast, for the inhibition the R_{mag} is expressed in absolute counts due to the low number of counts in the inhibition period. Expressing these low counts in the same way as positive R_{mags} induces artificially high values (i.e., percentages) and high variability. The count numbers during the inhibition period were normally distributed and

were comparable between the neurons. Finally, to determine the latency and the duration of each phase of the response, PSTH were generated from "2 minutes of" trials using 1-ms bins (Beyeler et al., 2010).

Statistical analysis was carried out using IBM SPSS Statistics 24 software (IBM, Portsmouth, UK). The raw data were checked for outliers and normality of distribution. Missing values were replaced using the multiple imputation function in SPSS using 5 imputations (Olivier et al., 2009). One-way repeated measures analysis of variance (ANOVA) followed by Sidak's post-hoc test was used. Data are depicted as means ± SEM.

RESULTS

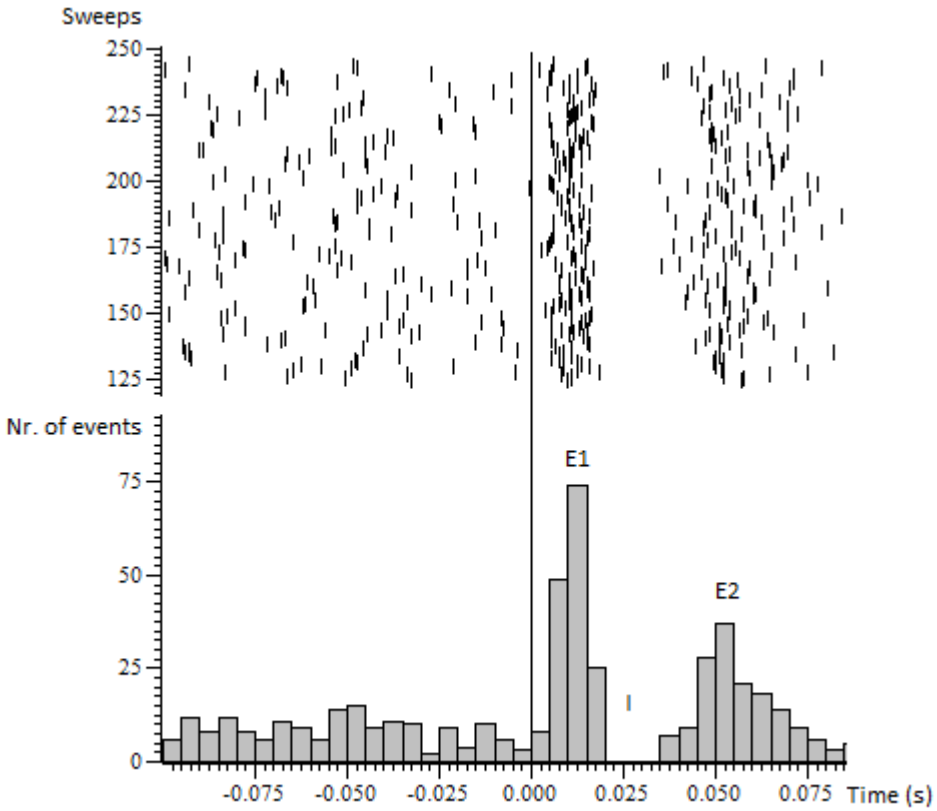

Figure 1. Raster plot and peristimulus-time histogram demonstrating a typical tri-phasic (excitation-inhibition-excitation) response of a SNr neuron to infralimbic cortex stimulation. Vertical line at T=0 represents the time of stimulation; E1= early excitation phase of the tri-phasic response; I= inhibition phase of the tri-phasic response; E2= late excitation phase of the tri-phasic response. Baseline firing properties of SNr neurons displaying a tri-phasic response (N=6): Average spike waveform width: 0.0013 ± 0.00021 (s); Firing rate (Hz):

25.89 ± 3.81; Coefficient of variation of the interspike interval: 0.25 ± 0.017; Latency first excitation(s): 0.0048 ± 0.0014.

Effects of roflumilast on basal SNr neuron activity

In order to determine the effect of roflumilast in the cognitive fronto-striatal circuit originating in the infralimbic cortex we recorded in 24 rats 65 neurons that exhibited an electrophysiological profile corresponding to the SNr criteria (see 'Experimental procedures' section). Figure 1 shows a typical tri-phasic response of the SNr after infralimbic cortex stimulation including baseline firing properties. Recordings of these neurons showed that about 50% of these neurons (n=33) responded to electrical stimulation of the infralimbic cortex (infralimbic cortex electrode location verified; see figure 2). Among these 33 neurons only 18% showed a tri-phasic response (n=6). This percentage corroborates earlier findings observed in earlier studies where the auditory-, motor-, and pre-limbic cortices were stimulated (Maurice et al., 1999; Kolomiets et al., 2003). Only SNr neurons exhibiting a tri-phasic response were included in the analysis because they represent activation of the full circuit of interest (see figure 2).

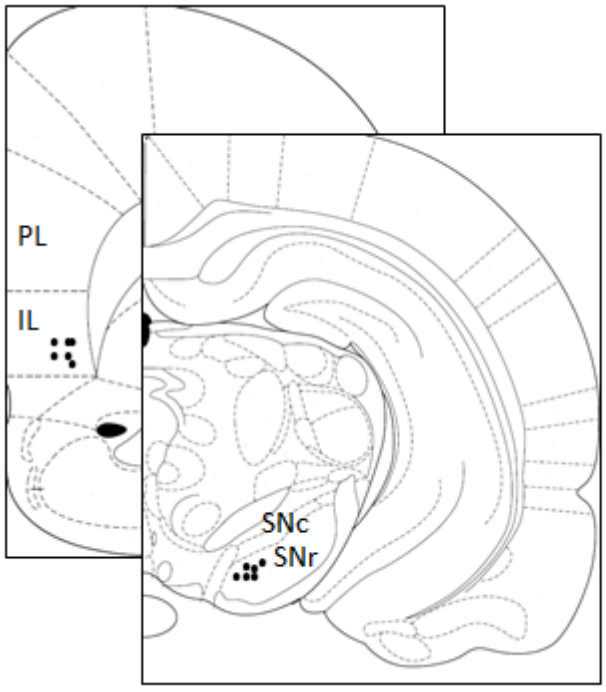

Figure 2. Graphical depiction of stimulation and recording electrode locations for the 6 neurons exhibiting a tri-phasic response (stimulation electrode: coronal section anterior: 3.2 mm to bregma; recording electrode: coronal section anterior: -5.3 mm to bregma). PL=prelimbic cortex; IL=infralimbic cortex; SNc= substantia nigra pars compacta (dorsal tier); SNr=substantia nigra pars reticulata

Before analyzing the effects of roflumilast on any of the baseline spike train parameters or the tri-phasic response we first verified that the (repeated) stimulation of the infralimbic cortex or the injection had no effect on any of the parameters investigated. Neither stimulation itself nor the injection had an effect on the baseline firing (data not shown). Subsequently, roflumilast had no effect on baseline firing properties of the recorded neurons (firing rate, or regularity (COV IS), data not shown).

The physiological response to stimulation of the SNr neurons was compatible with previous data (Ryan and Sanders, 1994; Kolomiets et al., 2003; Beyeler et al., 2010) regarding the delay of appearance and the duration of each phase of the tri-phasic response. Location of responding (cognitive) neurons within the SNr is in line with the division of motor, cognitive (associative) and limbic areas within the SNr (e.g. Kolomiets et al., 2003).

Effects of roflumilast on mPFC stimulation-evoked responses of SNr neurons

The tri-phasic response that was measured represents activation of the hyper direct, direct and indirect basal ganglia pathways. Each individual pathway is measured as early excitation, inhibition and late excitation, respectively. At stimulation (no treatment), latencies and durations were as follow (in ms±SEM; n=6) for the early excitation phase: 4.8±1.4 and 10.8±1.5, respectively. For the inhibition phase this was 20.17±1.8 and 12.67±0.99, respectively, and for the late excitation phase these values were 36.5±2.5 and 13.67±2.0, respectively. These values were not affected during the different stages of the experiment and did not change after roflumilast treatment. Thus, roflumilast had no effect on the latency or duration of the tri-phasic response.

No effects of roflumilast were observed for the R_{mag} of the early excitation phase (figure 3A). Roflumilast increased the firing rate during the inhibition phase (F (3,15) =6.294, p=0.006). Post-hoc test revealed a 117% increase in the firing rate after 0.01 mg/kg (figure 3B; p=0.049). The calculated effect size for this difference was 1.06 (Cohen's D), which can be considered as a large effect size. For the late excitation phase the R_{mag} the repeated measures ANOVA showed a marginal treatment effect (F (3,15) =3.05, p=0.06). However, post-hoc analysis revealed a 50% decrease in R_{mag} after 0.005 mg/kg roflumilast treatment when compared with vehicle (figure 3C; p=0.035). The calculated effect size for this difference was 1.58 (Cohen's D), which can be considered as a large effect size.

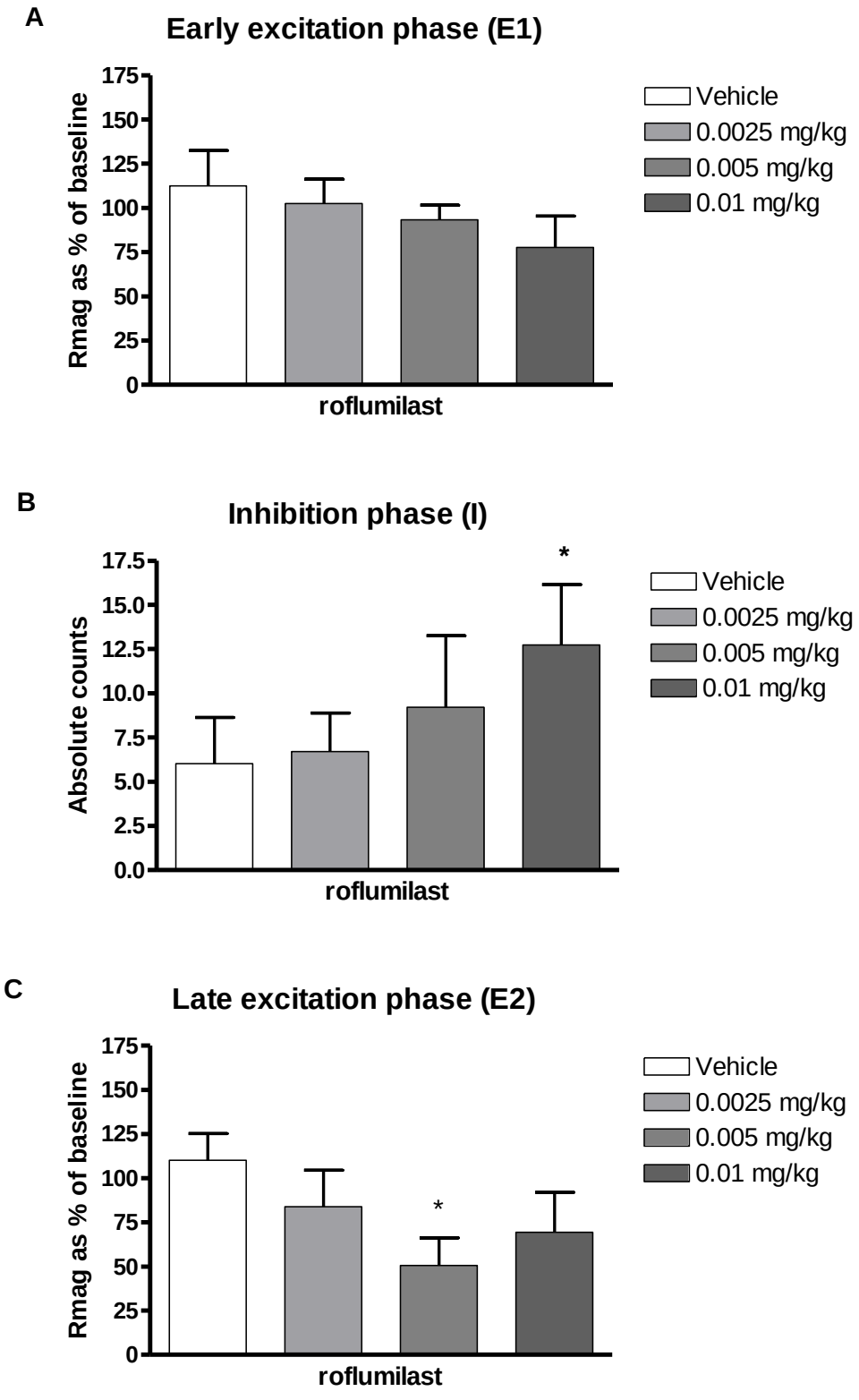

Figure 3. Effects of the PDE4 inhibitor roflumilast on the magnitude of the electrophysiological response of the SNr neurons to electrical stimulation of the infralimbic cortex. Each bar represents the magnitude of the response (R_{mag} as % of baseline for the early (E1) and late (E2) excitation phases, and absolute counts for the inhibition (I) phase). (**A**) Roflumilast did not affect the R_{mag} of the early excitation phase. (**B**) Roflumilast

decreased the magnitude of the inhibition as depicted by an increase in the absolute number of counts at the 0.01 mg/kg dose. (**C**) In the late excitation phase a decrease in R_{mag} was found at the 0.005 mg/kg dose.

DISCUSSION

The current study was conducted to address the distinctive effects of PDE4 inhibition on the three basal ganglia pathways: the hyperdirect, direct and indirect pathway. This was done by examining the effects on the tri-phasic (excitation-inhibition-excitation) response of the SNr after infralimbic cortex stimulation. Our results provide support for the hypothesis that PDE4 inhibition affects the direct and indirect pathways of the basal ganglia, but not the hyperdirect pathway. This conclusion is in line with previous findings supporting a role for PDE4 in the direct and indirect pathways originating in striatal medium spiny neurons (Nishi et al., 2008; Nishi and Snyder, 2010; Nishi et al., 2011; Heckman et al., 2016). The current data support the notion that PDE4 has a preferential role in the indirect pathway since PDE4 inhibition reduced the R_{mag} of the indirect pathway at a lower dose when compared to the neuronal activity in the direct pathway.

Previous studies showed that PDE4 predominantly regulates cAMP/PKA signaling at striatal dopaminergic terminals and associated dopamine synthesis and release (Schoffelmeer et al., 1985; Yamashita et al., 1997a; Yamashita et al., 1997b; Nishi et al., 2008). Secondary to this, PDE4 regulates cAMP/PKA signaling in medium spiny neurons. This is the case for both the striatopallidal as well as the striatonigral pathway as was observed by increased levels of phospho-DARPP-32 in neostriatal slices in both pathways after PDE4 inhibitor treatment (Nishi et al., 2008). These findings were supported by immunohistochemistry data. Of note, higher expression levels were observed in (indirect) striatopallidal neurons but only the PDE4B subtype was examined. Interestingly, PDE4 inhibition only potentiated adenosine A_{2a} receptor –induced phosphorylation of DARPP-32 by the adenosine A_{2a} receptor agonist CGS21680, whereas no additional effects of PDE4 inhibition were observed after stimulation of the dopamine D1 receptor with SKF81297 (Nishi and Snyder, 2010). The latter can be interpreted as a preferential effect of PDE4 inhibition in striatopallidal neurons as A_{2a} receptors are located on indirect pathway MSNs and D1 receptors on direct pathway MSN (Gerfen and Surmeier, 2011).

However, slices do not need to represent the *in vivo* situation as for instance, as mentioned earlier, the observed increase in DARPP-32 phosphorylation after rolipram treatment in neostriatal slices could not be observed *in vivo* by the same group (Nishi et al., 2008). Clearly, when studying the fronto-striatal circuitry the striatal slices can only model part of this circuitry and may therefore not

be the most suitable model for studying a fully intact functional circuit. Therefore, the current study is unique in providing support for a more sensitive indirect pathway for PDE4 inhibition.

The more potent effect of roflumilast in the indirect pathway can be hypothesized to occur from several possibilities. One option would entail lower levels of PDE4 expression in medium spiny neurons of the indirect striatopallidal pathway compared to the direct striatonigral pathway. This way, the same dose of the compound can induce larger effects since absolute lower number of PDE4 proteins in the indirect pathway compared to the direct pathway. Another possibility to explain the observed effect would be that the direct pathway contains higher levels of PDE4 when compared to the indirect pathway. That way a higher dose of roflumilast is needed to inhibit higher levels of PDE4 compared to the indirect pathway before cAMP and related signaling is affected.

Also, the tri-phasic response measured in the SNr originates in the frontal cortex and is modulated at many different levels throughout the system before reaching the output module of the basal ganglia. For instance, even though the same intracellular effectors are involved in frontal and striatal dopaminergic signaling, biosensor imaging in mouse brain slice preparations showed profound differences in the D1 response between pyramidal cortical neurons and striatal medium spiny neurons (Castro et al., 2013). Furthermore, it is unknown in which nigral and pallidal areas or where within the subthalamic nucleus PDE4 is expressed. The abundant number of feedback and feedforward connections within the circuits as well as their mediation and modulation by several neurotransmitter systems add to the complexity of predicting the net effect reaching the output modules of the basal ganglia (Gerfen and Surmeier, 2011; Surmeier et al., 2011; Calabresi et al., 2014). The latter aspects could be responsible for the unexpected finding in both pathways, i.e. increased firing in the inhibition phase and decreased firing in the late excitation phase after roflumilast treatment. Future studies will have to investigate these possible mechanisms at the cellular and molecular level further (e.g. Nishi et al., 2017; Nishi and Shuto, 2017).

Even though our results are in line with previous findings in neostriatal slices and on behavioral outcomes, some difference must be highlighted. For instance, in the current study we examined the effects of a PDE4 inhibitor in anaesthetized animals while behavioral studies discussed above examine fully awake and active animals. This means that, although the brain circuitry is intact and functional, it cannot be ruled out that different effects of roflumilast could be observed when conducting electrophysiological recordings in fully awake and freely moving animals. Since PDE4 is most prominently expressed at dopaminergic terminals affecting synthesis and release of dopamine in the striatum, a dopaminergic challenge of the brain circuitry may greatly affect fronto-striatal

signaling. The latter is more likely to occur in freely moving animals during behavioral tasks compared to anaesthetized animals (Sabeti et al., 2003). However, the former would also introduce more noise and thus variability in the data, when the research question reflects merely the examination of the effects of the drug. Another difference to address is that in the study examining striatal slices, the classic PDE4 inhibitor rolipram was used opposed to roflumilast in the current study, even though both inhibitors are selective for PDE4 over other PDE subfamilies and both have equal selectivity for the four PDE4 subtypes (Krause and Kuhne, 1988; Hatzelmann et al., 2010).

In line with previous studies (e.g. Deniau et al., 1994; Groenewegen et al., 1999; Vertes, 2004) cells exhibiting a tri-phasic response were located in the dorsomedial SNr. Results of the current study further add to the evidence that cognitive projections, or at least those originating in the infralimbic cortex, can induce a tri-phasic response in the SNr. As a result, it also implies the existence of a division within the basal ganglia into a hyper, direct and indirect pathway, in the cognitive fronto-striatal circuit originating in the infralimbic cortex. Thus, this also confirms the hypothesis of the existence of the three pathways in the cognitive fronto-striatal circuits as they do in the motor or limbic circuits (e.g. Maurice et al., 1999; Beyeler et al., 2010).

In conclusion, our results show that stimulation of the infralimbic cortex leads to a tri-phasic response in a subset of neurons in the SNr. This is topographically and functionally associated with the cognitive parts of the basal ganglia, that can be used to investigate distinctive effects of drugs within the basal ganglia circuitry. The temporal and topographic sensitivity of this tri-phasic electrophysiological response combined with the neuroanatomical markers directly addressed the distinctive effects of PDE4 inhibition by roflumilast in the three basal ganglia pathways of the cognitive fronto-striatal circuitry. PDE4 inhibition by roflumilast appears to affect both the direct pathway as well as the indirect pathway with a relative preference for the latter. These findings are in line with previous *in vitro* and *in vivo* studies. Further studies may reveal whether PDE4 inhibition could be considered as a possible treatment for cognitive deficits related to fronto-striatal disorders in which either the direct or indirect pathway is affected (e.g., schizophrenia and attention deficit hyperactivity disorder, Huntington's disease and Parkinson's disease).

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Chapter 6

The mediating role of phosphodiesterase type 4 in the dopaminergic modulation of motor impulsivity

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In preparation

Abstract

The current study investigates the mediating role of PDE4 in the dopaminergic modulation of premature responding (motor impulsivity). Response inhibition, which includes action restraint, finds its neurobiological origin in fronto-striatal circuitry and can be modulated by dopamine. Intracellularly, the effect of dopamine is largely mediated through the cAMP/PKA signaling cascade. It has been suggested that areas in the prefrontal cortex are very sensitive to their neurochemical environment, including catecholamine levels. Both high and low catecholamine release in the prefrontal cortex impairs prefrontal cortex function. As a result, we investigated the effects of PDE4 inhibition on premature responding in a hypo, normal and hyper dopaminergic state of the brain. As a hypo dopaminergic model we will induce a 6-OHDA lesion in the prefrontal cortex, more specifically the infralimbic cortex. For the hyperdopaminergic state we also turn to a well-established model of impaired action restraint, namely the systemic administration of d-amphetamine. Results indicated a role for PDE4 inhibitors in shifting performance on premature responding to the right on the U-shaped dose response curve. As a result, it would be interesting to test the effects of PDE4 inhibition in disorders affected by disrupted impulse control related to fronto-striatal hypodopaminergia including ADHD.

Introduction

Controlling impulsivity is of vital importance for decision making. A lack of control of your impulsive responding can have severe consequences and lead to various psychiatric disorders. For example, addiction, attention deficit hyperactivity disorder (ADHD), obsessive-compulsive disorder (OCD), eating disorders and pathological gambling (Aron 2007; Aron and Poldrack 2005; Bellgrove et al. 2006; Durston et al. 2008; Fillmore and Rush 2002; Fillmore et al. 2002, 2006; Gauggel et al. 2004; Monterosso et al. 2005; Nigg et al. 2004; Oosterlaan et al. 1998; Penadés et al. 2007; Rubia et al. 1998; Rubia et al. 2007; Rubia et al. 2005; Schachar et al. 2007; Schachar et al. 1995; van den Wildenberg et al. 2006; Dalley and Robbins 2017). Impulsivity is a multifaceted concept and in daily life it comes in many different forms (Evenden 1999; Cardinal et al. 2004; Winstanley et al. 2006). To make this multifaceted concept easier to study impulsivity, is generally divided into *impulsive actions*, i.e. an inability to inhibit a response, and *impulsive choices*, i.e. a distorted judgment with respect to choosing between two differential reward outcomes. A third main type of impulsivity is *reflection impulsivity*, making decisions before adequately sampling and evaluating available information. However, splitting the behavioral construct into six subcategories is perhaps more appropriate (see Table 1). Within *impulsive actions* there is a clear distinction between *action restraint* (inhibiting a prepotent, inappropriate response) and *action cancellation*, i.e. response inhibition or volitional control over responding once the response has been initiated. *Impulsive choice* may be subdivided into *delay-*, *uncertainty-*, and *effort-based decision making*. Impulsivity is a critical component of psychiatric symptoms within the class of 'inhibition' and is separated from compulsivity, perseveration, disinhibition, obsession, aggression, attention and mania (Aron 2007).

Table 1. Types of impulsivity

Type of impulsivity	Cognitive domain	Description
Impulsive action	Action restraint; motor impulsivity	Ability to inhibit prepotent, inappropriate responses
	Action cancellation; response inhibition	Ability to inhibit actions once initiated
Impulsive choice	Delay-based decision making	Decision making based on delay-aversion
	Uncertainty-based decision making	Decision making based on risk-seeking
	Effort-based decision making	Decision making based on effort-aversion
Reflection impulsivity	Information sampling	Ability to evaluate available information prior to making a decision

Current knowledge on the human neuroanatomical substrate of impulse control points to the fronto-striatal circuits (Alexander et al. 1986; Dalley et al. 2011; Chudasama and Robbins 2006). The fronto-striatal circuits comprise a complex mechanism of action and functionality, which is abundantly described elsewhere (e.g. Surmeier et al. 2007; Haber and Rauch 2010; Gerfen and Surmeier 2011; Surmeier et al. 2011; Calabresi et al. 2014; Calabresi et al. 2000). Although the general anatomical organization related to impulse control is known, the different subtypes of impulsivity arise from the dysfunction of different fundamental anatomical and neurochemical mechanisms within the fronto-striatal circuitry. The delicate control of neurotransmitter balance in these circuits and its effects on brain functioning and behavior are not yet fully understood. Studies using different drugs that interfere with this neurotransmitter balance have increased our understanding of the neurobiological regulation of impulse control (e.g. Winstanley 2011; Bari and Robbins 2013; Eagle and Baunez 2010; Dalley and Roiser 2012).

One of the key neurotransmitter systems involved in fronto-striatal physiology is the dopaminergic system originating in the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA)(Gerfen and Surmeier 2011). It is well accepted that dopamine plays an important regulatory role in many forms of impulsivity, especially impulsive action, as is supported by a wealth of preclinical and clinical studies (for a review see e.g. Dalley and Roiser 2012; Winstanley 2011; Dalley et al. 2008; Eagle and Baunez 2010; Dalley et al. 2011; Bari and Robbins 2013; D'Amour-Horvat and Leyton 2014). Psychostimulants such as amphetamine have opposing roles in both types of impulsive action as they improve performance on the stop-signal task (SST; action cancellation) as shown in both healthy rats and humans (de Wit et al. 2000; de Wit et al. 2002; Eagle and Robbins 2003), but impair performance on the choice serial reaction time task (CSRTT; action restraint) in rats (van Gaalen et al. 2006). Of note, the latter has not been tested yet in the CSRTT in healthy humans.

Intracellularly, the effect of dopamine is largely mediated through the cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) signaling cascade (Nishi et al. 2008; Nishi and Snyder 2010; Nishi et al. 2011; Kuroiwa et al. 2012; Heckman et al. 2016; Nishi and Shuto 2017). This cascade is thus a potential target for pharmacological intervention in several types of impulsivity-related disorders, including ADHD and addiction. Via this cascade the regulatory effect of dopamine on impulsivity can be modulated. The most prominent mean to target cAMP is via specific cyclic nucleotide phosphodiesterases (PDEs)(Beavo 1995; Bender and Beavo 2006). Especially, the PDE4 subfamily is involved in the regulation of dopaminergic cAMP/PKA signaling (Heckman et al. 2016; Richter et al. 2013; Hansen and Zhang 2015). For instance, within the striatum, the synthesis of dopamine by tyrosine hydroxylase (TH) (Harada et al. 1996; Dunkley et al. 2004) and the release of

dopamine from nigrostriatal dopaminergic terminals (Zhu et al. 2004; Seino and Shibasaki 2005; Schoffelmeer et al. 1985) are regulated by the cAMP/PKA signaling cascade and can be modulated by inhibitors of the PDE4 subfamily (Nishi et al. 2008). Additionally, PDE4 inhibitors modulate dopamine D1-receptor signaling in the frontal cortex, as D1 receptors are G_s-coupled which, in turn, stimulates cAMP (Kuroiwa et al. 2012).

Based on the above, we are interested in the effects of PDE4 inhibition on premature responding in a hypo, normal and hyper dopaminergic state of the brain (see figure 1). As a hypo dopaminergic model we induced a 6-OHDA lesion in the prefrontal cortex, more specifically the infralimbic cortex (IL), which is a well-known animal model for the induction of motor impulsivity related to action restraint as in ADHD (Lukkes et al. 2016; Freund et al. 2014). It has been suggested that the functioning of the areas in the prefrontal cortex is very sensitive to optimal levels of catecholamine (Arnsten 2009). High as well as low catecholamine release in the prefrontal cortex impairs prefrontal cortex function. For the hyperdopaminergic state we also turned to a well-established model of impaired action restraint, namely the systemic administration of d-amphetamine, which has proven to robustly increase premature responding (Winstanley et al. 2006). Taken together, we investigated the effects of the already approved PDE4 inhibitor roflumilast for human administration on premature responding in the CSRTT in a hypo, normal and hyper dopaminergic state of the animal. Main aim of the current study was to evaluate if roflumilast is able to normalize the motor impulsivity deficits induced by a hypo dopaminergic functioning, while probably deteriorating it in a hyper dopaminergic state as roflumilast will increase DA signaling in the frontostriatal circuit.

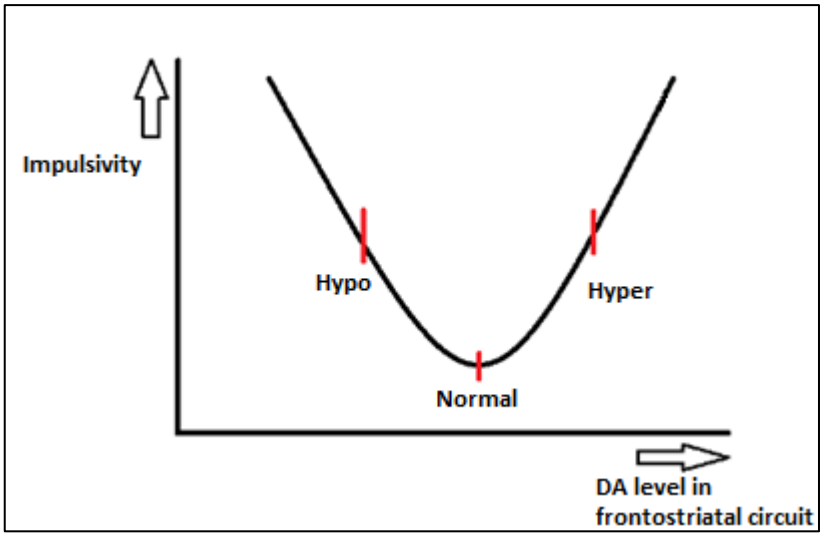


Figure 1. Hypothesized effects of infralimbic dopamine levels on premature responding (action restraint; motor impulsivity); DA=dopamine

Materials and Methods

Animals

All experimental procedures were approved by the local ethical committee for animal experiments of Maastricht University and met governmental guidelines. Sixty 3-month-old male Wistar rats (Charles River, The Netherlands) were used in three separate studies (20 animals per study) with average body weights of 386 g (±11.66). The animals were housed in pairs 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 h to 7:00 h). 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 h and 18:00 h in a shielded Skinnerbox. Animals had free access to water, but limited food access to reduce their body weight to 90-85 % of their free feeding weight.

Apparatus

The rats were tested in ten identical operant chambers (inner dimensions: 40x30x33 cm), which were equipped with two retractable levers and cue lights just above the levers. A food tray (5x5 cm and 2.5 cm above the grid floor), which was positioned equidistant between the two levers, could be accessed by pushing a hinged panel. The levers (4 cm wide) projected 2 cm into the conditioning chamber and were located 6 cm from each side of the food tray and 12 cm above the grid floor. A house light and a loudspeaker were fixed in the ceiling of the conditioning chamber. The operanda and manipulanda in the chambers were controlled by a personal computer and the data were stored on disk at the end of a session.

Experimental procedure

Magazine (MG) and Continuous reinforcement training (CRF)

Behavioral training started with magazine training (30 min sessions) in which the rats had to learn to push back the hinged panel in order to obtain a food reward (45 mg food pellets). The next stage of training consisted of continuous reinforcement training, in which either the left or right lever was inserted in the conditioning chamber after a rat had pressed the hinged panel of the food tray. Whenever a lever was inserted into the chamber the cue light above the lever was switched on. A food reward was provided when a rat had pressed the lever.

Fixed ratio 5 schedule of reward

Next the rats were trained on a fixed ratio schedule of reinforcement, in which they had to press a lever for five times (FR5) in order to obtain a 45 mg food reward. Reinforcement was continuous; i.e., each set of five lever presses was rewarded. A session was terminated after 60 trials or 30 min,

whichever applied first. Rats were trained once a day, Monday to Friday, and were given eight FR5 sessions before drug testing started. The measure used to evaluate performance on the FR5 schedule was interresponse time (i.e., time between consecutive lever presses which was averaged for each animal). The purpose of this task was to control for possible effects of any of the pharmaceutical agents used on sensorimotor functioning.

Progressive ratio 10 schedule of reward

After finishing drug testing in the FR5 task, rats immediately started training on a progressive ratio (PR10) schedule of reinforcement. The rats had to progressively increase the response requirement (steps of ten lever presses) to obtain a food reward. For the first food pellet they were required to press ten times, for the next reinforcement they had to press the lever twenty times, and so on. A session was terminated if a rat did not press the lever for 3 min. Rats were trained once a day, Monday to Friday, and were given eight PR10 sessions before drug testing started. The measure used to evaluate performance in the PR10 task was the breakpoint (i.e., total number of lever presses made during a session). The purpose of this task was to control for possible effects of any of the pharmaceutical agents used on food motivation.

Choice serial reaction time task (CSRTT; Figure 2)

Subsequently, rats did the CSRTT to test the effects of the PDE4 inhibitor on premature responding (Blokland 1998). In the CSRTT rats had to push the panel for a longer duration until one of the levers was made accessible. First, a randomly chosen duration of 0.5–1.0 s (steps of 0.1 s) was used. This variable period was called the hold duration. Also, an auditory stimulus was presented when the hold duration had elapsed. A high tone (10 kHz; 80 dB) predicted insertion of the left lever, and a low tone (2.5 kHz; 80 dB) predicted the insertion of the right lever. The insertion of the lever took about 2 s. This tone was turned off when the rat withdrew its nose from the food tray. When a rat did not succeed in pressing the panel for the entire hold duration (premature response), the same interval was started again upon pressing the panel. After the rats showed a steady performance during this stage they were required to push the panel for a randomly chosen duration of 0.5–1.5 s (steps of 0.1 s). These hold durations were used during all further testing. The inter-trial interval was 10 s. A session lasted 30 min or until a rat had completed 60 trials. After the rats showed a stable performance, which occurred after about 2 weeks of training in this task, the rats were subjected to the main test paradigm (drug treatment).

The following parameters were used to evaluate the responding of the rats:

Reaction time (RT): the main latency between the onset of the tone and the release of the hinged food tray panel (after pushing it away). It is generally accepted that response latencies shorter than 100 ms may not reflect true RT (Blokland and Honig 1999). These latencies were counted as premature responses. Response latencies longer than 1500 ms were not considered to be a task-related RT. The RT performance was evaluated in two ways: the mean reaction time of all responses and the mean RT per hold duration was examined.

Motor time (MT): the mean latency between the release of the hinged food tray panel and the lever press. It was assumed that MT of more than 2 s do not reflect true MT (Blokland and Honig 1999).

Number of trials: the total number of trials the rats completed in a session of maximum 30 min.

Premature responses (PR): the total number of times that the rat released the hinged panel before the hold duration had elapsed. In the present study, we always used the ratio of PR (i.e., the PR divided by the total number of completed trials, with a maximum of 60). Furthermore, a PR caused the trial to start over again.

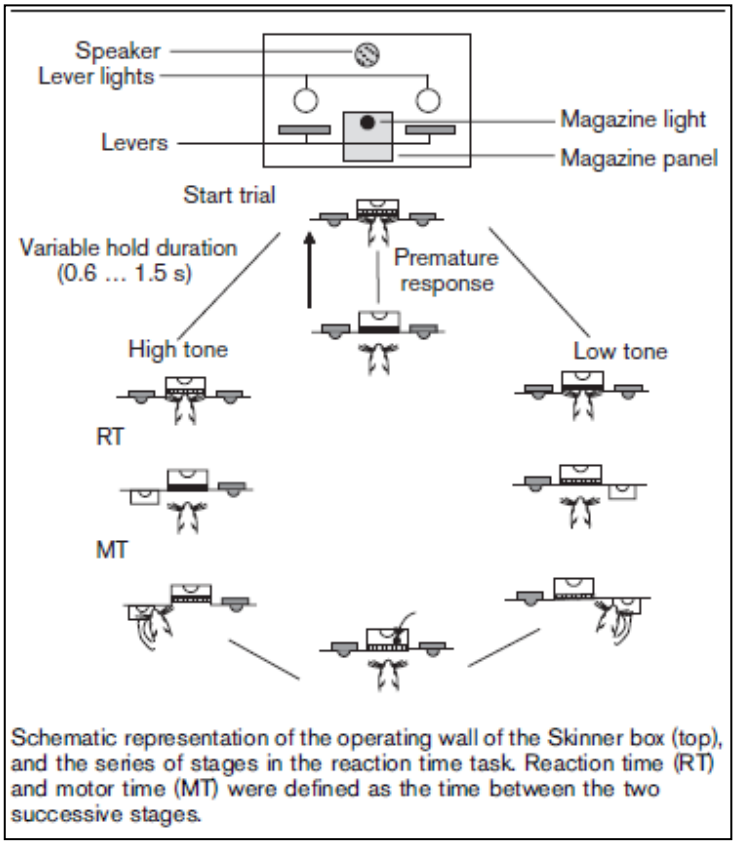


Figure 2. Schematic representation of the operating wall of the Skinner box (top), and the series of stages in the reaction time task. Reaction time (RT) and motor time (MT) were defined as the time between the two successive stages (adopted with permission from Blokland, Sik and Lieben, 2005).

Treatment

The PDE4 inhibitor roflumilast (kindly donated by Takeda, Konstanz, Germany) was tested in the FR5, PR10 and CSRTT. Roflumilast was freshly dissolved on the day of testing. Drug administration of the PDE inhibitor occurred 30 min prior to behavioral testing for all three paradigms (FR5, PR10 and CSRTT) in all three studies (hypo, hyper and normal dopaminergic state of the animal) and was done intraperitoneally (i.p.) for roflumilast (0.001, 0.003, 0.01, 0.03 and 0.1 mg/kg). The PDE inhibitor was dissolved in the same vehicle (98% methyl cellulose [tylose] solution (0.5%) and 2% tween80) and administered in a volume of 1 ml/kg (i.p.). In the second study, the hyper dopaminergic state was induced by administration of d-amphetamine (Sigma Aldrich, Zwijndrecht, The Netherlands). d-Amphetamine was administered i.p. 30 min before behavioral testing started (1.0 mg/kg) in an injection volume of 1 ml/kg. In the third study, a hypo dopaminergic state was induced in the IL as explained below.

Stereotactic surgery (study 3; 6-OHDA lesions)

In the third study, the effects of the PDE4 inhibitor roflumilast were tested in a hypo dopaminergic state of the IL. The hypo dopaminergic state was induced by means of 6-hydroxydopamine (6-OHDA) lesions. The animals (n=10) received stereotactic injections of 2 µl 6-OHDA (5 µg/µl dissolved in 0.9% saline and 0.2% ascorbic acid; Sigma Aldrich, Zwijndrecht, The Netherlands) or the same injection amount of the vehicle solution (n=10; 0.9% saline and 0.2% ascorbic acid) at four sites (two per hemisphere) in the IL. One hour before the surgery, rats received desipramine (30 mg/kg in 0.9% saline, injection volume 1 mg/kg i.p.). Desipramine was administered to protect noradrenergic neurons from possible damage by 6-OHDA and thus make the lesion more DA specific (Mason and Fibiger 1979). The animals were anaesthetized using isoflurane (induction 5%, maintenance 2%) and subsequently placed in a stereotactic frame. Surgery site was pretreated with lidocaine for local anesthesia. Corneal dehydration was prevented by applications of Vaseline. Body temperature was maintained at 37 ± 0.5 °C using a homeothermic heating blanket (Harvard Apparatus, Kent, UK). The injections of 6-OHDA or vehicle were performed in the IL at the following coordinates (Paxinos et al. 2009): AP: +3.2 mm, L: 0.6 and -0.6 mm, and DV: −4.8 and −5.4 mm (from bregma). Injection speed was 0.5 µl/min and the cannula was left in place for an additional 2 min before slowly retracting it. Pre-operatively and 8 hours post-operatively, Temgesic (0.05 mg/kg) was administered subcutaneous as analgesic. The animals were put back in their home cages for recovery, and weighted and handled daily. The animals had free access to food and water after surgery. Food and water was consumed without problems. Based on effects on performance, 5 out of 10 animals were selected to proceed with the roflumilast testing in the 6-OHDA condition.

Statistics

Statistical analysis was carried out using IBM SPSS Statistics 24 software (IBM, Portsmouth, UK). The raw data were checked for outliers using SPSS descriptive statistics and checked for normality of distribution. Paired samples T-test, GLM univariate ANOVA as well as GLM one-way repeated measures analysis of variance (ANOVA) followed by Sidak's post-hoc test was used. For the paired samples T-test equal variances were assumed unless the Levene's test for equality of variances was significant. P-values were corrected for multiple testing. Data are depicted as means ± SEM.

Results

Fixed ratio 5 schedule of reward

All animals completed the 60 trials within 30 minutes. Roflumilast, d-amphetamine and the IL 6-OHDA lesion did not affect the inter-response time in the FR5 schedule of reward (data not shown).

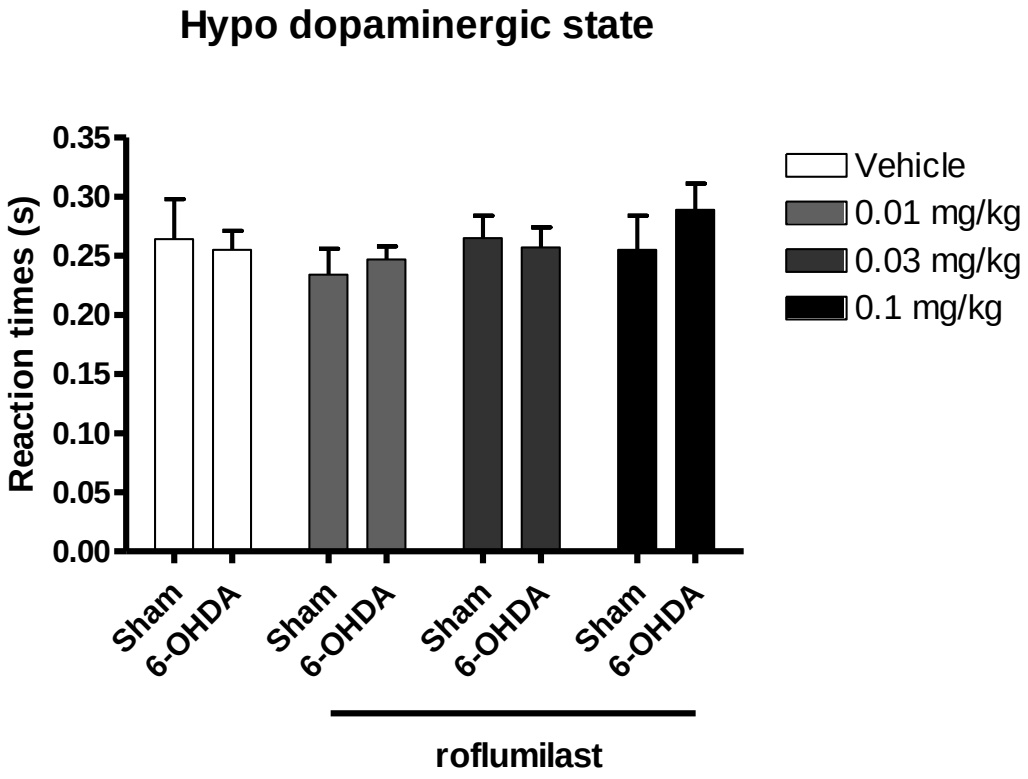
Progressive ratio 10 schedule of reward

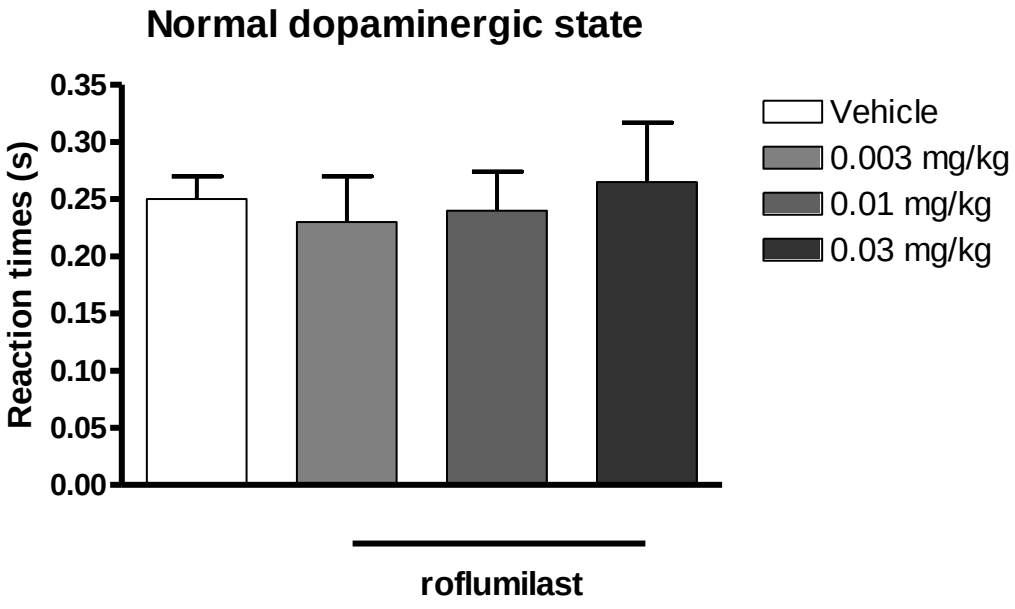
Roflumilast, d-amphetamine and the IL 6-OHDA lesion did not affect the breakpoint in the progressive ratio 10 schedule of reward (data not shown).

Choice serial reaction time task

Reaction times

For the hypo dopaminergic state, the mixed model ANOVA showed no significant dose x lesion interaction effect (F (3,55)=0.434, N.S.). Also, there was no main effect of dose (F (3,55)=0.751, N.S.) or lesion (F (1,55)=0.259, N.S.)(see figure 3A). There was no significant effect of roflumilast on the reaction times in a normal dopaminergic state (F (3,27)=0.632, N.S.; see figure 3B). Surprisingly, in the hyper dopaminergic state, treatment with d-amphetamine only showed a trend toward significance to affect the reaction times (t(19)=1.813; p=0.08) (see figure 3C). Roflumilast did not show any effect on the reaction times (F (4,76)=0.875, N.S.; see figure 3C).


Hypo dopaminergic state
Reaction times (s)
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Vehicle
0.01 mg/kg
0.03 mg/kg
0.1 mg/kg
Sham
6-OHDA
Sham
6-OHDA
Sham
6-OHDA
Sham
6-OHDA
roflumilast


Normal dopaminergic state
Reaction times (s)
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Vehicle
0.003 mg/kg
0.01 mg/kg
0.03 mg/kg
roflumilast

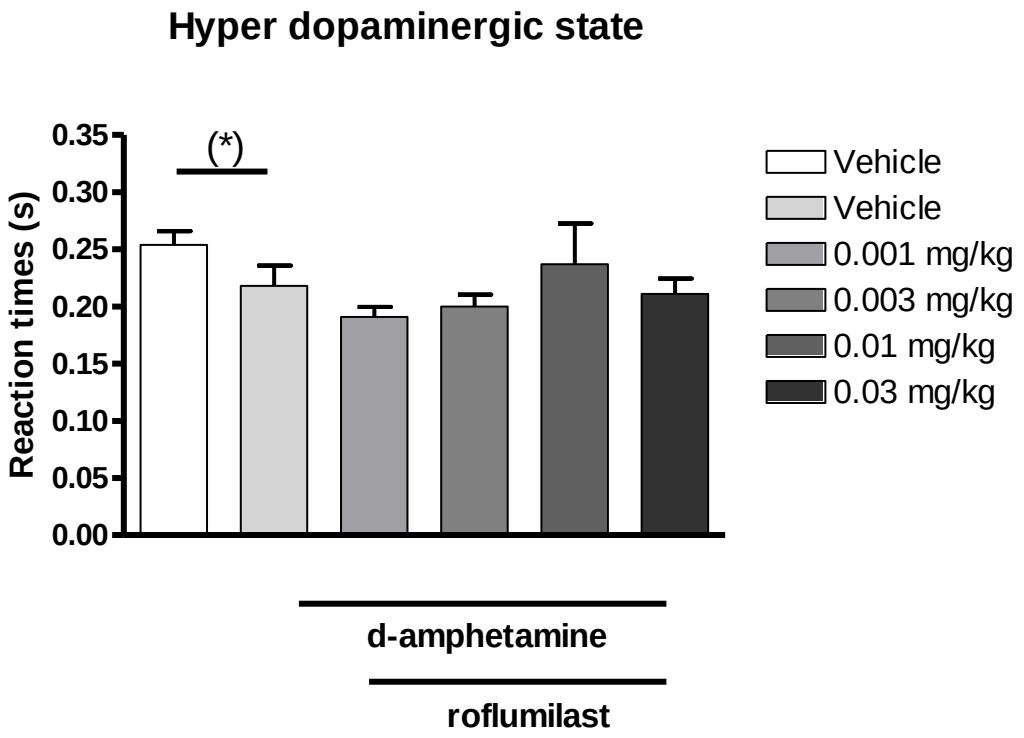


Figure 3. Effects of the PDE4 inhibitor roflumilast on reaction times (s) in the CSRTT in a hypo **(A)** and hyper **(B)** dopaminergic model of motor impulsivity (action restraint). (*)= p=0.08

Motor times

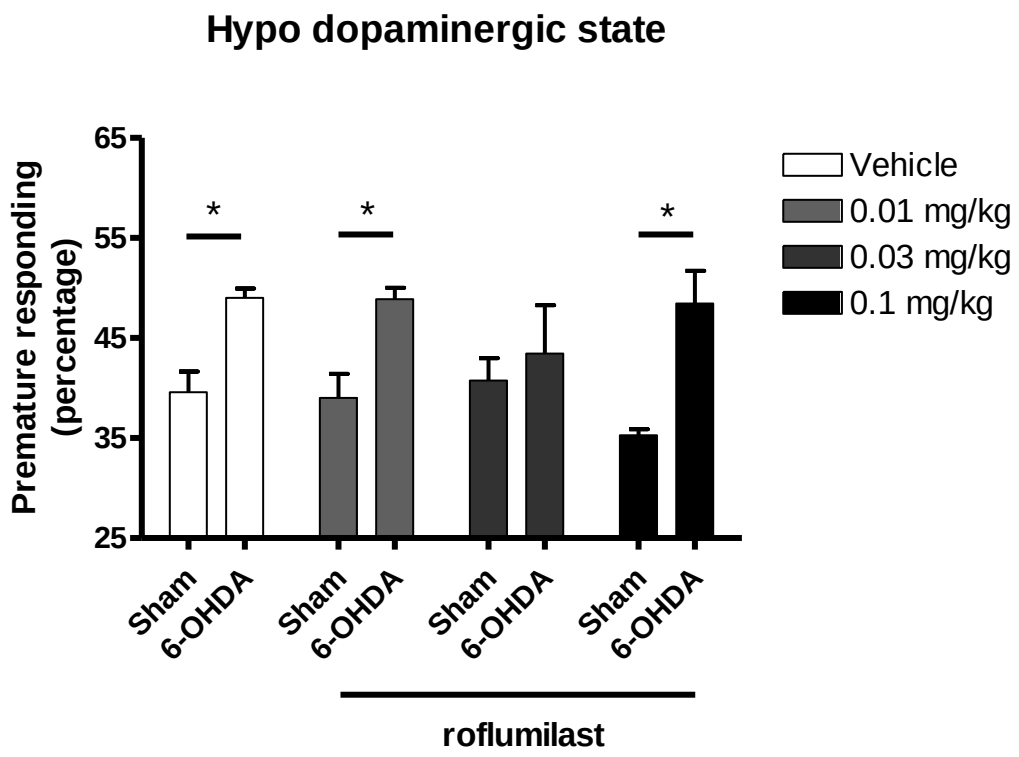
Roflumilast, d-amphetamine and the IL 6-OHDA lesion did not affect the motor times in the CSRTT (data not shown).

Premature responses

For the hypo dopaminergic state, the mixed model ANOVA showed no significant dose x lesion interaction effect (F (3,48)=1.289, N.S.). Also, there was no main effect of dose (F (3,48)=0.367, N.S.). However, there was a significant main effect of lesion (sham vs. 6-OHDA; F (1,48)=18.526, p<0.001; see figure 4A). Subsequently, we checked for differences between sham and 6-OHDA lesion per dose of roflumilast. For the 0.01 mg/kg (t(13)=2.384; p<0.05) and 0.1 mg/kg (t(4.261)=3.944; p<0.05) dosages the 6-OHDA lesioned animals showed significantly higher percentages of premature responses (see figure 4A; Sidak correction for multiple testing). Treatment with 0.03 mg/kg of roflumilast normalized the premature responding of the 6-OHDA lesioned animals to the level of sham lesioned animals (t(13)=0.586; N.S.).

There was no significant effect of roflumilast on the percentage of premature responses in a normal dopaminergic state (F (3,27)=0.723, N.S.; figure 4B).

In the hyper dopaminergic state, treatment with d-amphetamine increased the percentage of premature responses (t(18)=-4.748; p<0.001). Subsequent treatment with roflumilast further increased the percentage of premature responses as indicated by the overall treatment effect (F (4,76)=3.370, p<0.01; figure 4C). Post-hoc analyses (Sidak) revealed a significant increase in premature responding at the 0.01 mg/kg dose compared to amphetamine alone (figure 4C; p<0.01).



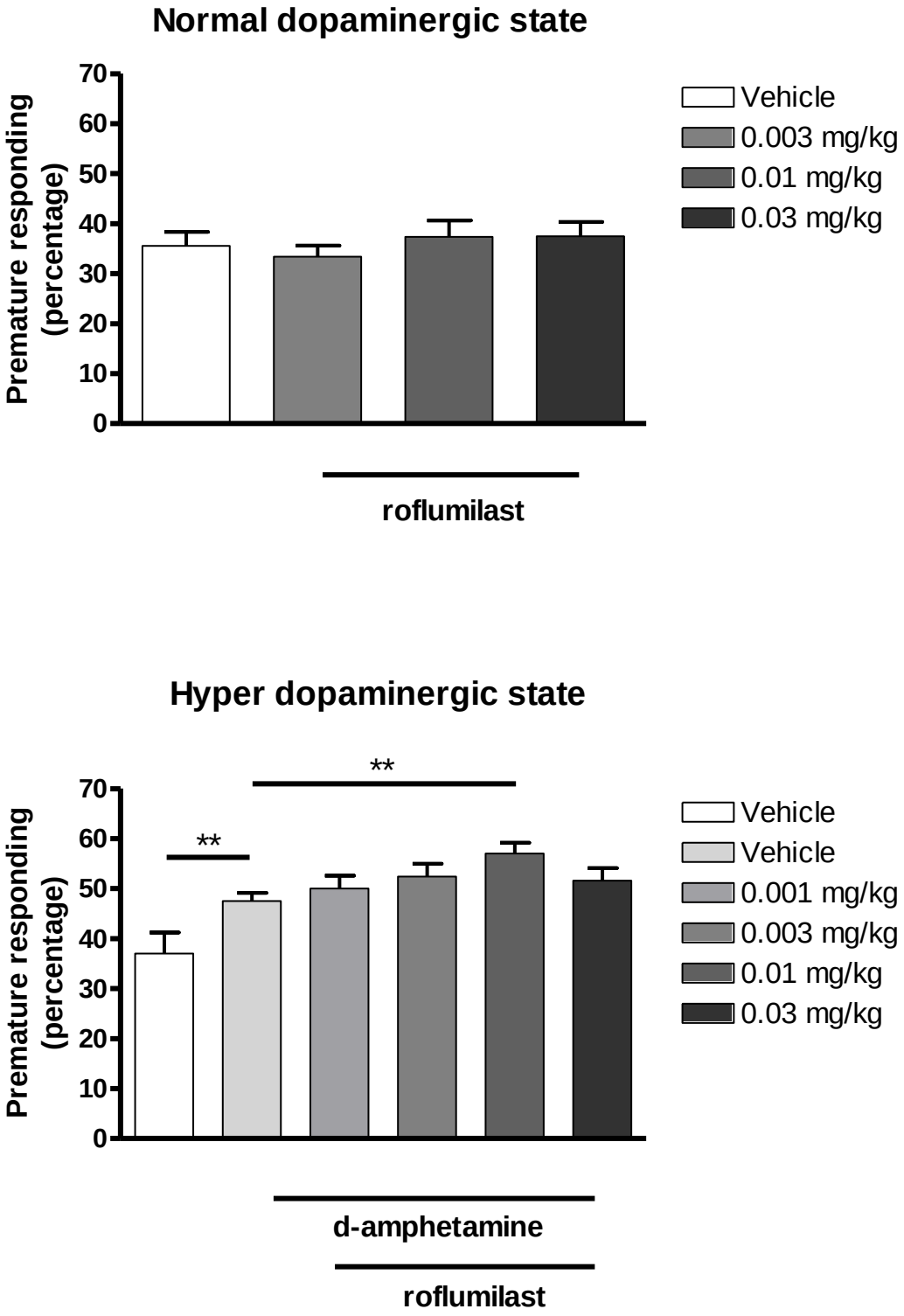


Figure 4. Effects of the PDE4 inhibitor roflumilast on premature responding in the CSRTT in a hypo **(A)**, normal **(B)** and hyper **(C)** dopaminergic model of motor impulsivity (action restraint). *= p<0.05; **=p<0.01

Discussion

The aim of the current study was to investigate the effects of the, for chronic obstructive pulmonary disease (COPD), clinically approved PDE4 inhibitor roflumilast on premature responding in the CSRTT in a hypo, normal and hyper dopaminergic state of the cognitive fronto-striatal circuit. Premature responding was used as a measure for action restraint, one of the two types of motor impulsivity. It was first checked whether the different treatments (i.e., amphetamine, IL 6-OHDA lesions, roflumilast) had an effect on sensorimotor functioning (FR5) and food motivation (PR10). None of the treatments showed any effect on these basic response rates in the operant chamber. Therefore, treatment effects in the CSRTT cannot be explained by differences in lever press responding or food motivation. In line with the notion of a U-shaped relation between dopamine and impulsive responding (Arnsten 2009), we found that both increasing and decreasing dopamine levels (by amphetamine and IL 6-OHDA lesion, respectively) resulted in an increase in premature responding in the CSRTT. Thus, interestingly, these results followed the U-shaped dose-response curve seen in figure 1. This finding indicates that 6-OHDA as well as d-amphetamine is a suitable tool to induce models of dopamine-related motor impulsivity (action restraint).

The release of dopamine from dopaminergic terminals is regulated by the cAMP signaling cascade and can be modulated by inhibitors of the PDE4 subfamily (Schoffelmeer et al. 1985; Nishi et al. 2008). Therefore, in the hypo dopaminergic state of the fronto-striatal circuit, the PDE4 inhibitor roflumilast was expected to enhance the release of dopamine, restoring low levels of dopamine to their original level. Linked to this assumption, treating the 6-OHDA lesioned animals with roflumilast indeed restored the level of premature responding back to the level of the sham-lesioned animals. Additionally, also following our hypothesis, roflumilast further increased the already heightened level of premature responding in the hyper dopaminergic state of the fronto-striatal circuit, induced by d-amphetamine. Thus, we again observed a shift to the right on the U-shaped dose-response curve. However, in the normal (untreated) dopaminergic state roflumilast did not exhibit any effects on the level of premature responding. Based on results of the current study, roflumilast thus seems to follow our hypothesis based on the dopaminergic dose-response curve except for the non-modulated dopaminergic condition. Possibly due to its effects on dopamine release, roflumilast seems to enhance the effects of dopamine (as was shown before by Nishi and Snyder 2010; Schoffelmeer et al. 1985), restoring depleted levels and further enhancing already heightened levels of dopamine. As a result, it would be interesting to test the effects of PDE4 inhibition in disorders characterized by impaired action restraint related to fronto-striatal hypodopaminergia like ADHD.

However, several points remain in need of further clarification/investigation. First of all, we observed no effects of roflumilast on premature responding in the normal dopaminergic state of the fronto-striatal circuit. This could be explained by the fact that the brain has a higher level of compensatory ability when catecholamine levels are at an optimum (or near optimum) level. On the other hand, it could also be that, since roflumilast affects the release of dopamine, the effects of roflumilast are most easily observed in experimental conditions in which a dopaminergic challenge is present (6-OHDA or d-amphetamine). It also seems likely that the peak of the U-shaped curve is rather wide due to compensatory mechanisms of the organism in physiological conditions. Thus, it could also just be that roflumilast shifted the performance on the U-shaped dose response curve from slightly left of the optimum to slightly right of the optimum while behavioral output remains unchanged. Only in pathophysiological conditions, when performance lies outside this compensatory window, a change in performance can be observed.

Pharmacokinetics of roflumilast have been established in previous studies by our group (Vanmierlo et al. 2016) and others (Jabaris et al. 2015) showing that roflumilast is brain penetrant and cognitively active. But despite brain penetration, a point of attention is that expression levels of the target of roflumilast, i.e. PDE4 itself, in the IL are currently unknown as are the expression levels at other key structures within the fronto-striatal circuitry, e.g. nigral and pallidal areas or the subthalamic nucleus. Finally, we like to discuss the fact that our findings in the hypo dopaminergic state of the IL, showed no significant interaction effect. This might be due to the high number of groups being tested occluding possible interaction effects. Importantly, on closer inspection we found no effects of roflumilast in the sham-lesioned animals as is in line with our findings in the normal dopaminergic state, while the 6-OHDA lesioned animals normalized their premature responding after treatment with an acute dose of 0.03 mg/kg roflumilast. Possibly, the lack of an overall roflumilast effect might be explained by the severity of the 6-OHDA lesion. Based on previous experience in our department, we used relatively high doses of 6-OHDA that induce reliable lesions in the striatum to mimic Parkinson's disease (Scholtissen et al. 2006). However, to our knowledge no literature exists regarding the exact dose of 6-OHDA to be used in the IL (e.g. Lukkes et al. 2016; Freund et al. 2014). Possibly, the strong lesions were not easily reversed by other doses besides the cognitively effective dose of roflumilast (Vanmierlo et al. 2016).

To our knowledge, the only other study investigating the effects of a PDE inhibitor on impulsivity is a study recently published using the PDE10 inhibitor TAK-063 (Suzuki et al. 2015). Based on the almost exclusive expression of PDE10 in the striatum (Lakics et al. 2010) and the preferential expression in the indirect D2 pathway, we hypothesize that a PDE10 inhibitor does the opposite of a PDE4 inhibitor

and should shift performance in the CSRTT to the left on the U-shaped dose-response curve. In contrast to our hypothesis, the PDE10 inhibitor TAK-063 showed positive effects in healthy animals. However, it should be noted that, as explained above, not all healthy subjects perform exactly at an optimal level on the U-shaped dose response curve. If a batch of animals or group of participants performs slightly right from the optimal performance on the U-shaped dose response curve, a shift to the left side may be beneficial. Therefore, it would be interesting to add next to a PDE4 inhibitor, a PDE10 inhibitor or PDE1 inhibitor, which has also high expression in the fronto-striatal brain areas, as references in future studies to obtain a more complete picture, especially when testing healthy animals.

Taken together, the current study looked at the unique role of the dopaminergic modulation of premature responding and investigated the mediating role of PDE4 by testing the effects of roflumilast in a hypo, normal and hyper dopaminergic state of the cognitive fronto-striatal circuit. Results indicate a role for PDE4 inhibitors in shifting performance on premature responding to the right on the U-shaped dose response curve. As a result, it would be interesting to test the effects of PDE4 inhibition in disorders affected by disrupted impulse control related to fronto-striatal hypodopaminergia including ADHD.

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Statement of interest

AB and JP have a proprietary interest in the PDE4 inhibitor roflumilast.

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Chapter 7

General Discussion

Aim of the dissertation

The main aim of the current dissertation was to investigate the function of PDE4 in cognitive fronto-striatal circuits. The focus was set on cognition, opposed to motor and limbic functions of the fronto-striatal circuits, and included cognitive functions such as attention, sensory gating, sensorimotor gating and impulsivity. After providing a critical review of the current status in the field we continued in twofold. On the one hand, we aimed to investigate the effects of PDE4 inhibition by means of roflumilast on behavioral outcomes in an auditory sensory gating paradigm (Chapter 4) as well as in a paradigm related to motor impulsivity (Chapter 6). On the other hand, we aimed to examine the functional output of the fronto-striatal circuit to the thalamus using an electrophysiological approach (Chapter 5). Also, we aimed to test both physiological (Chapter 4 and Chapter 5) as well as the pathophysiological (Chapter 6) conditions of the fronto-striatal circuit.

Overview main findings of the dissertation

PDEs appear to be a promising target in the field of cognition enhancement related to fronto-striatal functioning. Although this relatively new field in PDE research is just emerging, clear positive effects have already been found in animals and we are now awaiting translational confirmation by human data. As such, attention, sensory gating, sensorimotor gating and impulsivity are emerging cognitive domains in the field of PDE research. In Chapter 2 we discussed experimental studies and the potential beneficial effects of PDE inhibitors in these cognitive domains. Overall, PDE4 seems to be the most promising target for the four domains discussed in the chapter. Chapter 3 continues providing an overview of the current knowledge on the role of PDE1, PDE4 and PDE10 in the regulation of dopaminergic modulation of fronto-striatal circuits. The interplay between PDEs and dopamine in relation to the cognitive functions is discussed in more detail. In addition to a discussion on the neurobiological mechanisms of PDE inhibition, a clinical perspective is provided.

In Chapter 4 we investigated whether the PDE4 inhibitor roflumilast could enhance sensory gating in healthy young human volunteers. We aimed to find effects purely on gating without exhibiting an effect on overall auditory processing as indicated by auditory evoked potentials (AEP) to assure that PDE4 inhibition affects pure gating. Results showed that roflumilast significantly improved sensory gating in our participants. This effect was induced in a dose-dependent manner. No emetic side effects were reported by the participants after administration of the effective low dose. This means roflumilast shows a beneficial effect on gating at a dose that had no adverse effects reported following single-dose administration in the present study.

Studies discussed in chapter 5 were conducted to address the functional output of the fronto-striatal circuit to the thalamus at an electrophysiological level using rats. This was accomplished by studying the distinctive effects of PDE4 inhibition on the three basal ganglia pathways: the hyperdirect, direct and indirect pathway. This was done by examining the effects on the tri-phasic (excitation-inhibition-excitation) response of the SNr after infralimbic cortex stimulation. The tri-phasic response of the SNr represents activation of the hyper direct, direct and indirect basal ganglia pathways. Our results provide support for the hypothesis that, under normal conditions, PDE4 inhibition affects the direct and mainly the indirect pathways of the basal ganglia, but not the hyperdirect pathway.

In Chapter 6, the mediating role of PDE4 in the dopaminergic modulation of premature responding (motor impulsivity) was investigated in rats. We tested the effects of roflumilast on premature responding in the choice serial reaction time task (CSRTT) in a hypo, normal and hyper dopaminergic state of the cognitive fronto-striatal circuit. In line with the notion of a U-shaped relation between dopamine and impulsive responding, we found that both increasing and decreasing dopamine levels (by systemic d-amphetamine administration or a medial prefrontal 6-OHDA lesion, respectively) resulted in an increase in premature responding in the CSRTT. Treating the 6-OHDA lesioned animals with roflumilast restored the level of premature responding back to the level of the sham-lesioned animals. Additionally, in line with our hypothesis roflumilast further increased the already heightened level of premature responding in the hyper dopaminergic state of the fronto-striatal circuit, induced by d-amphetamine.

Main implication of the dissertation

The main aim of the current dissertation was to investigate whether PDE4 could be a relevant target for the treatment of cognitive dysfunctions in disorders related to the fronto-striatal circuits (Nishi and Shuto 2017; Nishi et al. 2011; Nishi et al. 2008). Besides cognitive behavioral therapy, there is hardly any efficacious pharmacological treatment without major side effects for these disorders, including attention deficit hyper activity disorder (ADHD), schizophrenia and Parkinson's disease. The focus was set on cognition and the role of dopamine (Gerfen and Surmeier 2011; Gerfen et al. 1990). As a result, we tried to regulate dopaminergic modulation of the fronto-striatal circuits via PDE4 inhibition examining the effects in several cognitive domains like sensory gating and impulsivity. Currently available dopaminergic treatments, like methylphenidate, are very nonspecific and induce many side effects (e.g., cardiac abnormalities, addiction, growth inhibition) resulting in low-compliance and high relapse rates in patients. Therefore, there is a clear need for more basic

knowledge regarding the role of dopamine in the physiology of the fronto-striatal circuits to generate new targets; and a clear need for the proper development of treatments for related diseases.

Roflumilast and the tri-phasic response

To increase our basic understanding of the role of PDE4 in the three basal ganglia pathways of the fronto-striatal circuits, we turned to *in vivo* electrophysiology due to its high spatial and temporal resolution. The first step encompasses the verification of the tri-phasic response in the cognitive fronto-striatal circuits, the same way it is observed in the motor circuits. An important difference between the rat brain and the human brain in this respect, involves the fact that cognitive areas in the prefrontal cortex project to the nucleus accumbens and leave the basal ganglia mainly via the ventral pallidum with less projections to the substantia nigra pars reticulata (SNr)(Groenewegen et al. 1999; Deniau et al. 1994; Maurice et al. 1999). In the current dissertation we showed that a tri-phasic response resembling the three basal ganglia pathways can be induced via infralimbic cortex stimulation.

Subsequently, we checked for effects of roflumilast on any of the three phases separately. Previous studies using immunohistochemistry provided us with an indication regarding expression of PDE4 in the three basal ganglia pathways (Nishi et al. 2011; Nishi et al. 2008; Nishi and Snyder 2010). However, the effects on the functional output of the pathways to the SNr remained to be elucidated. Our study showed for the first time the effects of roflumilast on the output of the three pathways. In line with the previous immunohistochemical data, we observed that roflumilast modulated output of the direct and indirect pathways but not of the hyperdirect pathway. The most potent effect was observed for the indirect pathway. In contrast to what was expected based on the immunohistochemical data, we observed an increase in action potentials from the direct pathway (indicative of reduced activation of the direct pathway). Likewise, we observed a reduction in action potentials coming from the indirect pathway (indicative of reduced activation of the indirect pathway). The absence of effects in the hyperdirect pathway indicates that the effects in the other two pathways may have originated in the striatum or globus pallidus pars externa (GPe). The indirect pathway shares its ending namely with the hyperdirect pathway, which remained unaffected. The exact intracellular compartmentalized signaling pathways responsible for the current findings remain to be elucidated in our future studies. We can, however, hypothesize in light of the current findings, that roflumilast is likely an interesting treatment for disorders benefiting from reduced indirect pathway activation like ADHD.

Our unique from “bench to bed and back again” approach in this dissertation also resulted in the tri-phasic response likely being able to be used as a biomarker (i.e. as electroencephalogram (EEG) and event-related potential (ERP) correlate). This further opens up possibilities for a translational approach as the tri-phasic response can be used as a translational electrophysiological measure. The rationale behind the tri-phasic response was inferred from patients exhibiting basal ganglia-related symptoms, subsequently tested in animals to result in an electrophysiological correlate known as “tri-phasic response of the SNr”, which, now that is has been validated, might be used for patient diagnosis and prognosis.

Roflumilast and sensory gating

Next to increasing our basic knowledge regarding the role of dopamine in the physiology of the fronto-striatal circuits, we investigated the potential of PDE4 inhibitors as efficacious treatment for impaired (dopaminergic) cognitive function related to fronto-striatal circuits. We investigated both physiological and pathophysiological conditions. In line with the translational approach we aspired, both rodents and humans were tested. Cognitive domains related to the fronto-striatal circuits investigated in the current dissertation include sensory gating and motor impulse control.

The effects of roflumilast on sensory gating were investigated in healthy human volunteers. Sensory gating is a process that prevents overstimulation of the brain and is measured by deriving ERPs from the EEG. Roflumilast 100 µg improved sensory gating at the P50 peak of the Fz electrode indicating improved frontal gating. Impairments in gating are characteristic of disorders like schizophrenia, ADHD and Alzheimer’s disease. As a result it would be interesting to continue future studies by testing the efficacy of roflumilast in a sensory gating paradigm in either a deficit model or in patients. The d-amphetamine deficit model is likely the most suitable deficit model as it mimics impaired D2 signaling in schizophrenia. Other options include phencyclidine (PCP) or biperiden depending on the neuropsychiatric disorder under investigation.

The classic PDE4 inhibitor rolipram has already shown to improve sensory gating in the d-amphetamine deficit model (Halene and Siegel 2008; Maxwell et al. 2004). However, this might only be indicative of antipsychotic potential of the drug. Anti-psychotic potential is defined here as the ability to reverse D2 indirect pathway hyperactivity. The biological mechanism behind improved gating in the frontal cortex is most likely explained via stimulation of γ-aminobutyric acid (GABA)-ergic interneurons, not via the D2 pathway. These interneurons terminate the response to the first stimulus via $GABA_A$ receptors and diminish to response to the second stimulus via activation of $GABA_B$ receptors (Smucny et al. 2015). Future studies will have to further elucidate the

compartmentalized role of PDE4 in intracellular signaling cascades within these inhibitory interneurons. Notably, PDE4 inhibition might thus not only benefit ADHD patients via decreased basal ganglia indirect pathway activation (as shown with the tri-phasic response) but also via frontal mechanisms.

Roflumilast and motor impulse control

Another cognitive domain investigated in the current dissertation is motor impulsivity. Current knowledge on the human neuroanatomical substrate of impulse control points to the fronto-striatal circuits (Alexander et al. 1986; Dalley et al. 2011; Chudasama and Robbins 2006). To investigate the role of dopamine in premature responding in the CSRTT we induced a hypo, normal and hyper dopaminergic state of the cognitive fronto-striatal circuit. Interestingly, results followed the U-shaped dose-response curve confirming our hypothesis that both low and high levels of dopamine induce motor impulsivity. PDE4 inhibition is known to induce several effects in the fronto-striatal circuits (Nishi et al. 2008). It affects both the direct and indirect pathway (with a more potent effect in the latter, as observed during our electrophysiological recordings) and the dopaminergic terminals in striatum and frontal cortex (Nishi and Snyder 2010). Effects at the dopaminergic terminals include increased release of dopamine (e.g. Schoffelmeer et al. 1985; Nishi and Snyder 2010). The shift to the right on the dose response curve after roflumilast treatment, depicted in Figure 1 of chapter 6, showing the relation between dopamine and impulse control, is in line with the assumption that PDe4 inhibition induces dopamine release.

ADHD patients are characterized by frontal hypodopaminergia (Arnsten 2009). By increasing their frontal dopamine levels via PDE4 inhibition, that is to say shifting dopamine levels to the right on the dose response curve (towards optimal levels); a PDE4 inhibitor potentially replaces the loss in cAMP signaling due to a loss in presynaptic dopamine. A similar mechanism (dopamine agonism of the postsynaptic receptor) applies to psychostimulants like methylphenidate, currently used to treat ADHD. However, PDE4 inhibitors including roflumilast have none of the severe side effects observed after psychostimulant treatment. Especially, if we combine the current findings on premature responding with findings from both other chapters, a PDE4 inhibitor seems a very promising treatment for ADHD. It improves several cognitive functions whose impairments are hallmarks of ADHD, such as motor impulse control and sensory gating. By decreasing direct pathway activation it can be hypothesized that roflumilast will also decrease hyperactivity. In fact, studies with the PDE4 inhibitor rolipram have shown a decrease of spontaneous and drug-induced (hyper)locomotion (Siuciak et al. 2007). The third major hallmark of ADHD is an attention deficit. Not much research has been conducted in the field of attention and PDE inhibition. However, two studies have shown that

rolipram reversed a MK-801-, bupropion-, amphetamine-, and GBR12783-induced latent inhibition deficit indicative of improved attentional performance (Davis and Gould 2005; Lipina and Roder 2009). A potential role for PDE4 inhibition in hyperactivity and attention remains to be further elucidated in future studies.

Of note, in the present dissertation we only investigated motor impulsivity (action restraint). However, the motor domain (impulsive action) also includes response inhibition. The latter is usually measured by means of the Stop-Signal Task, both in humans and rodents (Winstanley 2011). Additionally, there are two other major cognitive domains within the taxonomy of impulsivity next to impulsive action, i.e. impulsive choice (decision making → delay-, probability-, and effort-based discounting) and reflection impulsivity. Studies in both rodents and humans have shown that current ADHD psychostimulant treatment has opposite effects on impulsive action and impulsive choice. How this relates to PDE4 inhibition should also be further investigated in future studies.

As mentioned in the discussion chapter of the current dissertation, we focused on the cognitive fronto-striatal circuits. However, next to the cognitive circuits, the total fronto-striatal circuitry entails also motor and limbic circuits. These circuits are characterized by the same three basal ganglia pathways (hyperdirect, direct and indirect) including their modulation by dopamine. Hypothesizing, if we would apply the same hypo and hyper dopaminergic states to the motor circuits as we did to the cognitive circuits, it would result in hypokinetic movement disorders (like Parkinson's disease) and hyperkinetic movement disorders (like Huntington's disease), respectively. In the same way PDE4 inhibition can benefit ADHD, it might also benefit Parkinson's disease patients or any other movement disorder characterized by striatal hypodopaminergia. PDE4 inhibitors would target the same intracellular signaling pathways normally activated by dopamine, providing a substitute for the loss in dopamine due to neurodegeneration. More in detail, neurodegeneration of the presynaptic dopaminergic neurons results in dopaminergic treatments (e.g. levodopa) becoming ineffective over time. PDE4 inhibitors target the postsynaptic neuron enhancing signaling pathways usually activated by dopamine.

Along the same line of reasoning, it can be hypothesized that disorders with frontal hypodopaminergia benefit from PDE4 inhibition. Like the cognitive symptoms in ADHD, cognitive symptoms in, for instance, schizophrenia could be reduced via PDE4 inhibition. Cognitive symptoms in schizophrenia are believed to result from decreased dopamine D1 receptor signaling in the frontal cortex (Green 1996). D1 receptors activate the cAMP signaling cascade which is also stimulated by the inhibition of PDE4 (Kuroiwa et al. 2012). In fact, a clinical trial conducted at King's College London

showed positive results on cognitive function in schizophrenia patients after antipsychotic medication combined with roflumilast compared to antipsychotic medication alone (ClinicalTrials.gov Identifier: NCT02079844). Taken together, results of the current dissertation could potentially benefit several disorders related to impaired dopaminergic functioning of the fronto-striatal circuits.

General conclusion

Dopamine plays an essential role in the physiology of fronto-striatal circuits. The intracellular cascades involved in dopamine signaling involve cyclic nucleotide PDEs with an emphasis on PDE4. PDE4 inhibitors target dopaminergic signaling cascades in the frontal cortex and basal ganglia pathways, and induce the release of dopamine therein. Therefore, PDE4 seems to be a promising target regarding treatment for disorders affected by understimulated dopaminergic signaling in fronto-striatal circuits. In the current dissertation, we have increased our knowledge regarding the functional role of PDE4 in the dopaminergic modulation of fronto-striatal circuits. Results obtained in the dissertation provide us with enough data to expand the current research line.

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Chapter 8

Summary

The aim of the current PhD dissertation was to target phosphodiesterase type 4 for improving cognitive fronto-striatal functioning via a translational approach. The general introduction (**Chapter 1**) described the rationale of this dissertation and the aims of the studies discussed herein.

Chapter 2: PDE inhibitors enhance cAMP and/or cGMP signaling via reducing the degradation of these cyclic nucleotides. Both cAMP and cGMP signaling are essential for a variety of cellular functions and exert their effects both pre- and post-synaptically. Either of these second messengers relays and amplifies incoming signals at receptors on the cell surface making them important elements in signal transduction cascades and essential in cellular signaling in a variety of cell functions including neurotransmitter release and neuroprotection. Consequently, these processes can be influenced by PDE inhibitors as they increase cAMP and/or cGMP concentrations. PDE inhibitors have been considered as possible therapeutic agents to treat impaired cognitive function linked to fronto-striatal circuits, including ADHD, schizophrenia and Parkinson's disease. In Chapter 2 we discussed the involvement of PDEs on four related domains: attention, information filtering (sensory- and sensorimotor gating) and response inhibition. Currently, these are emerging cognitive domains in the field of PDE research. We discussed experimental studies and the potential beneficial effects of PDE inhibitors on these cognitive domains. Overall, PDE4 seems to be the most promising target for all domains discussed in the chapter.

Chapter 3: Chapter 3 provides a detailed discussion of the relation between PDEs and dopamine in relation to the cognitive functions. Subsequently, an overview is provided of the current clinical status. Clinical trials investigating the effects of PDE inhibitors in neuropsychiatric disorders are overall very sparse and the wealth of positive preclinical data could not yet be translated into clinical efficacy.

Chapter 4: Research has shown that the process of sensory gating is disrupted in patients suffering from clinical disorders including ADHD, schizophrenia and Alzheimer's disease. PDE inhibitors have received an increased interest as a tool to improve cognitive performance related to fronto-striatal functioning in both animals and man. One of the cognitive areas investigated is sensory gating. Therefore, we investigated the effects of the PDE4 inhibitor roflumilast in a sensory gating paradigm in 20 healthy young human volunteers (age range 18 – 30 years). We applied a placebo-controlled randomized cross-over design and tested 3 doses (100, 300, 1000 µg). Results discussed in this chapter showed that roflumilast improved sensory gating in healthy young human volunteers only at the 100 µg dose. This means roflumilast shows a beneficial effect on gating at a dose that had no adverse effects reported following single-dose administration. This indicates that roflumilast 100 µg

has a favorable side-effect profile. Roflumilast and PDE4 inhibition in general could therefore be seen as a promising treatment in disorders affected by disrupted sensory gating.

Chapter 5: In Chapter 5 we examined the functional output of the fronto-striatal circuit to the thalamus at an electrophysiological level by studying the distinctive effects of PDE4 inhibition on the three basal ganglia pathways: the hyperdirect, direct and indirect pathway. Effects of roflumilast on the three pathways were studied via the tri-phasic (excitation-inhibition-excitation) response of the SNr after infralimbic cortex stimulation. Results show for the first time that stimulation of the infralimbic cortex leads to a tri-phasic response in the SNr, topographically and functionally associated with the cognitive parts of the basal ganglia. Interestingly, we found that PDE4 inhibition resulted in inhibition of the direct pathway and reduced activation of the indirect pathway at the level of the SNr. This finding is likely due to the complexity of the system already at hand at the level of the SNr, i.e. the abundant number of feedback and feedforward connections within the circuits as well as their mediation and modulation by PDE4 and several neurotransmitter systems. Most importantly, in line with previous studies, PDE4 inhibition by roflumilast affects both the direct pathway as well as the indirect pathway of which the latter appears more affected than the former.

Chapter 6: In Chapter 6, the mediating role of PDE4 in the dopaminergic modulation of premature responding (motor impulsivity) was studied. We investigated the effects of roflumilast on premature responding in the choice serial reaction time task (CSRTT) in a hypo, normal and hyper dopaminergic state of the cognitive fronto-striatal circuit. Results showed that both increasing and decreasing dopamine levels resulted in an increase in premature responding in the CSRTT. Results indicated a role for PDE4 inhibitors in shifting performance on premature responding to the right on the U-shaped dose response curve. As a result, it would be interesting to test the effects of PDE4 inhibition in disorders affected by disrupted impulse control related to fronto-striatal hypodopaminergia including ADHD.

Appendices

Valorization addendum

About the author

List of publications

Acknowledgements

Valorization addendum

Relevance for society and Target groups

The dopaminergic fronto-striatal circuits constitute the neurobiological basis of several neuropsychiatric disorders, including neurodegenerative disorders like Parkinson's disease and Huntington's disease, psychiatric illnesses such as schizophrenia and obsessive-compulsive disorder (OCD), and pervasive developmental disorders like attention deficit hyperactivity disorder (ADHD) and autism spectrum disorder (Alexander et al. 1986; Haber and Rauch 2010; Gunaydin and Kreitzer 2015). Dysfunction of these circuits produces the wide range of motor, cognitive and affective symptoms observed in related neuropsychiatric disorders (Chudasama and Robbins 2006).

The burden that cognitive impairments impose on the quality of life is enormous. Not only for those who suffer from it but also for their families and caregivers. For instance, as many as one million Americans live with Parkinson's disease, which is more than the combined number of people diagnosed with multiple sclerosis, muscular dystrophy and Lou Gehrig's disease. Approximately 60,000 Americans are diagnosed with Parkinson's disease each year, and this number does not reflect the thousands of cases that go undetected. More than 10 million people worldwide are living with Parkinson's disease. Incidence of Parkinson's increases with age, but an estimated four percent of people with Parkinson's disease are diagnosed before the age of 50.

Schizophrenia is another devastating disorder of the fronto-striatal circuits for most people who are afflicted, and very costly for families and society. The overall U.S. 2002 cost of schizophrenia was estimated to be $62.7 billion, with $22.7 billion excess direct health care cost ($7.0 billion outpatient, $5.0 billion drugs, $2.8 billion inpatient, $8.0 billion long-term care). The Prevalence Rate for schizophrenia is approximately 1.1% of the population over the age of 18 or, in other words, at any one time as many as 51 million people worldwide suffer from schizophrenia, including 2.2 million people in the United States (US), 285,000 people in Australia, over 280,000 people in Canada and over 250,000 diagnosed cases in the United Kingdom (UK).

5.1 million Children in the US (8.8% or 1 in 11 of the age group 4-17 years) have a current diagnosis of ADHD: 6.8% of children ages 4-10 (1 in 15); 11.4% of children ages 11-14 (1 in 9); 10.2% of children ages 15-17 (1 in 10). Additionally, 6.4 million children (11% of the age group 4-17 years) have ever been diagnosed with ADHD, and rates of ever-diagnosed ADHD increased an average of approximately 5% per year from 2003 to 2011. Estimated cost to US society entails $42.5 billion dollar annually.

Without going into the statistics of the other disorders, it becomes clear from the numbers provided for the three examples above, that the need to increase our understanding of the physiology and pathophysiology of the fronto-striatal circuits is of utmost importance. Increasing our understanding of fronto-striatal circuits' biology will not only aid in better understanding the many different disorders related to the circuits, but it will also create new targets for pharmacological treatment and generate possible biomarkers as is further explained below.

Besides cognitive behavioral therapy, there is currently hardly any pharmacological treatment for disorders related to dysfunctional fronto-striatal circuits. Presently available dopaminergic treatments for ADHD, Parkinson's disease and schizophrenia are very nonspecific and induce many side effects (cardiac arrhythmias, addiction, growth inhibition, dyskinesias, dopamine dysregulation syndrome, etc.). This results in low-compliance and high relapse rates, again stressing the need for more basic knowledge regarding the role of dopamine in the physiology of the fronto-striatal circuits and the possible impact of the current dissertation. By modulating the same intracellular machinery via phosphodiesterase type 4 (PDE4) inhibitors as would dopamine itself via the extracellular receptor, we tried to regulate the dopaminergic modulation of the fronto-striatal circuitry. Thereby, we reverse the decrease in intracellular signaling due to the loss in dopamine. Main benefit of PDE4 inhibitors compared to dopaminergic medication includes increased efficacy and improved side-effect profile. From the results of the current dissertation it seems that especially disorders characterized by hypodopaminergia, including ADHD and Parkinson's disease, will benefit from PDE4 inhibition.

Cognitive symptoms observed in ADHD, including sensory gating and attention deficits, as well as impulsivity and hyperactivity seem to be beneficially affected by PDE4 inhibition. Roflumilast is currently the only clinically approved PDE4 inhibitor available for oral administration with a unique side effect profile compared to both, for PDE4 inhibitors typically observed, emetic effects and psychostimulant-induced side effects.

Effectiveness and feasibility for use of knowledge to meet the needs of others

Through publications and presentations at conferences I will inform the scientific community in the field of our results and outcomes of translational research. In the case of positive results, I will also explore the possibilities of patenting new treatments of ADHD based on the newly generated targets using our Technology Transfer Office. On the one hand for monotherapy but also for combination therapy with existing dopaminergic drugs (e.g. Ritalin) to reduce their side effects. This would greatly help my research to bring in extra funding. Further, I will contact relevant patient organizations (like

the foundation Attention Deficit Disorders (ADD)) as well as the Trimbos. Patient organizations would like to learn more about the latest treatment options and the Trimbos focuses on improving health care through sharing knowledge. This way, we will also reach health care professionals to share our knowledge and possibly recruit volunteers for future studies.

Activity/Products and Innovation

Our unique from "bench to bed and back again" approach has the potential to result in the generation of new targets to develop improved treatments for patients with ADHD. PDE4 seems to be a very promising target based on results of the current dissertation. Next to the generation of new treatments, the current project also has the potential to generate new biomarkers including electroencephalogram (EEG) and event-related potential (ERP) correlates. The latter will lead to better and faster diagnosis improving prognosis. Improved prognosis will lead to reduced cost for society and increased quality of life for patients and family.

Additionally, results of the current dissertation further add to the evidence that cognitive projections, or at least those originating in the infralimbic cortex, can induce a tri-phasic response in the SNr. As a result, it also implies the existence of a division within the basal ganglia into a hyper, direct and indirect pathway, in the cognitive fronto-striatal circuit originating in the infralimbic cortex. Thus, this also confirms the hypothesis of the existence of the three pathways in the cognitive fronto-striatal circuits as they do in the motor circuits (e.g. Maurice et al. 1999; Beyeler et al. 2010).

Finally, we have implemented and verified a new model for motor impulsivity as seen in ADHD. Currently, there is no good model for ADHD available in animals except for the Spontaneously Hypertensive Rat. This model shows high face validity but moderate construct validity (Sagvolden et al. 2005) and induces confounding results due to inattentiveness and hyperactivity. In our experiments we wanted to focus purely on motor impulsivity. Therefore, we induced hypodopaminergia via a 6-hydroxy dopamine (6-OHDA) lesion in the medial prefrontal cortex to target the cognitive circuits of the fronto-striatal circuitry. Since the levels of dopamine seem to follow a U-shaped dose-response curve, this model is suited to be used in future studies unraveling the pathophysiology of ADHD-related motor impulsivity an testing promising treatments (Arnsten 2009; Arnsten and Pliszka 2011).

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About the author

Pim Heckman was born in Heerlen on November 24th 1986 and grew up in Kerkrade, the Netherlands. In 2006 he finished his high school education (Gymnasium) at College Rolduc in Kerkrade. In September 2006, he started studying Psychology at Maastricht University. In 2009 he obtained his Bachelor's Degree in 'Biological Psychology'. He continued his scientific career by obtaining his Master Degree in 'Neuropsychology' cum laude in 2011 at Maastricht University. Because he voluntarily extended the research period for his Master Thesis (to finish the study that was still ongoing when the internship period ended) he worked the remaining part of the academic year as a research assistant at the department of 'Neuropsychology and Psychopharmacology'. In September 2011 he started a Research Master in 'Cognitive and Clinical Neuroscience', for which he obtained his degree in 2013. For the Research Master he conducted research for his internship at the department of 'Psychiatry and Neuropsychology'. During his first master he worked in the group of Prof. J.G. Ramaekers, Dr. E. Theunissen and Dr. K. Kuypers on the effects of Vardenafil and Rivastigmine on THC-induced memory impairment. For his second (research) master he worked in the group of Dr. J. Prickaerts and Dr. A. Blokland on the effects of PDE2 and PDE4 inhibition on cognitive functioning related to cortico-striatal-thalamic circuitry. In 2013 Pim started working as a PhD student for Dr. J. Prickaerts and Dr. A. Blokland at the School for Mental Health and Neuroscience at Maastricht University. The latter resulted in the current thesis.

List of publications

Peer-reviewed publications:

De Sousa Fernandes Perna EB, Theunissen EL, Kuypers KPC, **Heckman PRA**, De La Torre R, Farre M & Ramaekers JG (2014) Memory and mood during MDMA intoxication, with and without memantine pretreatment. *Neuropharmacology, 87*, 198-205

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Heckman PRA, Blokland A & Prickaerts J (2017) The mediating role of phosphodiesterase type 4 in the dopaminergic modulation of motor impulsivity. (in preparation)

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Heckman PRA, Blokland A & Prickaerts J (2016) From age-related cognitive decline to Alzheimer's disease: a translational overview of the potential role for phosphodiesterases. In H.T. Zhang (Ed.), *Phosphodiesterases: CNS Functions and Diseases* (pp. 88-135). London: Springer

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TO JOS AND ARJAN

THE RABBIT, THE FOX, AND THE WOLF - A GRADUATE STUDENT FABLE

Chapter One

One sunny day, a rabbit came out of her hole in the ground to enjoy the weather. The day was so nice that the rabbit became careless, and a fox sneaked up behind her and caught her.

"I am going to eat you for lunch!" said the fox.

"Wait!" replied the rabbit. "You should at least wait a few days."

"Oh yeah? Why should I wait?" sneered the fox.

"I am almost finished writing my Ph.D. thesis," the rabbit said

"Hah! That's a stupid excuse. What is the title of your thesis anyway?"

"I am writing a thesis on `The Superiority Of Rabbits Over Foxes And Wolves.'"

"Are you crazy? I should eat you right now!" the fox snarled. "Everybody knows that a fox will always win a fight with a rabbit."

"Not really, according to my research," said the rabbit. "If you'd like, you can come to my hole and read it for yourself. If you're not convinced, you can go ahead and have me for lunch."

"You really are crazy!" replied the fox. But the fox was pretty curious, and figured he had nothing to lose, so he went with the rabbit into its hole. The fox never came back out.

Chapter Two

A few days later the rabbit was again taking a break from writing. Sure enough, a wolf came out of the bushes, caught the rabbit, and was getting ready to eat her.

"Wait!" yelled the rabbit, "you cannot eat me right now."

"And why might that be, you fuzzy appetizer?"

"I am almost finished writing my Ph.D. thesis on "The Superiority Of Rabbits Over Foxes And Wolves.'"

The wolf laughed so hard it almost lost its hold on the rabbit. "Maybe I shouldn't eat you-- you really are sick in the head and you might have something contagious!" the wolf opined.

"Come read for yourself. You can eat me after that if you disagree with my conclusions."

So the wolf went into the rabbit's hole... and like the fox, he never came back out.

Chapter Three

A few weeks later, the rabbit finished writing her thesis and was out celebrating in the lettuce fields. Another rabbit came by and asked, "What's up? You seem to be very happy."

"Yup, I just finished writing my dissertation."

"Congratulations! What is it about?"

"It is entitled `The Superiority Of Rabbits Over Foxes And Wolves.'"

"Are you sure? That doesn't sound right."

"Oh, yes, you should come over and read for yourself."

So they went off together to the rabbit's hole.

As they entered, the friend saw what looked like a typical graduate student abode-- albeit a rather messy one after writing a thesis. The computer with the controversial dissertation was in one corner of the room. On the right there was a pile of fox bones, on the left was a pile of wolf bones, and in the middle was a lion.

And the moral of the story is:

Who you are doesn't matter. What the title of your dissertation is doesn't matter.

All that matters is who your thesis supervisor is.

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