Neuromodulation in corticostriatal circuits: On deep brain stimulation and dopamine

Klanker, M.

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In our everyday life, we are constantly performing actions with a certain goal in mind, but we are also able to adjust our behavior to a constantly changing environment. Adaptation of goal-directed behavior relies on integrity of a network that consists of connections between the prefrontal cortex and striatum. Dopamine is an important neuromodulator in this network.

The first part of this thesis investigates the role of dopamine in the control of adaptive behavior. Enhanced understanding of the neurobiological mechanisms that control adaptive behavior will not only increase our understanding of our everyday functioning, but may also provide insight in the dysfunctions underlying cognitive disturbances in psychiatric disorders.

The second part of this thesis investigates the cognitive and neurobiological effects of deep brain stimulation, a relatively novel treatment option in psychiatry. These studies show how preclinical studies can be used to enhance our understanding of the working mechanisms of deep brain stimulation in psychiatry.
Neuromodulation in corticostriatal circuits

On deep brain stimulation and dopamine

Marianne Klanker
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Author: Marianne Klanker

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Neuromodulation in corticostriatal circuits
On deep brain stimulation and dopamine

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Universiteit van Amsterdam

Copromotores: Dr. M.G.P. Feenstra
NIN-KNAW
Dr. I. Willuhn
Universiteit van Amsterdam

Overige leden: Prof. Dr. J. Booij
Universiteit van Amsterdam
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Dr. C.S. Lansink
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University of Washington, USA

Faculteit der Geneeskunde
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General introduction
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General introduction and thesis outline

Cognitive flexibility

Sometimes, things don’t go as expected. When I walk to the coffee machine in the afternoon, it sometimes happens that when pressing the button for a coffee with milk, no beverage comes out. Further inspection of the machine’s display then tells me that the supply of milk has run out. This leaves me with several options (the choice of which may depend on how much I actually want the coffee) – I can walk to another building in search of a coffee machine that hasn’t run out of milk, I can use this as an opportunity to acquire a taste for black coffee, or I can settle for tea instead.

These types of experiences are a hallmark of everyday life – we pursue certain actions in order to get things we desire (and we refrain from acting in order to prevent certain adverse or unwanted events). When a certain action no longer gives us the desired outcome, we are able to change our behavior. This adaptation of goal-directed behavior in response to changes in the environment is also known as cognitive flexibility. Impaired behavioral adaptation to environmental changes can result in behavioral rigidity and maladaptive behavior as observed in various neurological and psychiatric disorders, such as Parkinson’s disease, drug addiction and obsessive-compulsive disorder (OCD) (Chamberlain et al., 2006; Cools et al., 2001b; Ceaser et al., 2008; Yerys et al., 2009; Verdejo-Garcia et al., 2006). These disorders are also characterized by altered activity in corticostriatal circuits. Corticostriatal circuits consist of reciprocal connections between cortex, striatum and thalamus and are critically involved in the regulation of motivational and goal-directed behavior, for example when learning that certain actions are associated with wanted outcomes, such as obtaining rewards, or with avoiding unwanted outcomes, such as preventing punishments. Thus, studying the underlying neurobiological mechanisms underlying cognitive flexibility will not only increase our understanding of our everyday functioning, but may also provide an indication of the neurobiological changes underlying psychiatric symptoms.

Integrity of corticostriatal circuits is essential for cognitive flexibility. Studies in rodents, non-human primates and humans have shown that both prefrontal and striatal regions are important for the regulation of cognitive flexibility (Castane et al., 2010; McAlonan and Brown, 2003; Dias et al., 1996; Clarke et al., 2008; Bellebaum et al., 2008). Because there is homology in the brain circuits that regulate adaptive behavior between different species, we can use certain behavioral paradigms in translational research to investigate the neurobiology underlying this type of behavior. The use of rodents as our research subject permits direct (and invasive) manipulations and measurements during execution of these behavioral paradigms, which can extend and specify findings obtained in human subjects. For example, rats are willing to perform certain actions, such as pressing a lever, in order to receive a sugar pellet. Thus, we can put rats in a controlled environment (operant chamber) and directly monitor their neurotransmitter levels whilst they perform behavioral tasks.

A variety of behavioral tasks that have been developed to study cognitive flexibility in controlled settings is available (see Chapter 2 for a short description of different tasks available). For the experiments described in this thesis we have used a spatial reversal learning task to measure cognitive flexibility (De Bruin et al., 2000). In reversal learning, subjects first learn that an action results in reward presentation. Subsequently, they learn to discriminate between two options – one option is linked to a positive outcome, and the other option is linked to
a negative or neutral outcome. For example, a press on the right lever gives subjects a sugar pellet, whereas a response on the left side leaves them with nothing. In this discrimination phase, subjects learn to associate a certain action (respond on right side) with a corresponding outcome (sugar pellet; reward). When subjects have mastered this discrimination, a reversal is presented during which the response-reward contingencies are reversed: The originally rewarded response is no longer rewarded, whereas the previously unrewarded response is now rewarded. This means that if the subject wants to keep getting the reward, it has to adapt its behavior and start responding to the previously unrewarded site. Successfully adapting behavior in response to these changing task demands requires the ability to use negative feedback to switch responding and the use of positive feedback to consolidate the newly rewarded action. Therefore, it is important to identify the neural systems that underlie this feedback mechanism.

**Dopamine**

Dopamine (DA) is an important neuromodulator in corticostriatal circuits. The cell bodies of DA neurons are located in midbrain regions such as the ventral tegmental area (VTA) and substantia nigra (SN). From these two regions, DA neurons send widespread connections to cortical (prefrontal cortex) as well as subcortical regions (striatum, amygdala, hippocampus). DA neurons show a low frequency baseline firing rate (Hyland et al., 2002; Grace and Bunney, 1984) that ensures a relatively stable concentration of DA in cortical and limbic projection regions. The constant release of DA in projection regions is required for the performance of motor actions, but also for cognitive functions such as working memory, decision making and motivation (Wise, 2004). The low, baseline frequency firing of DA neurons and the associated slower changes in the concentration of DA in projection regions is often referred to as tonic DA.

Deviations from the basal level of DA occur when DA neurons show a short lasting increase in firing frequency (burst firing) (Freeman et al., 1985). Such burst firing can be observed spontaneously or in response to salient events, such as unexpected food rewards or other sensory stimuli (Freeman and Bunney, 1987). Burst firing of DA neurons leads to a rapid, but short lasting, increase in the extracellular DA concentration in projection regions, called a DA transient. Similar to burst firing of DA neurons, these transients are observed spontaneously and in response to salient events (Robinson et al., 2002; Cheer et al., 2004). The burst firing of DA neurons and the associated rapid changes in extracellular DA in projection regions is referred to as phasic DA.

The phasic changes in activity of DA neurons are often associated with reward-related learning. This idea originated from electrophysiological studies in primates, where the activity of DAergic cell bodies was directly measured. DA neurons show increased burst firing in response to unexpected reward and reward-predicting stimuli, and decrease firing when an unexpected reward is omitted (Schultz et al., 1997), suggesting that phasic DA may serve as a teaching signal to guide behavior during learning (Montague et al., 1996; Schultz et al., 1997; Waelti et al., 2001). Very similar results were more recently obtained in rodents (Pan et al., 2005; Steinberg et al., 2013). In human subjects, DA neuronal activity cannot be measured directly, but changes in BOLD-levels in striatal and brainstem regions during presentation of rewards and punishments have been consistently reported (Pessiglione et al., 2006; O’Doherty et al., 2002; McClure et al., 2003). Patients with MDD or OCD show blunted activity to rewards and during reward anticipation (Greenberg et al., 2015; Figee et al., 2011; Jung et al., 2011; Marsh et al., 2015; Pizzagalli et al., 2009), suggesting that they show impairments in phasic DA reward processing.
Deviations from the baseline level of DA may also be important during the reorganization of response patterns during adaptive behavior (Frank and Claus, 2006; Hong and Hikosaka, 2011). The presence or absence of DA may influence the effect of excitatory input from cortical or limbic regions to the striatum, which allows restructuring of neuronal networks that may be necessary for the reorganization of response patterns (Goto and Grace, 2005; Kelle et al., 2003; Hong and Hikosaka, 2011). Although the idea that DA facilitates cognitive flexibility has already been proposed 30 years ago (Cools, 1980; van den Bos and Cools, 1989; Oades, 1985), only a few reports on the measurement of extracellular levels of DA in the brain (reflecting DA release) are available. In addition, it is not well known how DA release in different subregions of the striatum alters during adaptive behavior. Ventromedial and dorsolateral striatum receive input from separate cortical regions (Webster, 1961; McGeorge and Faull, 1989) and these parallel corticostriatal circuits are differently involved in the control of behavior (Voorn et al., 2004; Yin and Knowlton, 2006).

**Fast-scan cyclic voltammetry**

Studying the relation between release of neurotransmitters and specific behavioral events requires a detection method with sufficient time resolution. This enables the direct coupling of specific behavioral events or stimuli presented to fluctuations in neurotransmitter release. Fast-scan cyclic voltammetry (FSCV) is a neurochemical detection technique with a decent time resolution. It uses the redox-properties of molecules to detect changes in neurotransmitters, allowing distinct measurements of release during separate behavioral events. A potential is applied between a carbon fiber electrode (the working electrode) and an Ag/AgCl reference electrode. Every 100 ms (10 Hz scan rate), the potential is driven from -0.4 V to 1.3 V and back in a triangular waveform. Molecules present in close proximity to the surface of the electrode will be oxidized and reduced at a specific potential, and plotting the applied potential versus the current that is measured will result in a cyclic voltammogram that can be used to identify the molecule of interest (Phillips et al., 2003; Millar et al., 1985). Because FSCV is a technique that requires background subtraction, it is most suited to track relative changes in neurotransmitter concentration, but less suited to measure the absolute concentrations (Phillips et al., 2003; Robinson et al., 2003).

The first aim of this thesis is to investigate the involvement of phasic DA in the striatum in the control of cognitive flexibility.

**Deep Brain Stimulation**

The studies described in the first part of this thesis investigate the neurobiology of adaptive behavior, which may ultimately enhance our understanding of the dysfunctions underlying cognitive disturbances in psychiatric disorders. The second part of this thesis explores the neurobiological and cognitive effects of a relatively new treatment option in psychiatry: Deep Brain Stimulation (DBS). DBS is a reversible neurosurgical intervention technique. One or two electrodes are implanted in the brain to deliver electrical pulses. Although it was originally developed for movement disorders, in recent years, DBS has been applied to psychiatric disorders as well. Successful trials have been described for OCD and MDD (Denys et al., 2010; Greenberg et al., 2010; Mayberg et al., 2005). These studies suggest that DBS shows promising treatment effects in a group of patients that does not respond to other available treatment options. Although DBS can successfully reduce symptoms in approximately 50%
of treatment resistant patients, the working mechanisms of DBS for psychiatric disorders are not fully understood.

For both MDD and OCD, successful remission of symptoms can be achieved with stimulation in different target regions, with similar treatment effects. These target regions have in common that they are all part of the corticostriatal circuit. For OCD, targets include: the nucleus accumbens, ventral internal capsule/ventral striatum and subthalamic nucleus (De-nys et al., 2010; Greenberg et al., 2010; Nuttin et al., 1999; Mallet et al., 2008), whereas for MDD stimulation of the nucleus accumbens, subgenual cingulate cortex, lateral habenula and medial forebrain bundle have resulted in symptom reduction (Malone, Jr. et al., 2009; Schlaepfer et al., 2008; Schlaepfer et al., 2013; Mayberg et al., 2005). DBS not only influences neural activity in the target region that immediately surrounds the electrode, but can also influence activity in distal regions (Mayberg et al., 2005; Fige et al., 2013; McCracken and Grace, 2007). These findings suggest that DBS can influence activity throughout a neural network. Thus, it has been hypothesized that DBS may exert its treatment effect by ‘resetting’ dysfunctional activity throughout the corticostriatal circuit (Lujan et al., 2008; McIntyre and Hahn, 2010; Deniau et al., 2010; Anderson et al., 2012).

Although we may have a general idea that DBS influences activity within a neural network, this still leaves the question of how this activity is modulated. Preclinical animal studies can be used to more directly study neurobiological effects of deep brain stimulation. One way by which deep brain stimulation may modulate activity within a neural network might be by influencing the release of neurotransmitters.

The second aim of this thesis was to characterize the effect of DBS on phasic DA release by combining DBS with FSCV in awake rodents.
THESIS OUTLINE

PART I – Dopaminergic control of cognitive flexibility

The first part of this thesis describes studies that were initiated to increase our insight in the neurobiological mechanisms underlying cognitive flexibility.

Chapter 2 provides a general overview of the involvement of the dopamine system in cognitive flexibility. This chapter summarizes a wealth of human and animal studies that have used genetic and pharmacological approaches to study the role of dopamine in adaptive behavior, as well as the influence of these manipulations on neural activity during the execution of these tasks. Because cognitive inflexibility has been associated with obsessive-compulsive disorder, we examined whether this inflexibility in obsessive-compulsive disorder is associated with dopamine dysfunction in the second part of this chapter.

Although studies using pharmacological and genetic techniques can provide a general idea of the role of dopamine in the regulation of adaptive behavior, these type of manipulations result in long-term changes in the dopamine system and thus cannot delineate fast changes in dopamine that occur in response to specific events when adapting an established behavioral response. The two following chapters describe a set of experiments that were initiated to investigate the involvement of phasic dopamine in the control of cognitive flexibility. Specifically, we used fast-scan cyclic voltammetry to monitor phasic changes in dopamine release in the ventromedial and dorsolateral striatum whilst rats performed a reversal learning task. The use of this detection technique, with its high time resolution, allowed us to delineate changes in striatal dopamine release in direct relation to stimuli presented and behavioral actions.

Dopamine in the striatum is central to reward-based learning. Less is known about the contribution of dopamine to the ability to adapt previously learned behavior in response to changes in the environment, such as a reversal of response-reward contingencies. Chapter 3 describes how dopamine signaling in the ventral striatum changes when animals adapt their behavior in a reversal learning task. Rapid updating of information about response-reward contingencies is essential for successful adaptation of behavior during reversal learning. We therefore hypothesized that dopamine is involved in the rapid updating of response-reward information. Modelling studies propose that fast bidirectional fluctuations in striatal dopamine immediately following positive and negative feedback are important for adaptation of behavior (Frank and Clauss, 2006; Hong and Hikosaka, 2011). Therefore, we specifically looked at whether measurements of extracellular dopamine can indeed reflect the receipt of feedback on a trial-by-trial basis.

A large body of work investigated dopamine release in the ventromedial striatum during reward-related learning and motivation, whereas relatively little attention was given to the dorsolateral striatum. Although there is evidence that dopamine in the dorsal striatum is also involved in the regulation of reward-related learning and cognitive flexibility (Faure et al., 2005; Amalric and Koob, 1987; Beninger and Ranaldi, 1993; Robbins et al., 1990; Robinson et al., 2007; Cools et al., 2001a; Sawamoto et al., 2008; Clarke et al., 2011), it is not clear how phasic dopamine in the dorsal striatum is modulated during reward-related learning and...
adaptation of behavior. Dopamine release patterns are not uniformly broadcast throughout the entire striatum. Instead, there are regional differences in dopamine release in response to natural rewards and reward-related stimuli between separate striatal subregions (Willuhn et al., 2012; Brown et al., 2011; Shnitko and Robinson, 2015). We therefore expected to see different release patterns in the dorsolateral and ventromedial striatum. The study described in Chapter 4 describes phasic dopamine characteristics in the dorsolateral striatum during reversal learning with a direct comparison to the results of chapter 3.

PART II – Deep brain stimulation in corticostriatal circuits – effects on dopamine and cognition

The second part of this thesis contains two examples of how preclinical studies can be used to enhance our understanding of the working mechanism of deep brain stimulation. The first chapter investigates if deep brain stimulation can influence neurotransmitter release in the striatum. The second chapter investigates cognitive effects of deep brain stimulation in a potential novel target region.

Deep brain stimulation in the medial forebrain bundle was recently proposed as a novel target for deep brain stimulation in major depressive disorder. This target appears promising, as the antidepressant effect occurred rapidly after onset of stimulation and applying a low current was sufficient to reach treatment effect. The authors of this clinical report hypothesized that recruitment of the dopamine system may contribute to the anti-depressant effect of medial forebrain bundle stimulation (Schlaepfer et al., 2013; Schlaepfer et al., 2014). Although older studies suggest that brief stimulus trains can induce striatal dopamine release (Gratton et al., 1988; Nakahara et al., 1989; Young and Michael, 1993; Owesson-White et al., 2008; Hernandez et al., 2006; Garris et al., 1997), the effect of continuous stimulation with clinically relevant stimulation parameters on striatal dopamine release in freely moving rodents had not been described before. Thus, we set up an experiment to directly test if deep brain stimulation of the medial forebrain bundle influences dopamine release dynamics in the striatum. Chapter 5 describes the results of this experiment.

In Chapter 6, the cognitive effects of deep brain stimulation in the orbitofrontal cortex are explored. Patients with obsessive-compulsive disorder show hyperactivity in the orbitofrontal cortex in rest and during provocation of symptoms. This hyperactivity is normalized after pharmacological or behavioral therapy (Nakao et al., 2005; Saxena et al., 1999) and following deep brain stimulation, suggesting that modulation of activity in the orbitofrontal cortex is an important component in the successful treatment of obsessive-compulsive disorder. Preliminary results suggest that deep brain stimulation in the orbitofrontal cortex can rescue compulsive behavior in a mouse homolog of obsessive compulsive disorder (de Haas, 2012). We used a cognitive task that depends on integrity of the orbitofrontal cortex to test the cognitive consequences of stimulation in this region.

In final Chapter 7, the main results of each of the above chapters are summarized, followed by a general discussion and suggestions for future research.
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PART I

Dopaminergic control of cognitive flexibility
Dopaminergic control of cognitive flexibility in humans and animals

Marianne Klanker, Matthijs Feenstra, Damiaan Denys

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ABSTRACT

Striatal dopamine is thought to code for learned associations between cues and reinforcers and to mediate approach behavior towards a reward. Less is known about the contribution of dopamine to cognitive flexibility – the ability to adapt behavior in response to changes in the environment. Altered reward processing and impairments in cognitive flexibility are observed in psychiatric disorders such as obsessive compulsive disorder (OCD). Patients with this disorder show a disruption of functioning in the frontostriatal circuit and alterations in dopamine signaling. In this review we summarize findings from animal and human studies that have investigated the involvement of striatal dopamine in cognitive flexibility. These findings may provide a better understanding of the role of dopaminergic dysfunction in cognitive inflexibility in psychiatric disorders, such as OCD.
INTRODUCTION

In a constantly changing environment behavior has to be adaptive and flexible. Cognitive flexibility is the ability to adapt goal-directed behavior in response to changing situational demands. Cognitive flexibility is one of the cognitive domains that are grouped together as executive functions or executive control (Gilbert and Burgess, 2008). Despite the necessity of cognitive flexibility for everyday functioning there is a substantial variation within the healthy population (Miyake and Friedman, 2012) that can be related to variations in dopamine (DA) related genes in humans (Barnes et al., 2011; Braver et al., 2010) and mice (Laughlin et al., 2011). Specific deficits in the ability to flexibly update behavior are observed in various neurological and psychiatric disorders such as Parkinson’s disease (PD), schizophrenia, autism, addiction and obsessive compulsive disorder (OCD) (Chamberlain et al., 2006; Cools et al., 2001b; Ceaser et al., 2008; Yerys et al., 2009; Verdejo-Garcia et al., 2006).

Here, we intend to provide an overview of animal and human studies on the relation between cognitive flexibility and DA neurotransmission and relate this to OCD, a psychiatric disease that combines defects in cognitive flexibility and alterations in DA processes.

Testing cognitive flexibility

The successful adaptation of behavior following changes in the environment encompasses several cognitive processes, such as associative learning, decision making, response selection and inhibition, working memory and attention. Several neuropsychological tests have been constructed to study different types of cognitive flexibility, which may recruit varied cognitive functions and depend on parallel neurobiological substrates. The use and translational applicability of a number of these tasks was discussed by Barch et al (2009). One set of tasks probes flexibility of choice behavior, where selection of one from two or more options leads to a wanted outcome. For a specific response to be adapted, the behavior has to be acquired first. During discrimination learning, subjects learn to discriminate between a certain rewarded/correct stimulus, strategy or response rule and another one that is not rewarded/correct. When task demands change, the response that has been successful so far no longer yields reward and has to be inhibited, whilst another response/stimulus/strategy has to be chosen, initiated and maintained. This requires extinction of the old association and acquisition of a novel association. Classical reversal learning and intra- and extradimensional attentional set-shifting fall in this category.

Reversal learning

With reversal learning, the ability to adapt behavior in response to a reversal of reinforcement contingencies is studied. This requires a shift in valence between stimuli or locations that have been associated with a specific outcome (e.g. a reward) previously. Depending on the operationalization of the reversal task used, this can be a reversal of all sorts of cues, but the choice options remain the same.

Attentional set-shifting and strategy shifting

Attentional set-shifting requires adaptation of behavior following changes in the relevance of perceptual categories or dimensions. In an intradimensional set-shift, new stimulus exemplars (i.e. novel choice options) are presented but the relevant stimulus dimension does not change between trials. Successful shifting requires maintenance of the current rule (atten-
tional set) and adapting behavior accordingly. In an *extradimensional set* shift, not only are the stimulus exemplars novel, but the reinforced dimension has also changed. This requires a response shift to a dimension that has previously been irrelevant and bypassing of an acquired attentional bias (Rogers et al., 2000).

In human subjects, the ability to shift cognitive sets is commonly tested with the Wisconsin Card Sorting Test (WCST). The WCST requires matching of a multi-dimensional cue card to one of four reference cards according to a specific stimulus aspect. The attentional set-shifting task has been developed as a non-human primate version of the WCST (Roberts et al., 1988). Because it is a more direct measure of the ability to shift cognitive set and a better measure for frontal lobe impairments (Rogers et al., 2000), it is now often used in human subjects as well.

Both reversal learning and attentional set-shifting paradigms have been developed for humans, non-human primates and rodents. Stimulus dimensions consist of different visual stimulus sets that can be simple or compound in nature (human, non-human primate, rodent) or stimulus sets consisting of multiple sensory dimensions (spatial, odour, touch, visual; rodent bowl digging procedure (Birrell and Brown, 2000; Garner et al., 2006). Discriminations based on stimulus valence have been classified as representing a lower order of abstraction, whereas discriminations based on stimulus components or abstract rules may represent a higher order of abstraction (Wise et al., 1996; Ragozzino, 2007).

Another example of a procedure based on a response rule or strategy and an announced switch to a different rule or strategy is response-based vs cue-based responding on a T-maze, often applied in rodents (Packard, 2009).

A general problem with switching responses in these tasks is that several processes occur simultaneously and that incorrect responses may reflect different mechanisms, i.e. resistance to extinction versus learned irrelevance (Maes et al., 2004). Task adaptation (Tait and Brown, 2007) or detailed analysis (e.g. (Dias et al., 1996a)) lead to more informative outcomes. Three-choice paradigms have been used in non-human primates and may offer superior experimental approaches as they allow testing of more variable conditions and require animals to trace the value of several alternative options, as a change in one option does not automatically imply a change in the other alternative options (Walton et al., 2010).

**Task switching**

Task switching is a paradigm that is mostly, but not exclusively (Stoet and Snyder, 2003; Lee-naars et al., 2012) used in human subjects and requires the rapid switching between stimulus-response sets that have been acquired previously (Monsell, 2003; Sohn et al., 2000). Presentation of an external cue indicates which task (stimulus-response set) has to be executed in a given trial. This differs fundamentally from reversal learning and set-shifting procedures, where the presentation of altered contingencies (i.e. ‘the switch’) is not cued and subjects have to use the change in reinforcing feedback to adapt behavior accordingly.

**Control over prepotent or automatic responses**

Another category incorporates tasks that probe the ability to behave flexibly in conditions that previously allowed automatic or habitual performance. A well-known example is the countermanding or stop-signal task (Logan et al., 1984; Eagle et al., 2008), testing inhibitory control over actions. Another example is the anti-saccade task where a more or less automatic action needs to be suppressed to allow flexible responding (Munoz and Everling, 2004).
In the present review we focus on studies using reversal learning, attentional set-switching (including WCST) and task-switching as these tasks have received most translational interest, have been related to DA function and have been performed in OCD patients.

**Neural circuitry supporting cognitive flexibility**

**Prefrontal cortex**

Within the prefrontal cortex (PFC), damage to different prefrontal areas results in dissociable deficits in separate forms of cognitive flexibility. Damage to the orbitofrontal cortex (OFC) is thought to specifically impair reversal learning, but not attentional set-shifting (Hornak et al., 2004; Boulougouris et al., 2007; McAlonan and Brown, 2003; Dias et al., 1996a). Damage to the lateral PFC (or medial PFC in rodents, suggested to be functionally equivalent; (Uylings et al., 2003)) specifically impairs (extradimensional) shifting of attentional sets but not reversal learning (Dias et al., 1996a; Dias et al., 1997; Birrell and Brown, 2000; Bissonette et al., 2008; Owen et al., 1991). However, the proposed unique role of the OFC in reversal learning is under discussion and alternative views have been presented (Schoenbaum et al., 2009). Recent findings suggest that impaired reversal learning in Rhesus monkeys is only observed following aspiration but not excitotoxic OFC lesions (Rudebeck et al., 2013), suggesting that reversal learning does not depend on an intact OFC but instead on intact communication between other prefrontal areas and more caudal structures. While human brain lesions generally involve passing fibers and brain parenchym, many studies in rodents and new world monkeys report deficits after fiber-sparing lesions. The transient character of impairments in these studies may reflect evolution-related differences in neurobiological and/or anatomical substrates of reversal learning (Rudebeck et al., 2013).

**Striatum**

Reciprocal projections from PFC to the striatum and thalamus form parallel frontostriatal loops, suggesting striatal regions also contribute to the regulation of cognitive flexibility (Castele et al., 2010; Clarke et al., 2008; Ragozzino, 2007; Rogers et al., 2000; Floresco et al., 2006). Combined results from lesion and functional imaging studies suggest that different types of cognitive flexibility are regulated by segregated fronto-striatal circuits: OFC and dorsomedial striatum (human/non-human primate: caudate nucleus; functional equivalent rodent area: dorsomedial striatum) are implicated in reversal learning (Ghahremani et al., 2010; Rogers et al., 2000; Bellebaum et al., 2008; Castane et al., 2010; Clarke et al., 2008; Dias et al., 1996a; McAlonan and Brown, 2003a; Divac, 1971). Set- and task switching performance relies on connections between the dorsolateral PFC (or the medial PFC in rodents which is in this task functionally equivalent) and striatum (Dias et al., 1996a; Dias et al., 1996c; Sohn et al., 2000; Ragozzino, 2007; Graham et al., 2009; Owen et al., 1991; Manes et al., 2002; Birrell and Brown, 2000). It should be noted that these circuits are not fully segregated but overlapping. Importantly, these circuits show consistent similarities between primates and rodents (Mailly et al., 2013).

**Dopamine**

DA is an important neuromodulator in fronto-striatal circuits. A substantial amount of work has described a role for DA in reward-related learning and motivated behavior. More specifically, burst firing of DA neurons (associated with phasic DA release) may code a quantitative
prediction error that serves as a teaching signal to guide behavior and is essential for a range of learning situations (Montague et al., 1996; Schultz et al., 1997; Schultz, 2013; Steinberg et al., 2013). Yet not much is known about the contribution of DA to the adaptation of behavioral following changing task demands, such as a reversal of contingencies. A common factor in all tests of cognitive flexibility is the expectation of a reward (or absence of punishment) when a correct response is made. The absence of an expected reward and presence of an unexpected reward following a reversal or shift is the archetypal situation for the occurrence of reward prediction errors coded by DA. Therefore, one would expect that DA is in some way involved in the regulation of cognitive flexibility. However, in the past decade the role of the PFC and its serotonergic innervation in cognitive flexibility received most attention (e.g. (Robbins and Arnsten, 2009)).

In this review, we summarize findings from animal and human studies that investigated whether DA contributes to the regulation of cognitive flexibility. First, we will describe pharmacological manipulations to the DA system in humans and animals, then DA-related genetics in humans and animals. Next, we report on DA changes and cognitive flexibility in OCD, to investigate whether alterations in DA signaling contribute to cognitive inflexibility in this disorder. Previously, OCD has been proposed to be characterized by a hyperdopaminergic state (Denys et al., 2004b) and similar states in animals have repeatedly been described as leading to OCD-like behaviors (see further). This, combined with the suggestion that impairments in the ability to flexibly adapt behavior may be an endophenotype for OCD (Robbins et al., 2012) drove us to review the evidence for a relation between the two.

PHARMACOLOGICAL MANIPULATIONS AND IMAGING STUDIES IN HUMAN SUBJECTS

DA synthesis

DA synthesis capacity in humans is determined after administration of radio labeled F-DOPA or F-tyrosine and imaging the resulting fluorinated amines using PET. The observed variations in DA synthesis capacity may relate to variations in DA neurotransmission, as a significant negative correlation between synthesis capacity and D2-receptor availability was reported (Ito et al., 2011). Decreasing DA synthesis by dietary omission of DA precursors tyrosine and phenylalanine reduces occupation of D2 receptors by endogenous DA, suggesting decreased DA transmission (Montgomery et al., 2003). Administration of the tyrosine hydroxylase inhibitor alpha-methyl-paratyrosine also reduces D2 occupation by endogenous DA (Verhoeff et al., 2003), but affects noradrenergic signaling as well (Krahn et al., 1999).

The small number of studies using these approaches does not support a general relation between DA synthesis and flexible updating of task information: no correlation was observed between DA synthesis capacity and task performance on the WCST (Vernaleken et al., 2007), and reward- and punishment-based reversal learning was not impaired following DA depletion in males (Robinson et al., 2010). In contrast, catecholamine depletion (affecting both DA and NA) impaired performance during probabilistic reversal learning (Hasler et al., 2009).

Other studies suggest that when tasks are used that allow more selective approaches, a differential involvement of DA synthesis is observed. Thus, subjects with high DA synthesis capacity perform worse compared to subjects with low DA synthesis capacity when presented with shifts in object features but not in abstract rules in a task-switching paradigm (Dang et al., 2012). Cools et al (2009) reported that individuals with high DA synthesis capacity per-
DAergic control of cognitive flexibility

form better when presentation of an unexpected reward signals reversal compared to reversals that are signaled by presentation of an unexpected punishment, whereas the opposite is observed for individuals with low DA synthesis capacity. Females tend to have a higher DA synthesis capacity (Laakso et al, 2002) and this may explain gender-related differences such as the DA depletion-induced improvement of punishment-based but not reward-based reversal learning in females (Robinson et al, 2010).

In conclusion, DA synthesis is differentially associated with task features in cognitive flexibility and variations in synthesis capacity affect performance only in some task conditions, probably depending on specific DA homeostasis parameters in cortical and striatal areas (cf Cools & D’Esposito, 2011).

DA receptor/transporter binding

Using imaging techniques, baseline availability of DA receptors and transporters can be investigated and related to task performance. Receptor availability in resting conditions provides an index of the number of receptors unoccupied by the endogenous transmitter. Subjects with higher availability of DA transporters in the striatum make less perseverative errors in the WCST (Hsieh et al., 2010) but the interpretation of this finding depends on whether the higher availability reflects the density of the DA innervation or a possible substrate-induced adaptation (Chen et al, 2010).

WCST performance has also been linked to differences in DA receptor availability (see Table 1). Decreased striatal D2 availability is associated with impaired performance (Volkow et al., 1998), but D2/D3 receptor binding in the anterior cingulate cortex correlates positively with the number of errors made in the WCST (Lumme et al., 2007).

For DA transmission through D1 receptors, an optimal level of DA activity is required for best working memory performance (Zahrt et al., 1997; Williams and Goldman-Rakic, 1995; Vijayraghavan et al., 2007). Similar results were obtained for flexible responding in the WCST where impaired performance is observed for both high and low prefrontal D1 (but not D2) binding (Takahashi et al., 2008), but see (Karlsson et al., 2011)).

When receptor availability is assessed during task performance, it provides a measure of task-related release of endogenous DA. Reduced binding to D2 receptors in the dorsal striatum (Monchi et al., 2006a) and anterior cingulate cortex (Ko et al., 2009) during set-shifting (see Monchi et al., 2006b) suggests that DA is indeed released during tasks requiring flexibility. Transient inactivation of dorsolateral PFC activity impaired striatal DA release as well as task performance, suggesting both are under top-down control by the dorsolateral prefrontal cortex (Ko et al., 2008).

Taken together, these findings indicate that DA is activated and can influence performance on set-shifting tasks through D2 receptors in the striatum and anterior cingulate cortex, whereas in the prefrontal cortex, DA activity through D1 receptors can modulate performance. In addition, optimum values may exist for both extracellular DA concentrations and DA receptor numbers. The majority of studies relating performance on cognitive flexibility tasks to DA-receptor binding potential have specifically focused on binding to D2 receptors in specifically delineated brain areas. Therefore, although this provides evidence that D2 receptors modulate performance in these types of tasks, one cannot exclude the involvement of D1 receptors.
Pharmacological manipulations affecting DA signaling
DA neurotransmission during task performance can be influenced by administration of pharmacological agents that directly bind to DA receptors or by drugs that induce DA release. Combining the administration of pharmacological agents with functional imaging during task performance indicates in which brain areas modulation by DA is most pronounced.

DA antagonist
Systemic administration of the D2 receptor antagonist sulpiride slows response times during task-switching (Mehta et al., 2004) and impairs performance of an extra-dimensional set-shift, without affecting intra-dimensional set-shifting (Mehta et al., 1999; Mehta et al., 2004). Sulpiride enhances performance on reward-based reversal learning (van der Schaaf et al., 2012). This behavioral effect was stronger in subjects with higher working memory capacity (which is assumed to reflect higher striatal DA synthesis capacity (Cools et al., 2008)). In addition to behavioral effects, sulpiride also increased striatal BOLD signals during unexpected outcomes, irrespective of whether the unexpected outcome was a reward or a punishment (van der Schaaf et al., 2012).

Indirect DA agonist
Methylphenidate is a psychostimulant that increases striatal extracellular DA levels (Volkow et al., 2001), but also affects serotonin (5-hydroxytryptamin, 5-HT) and noradrenaline (Kuczenski and Segal, 1997). Administration of methylphenidate leads to displacement of raclopride binding to D2 receptors (Clatworthy et al., 2009). These changes in the post commissural part of the caudate nucleus were associated with effects on reversal learning, such that a large displacement following methylphenidate was associated with impaired performance and a small displacement with improved performance (Clatworthy et al., 2009). As these effects may depend on individual variation in receptor availability and DA synthesis capacity, behavioral effects of the psychostimulant on measures of flexibility are likely to be averaged out when the individual variation is not taken into account – which may explain the negative results on attentional set-shifting (Elliott et al 1997).

Administration of methylphenidate influences brain activation in ventral striatal regions during behavioral adaptation and modulates activity in frontal regions during cognitive control. Thus, activation in ventral striatal regions was reduced during reversal errors (even in the absence of behavioral effects), whereas in prefrontal regions, increased activation was observed following correct responses (Dodds et al., 2008). The balance of DA in frontal and striatal regions may therefore be crucial in regulating the balance between cognitive control and cognitive flexibility.

DA agonist
Interestingly, DA synthesis capacity also influences the effect of direct DA agonists on task performance. While Mehta et al (2001) originally observed an increase in non-perseverative errors and slowed reaction times during probabilistic reversal learning after administration of the D2 agonist bromocriptine, Cools et al (2009) later showed that this drug impaired reversal learning from unexpected rewards in subjects with high DA synthesis capacity, but improved the same parameter in subjects with low synthesis capacity in striatal regions.

The beneficial effect of D2 receptor stimulation in subjects with low DA synthesis capacity is not limited to reversal learning. Bromocriptine can also improve performance on the WCST
(Kimberg et al., 1997) and task-switching performance (van Holstein et al., 2011) in subjects with low DA synthesis capacity, whereas no effects are observed following administration of pergolide, which differs from bromocriptine in that it also activates D1 receptors (Kimberg and D’Esposito, 2003). That the improvement on task switching after bromocriptine can be specifically related to the function of D2 receptors was shown by van Holstein et al. (2011), as pre-treatment with the D2 antagonist sulpiride blocked the beneficial effect. Therefore, performance of subjects with high DA synthesis capacity is impaired following administration of bromocriptine, and increases following administration of sulpiride.

Summary and conclusion
To conclude (see Table 1), flexible updating of behavior in set-shifting tasks (WCST and attentional set-shifting) as well as task switching is associated with increased DA neurotransmission through D2 receptors. In particular, the mediating effects of D2 signaling on task performance have been observed in the dorsal striatum and anterior cingulate cortex, which is in line with observations from imaging and lesion studies suggesting the involvement of the connections between PFC and dorsal striatum in the regulation of these types of flexibility (Sohn et al., 2000; Owen et al., 1991). This also concurs with observations in patients with PD. In the early stages of PD, when DA depletion is largely limited to the dorsal striatum, patients show impairments in task switching whereas reversal learning performance is spared. Administration of levodopa reverses the impairments in task switching, whilst it impairs performance on reversal learning probably due to overstimulation of DA receptors in ventral striatal regions (Cools, 2006; Kehagia et al., 2010). In control subjects increased D2-mediated transmission also impairs reversal learning, although this may turn into an improvement when DA synthesis capacity is low.

Human studies have particularly shown the importance of individual differences in the DA system. Individual differences in DA synthesis capacity influence both task performance and effects of manipulations to the DA system in different types of flexibility. Individual differences in D2 receptor availability also influence stimulation-induced changes in performance during reversal learning. The combined study of manipulations to the DA system with performance on behavioral tasks, indicate that DA transmission in the ventral striatum changes during reversal learning.

These results also indicate that there may be differences in the involvement of DA in reversal learning compared to set-shifting and task switching. As noted before, these paradigms are thought to represent different levels of complexity and may depend on different brain areas. However, studies differ in the task designs used to study one type of cognitive flexibility. Therefore, replication of effects of DAergic manipulations using similar task designs would help in delineating the possible differences in DA contribution to reversal, set-shifting and task switching.

A question remains in what way D1 receptors contribute to behavioral performance during cognitive flexibility tasks. Direct manipulations of D1 signaling or studies relating performance on behavioral task to D1 receptors availability are scarce. Combining the administration of pharmacological agents with functional imaging during performance of different behavioral paradigms may provide more insight on the effects of DA on cognitive flexibility in prefrontal and striatal regions.
PART I – DAergic control of cognitive flexibility

<table>
<thead>
<tr>
<th>Paradigm</th>
<th>Manipulation</th>
<th>Performance</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
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<td>Nagano-Saito et al., 2008</td>
</tr>
<tr>
<td></td>
<td>Synthesis capacity</td>
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<td>D1/D2 agonist</td>
<td>=</td>
<td>Kimberg and D’Esposito, 2003</td>
</tr>
<tr>
<td></td>
<td>DAT availability striatum</td>
<td>↑ reduced errors</td>
<td>Hsieh et al., 2010</td>
</tr>
<tr>
<td></td>
<td>D2/D3 binding anterior cingulate</td>
<td>=</td>
<td>Lumme et al., 2007</td>
</tr>
<tr>
<td></td>
<td>D1 binding dorsolateral PFC</td>
<td>=</td>
<td>Takahashi et al., 2008</td>
</tr>
</tbody>
</table>

| Reversal learning | DA Depletion Catecholamine depletion | ↑ punishment based reversal | Robinson et al., 2010 |
|                  | Synthesis capacity High | ↑ reward based reversal | Cools et al., 2009 |
|                  | Low | ↑ punishment based reversal | Cools et al., 2009 |
|                  | D2 agonist High DA synthesis | ↓ more errors, longer RT | Mehta et al., 2001 |
|                  | Low DA synthesis | ↓ reward based reversal | Cools et al., 2009 |
|                  | D2 antagonist | ↑ reward based reversal | van der Schaaf et al., 2012 |

| Task Switching | Synthesis capacity | = abstract rule shift | Dang et al., 2012 |
|               | Low DA synthesis | ↑ | van Holstein et al., 2011 |
|               | D2 antagonist | ↓ longer RT | |

| Attentional set-shift | D2 binding Dorsal striatum Binding reduced during shifts | = | Monchi et al., 2006a; Ko et al., 2009 |
|                       | Anterior Cingulate | ↓ EDS performance | Mehta et al., 1999; Mehta et al., 2004 |
|                       | D2 antagonist | = IDS performance | |
|                       | D2 agonist Methylphenidate | = | Elliott et al., 1997 |

Table 1 Summary of effects of pharmacological manipulations to the dopamine system on cognitive flexibility in human subjects. = no effect, ↑ increased performance, ↓ decreased performance, DA dopamine, RT reaction time, EDS extra dimensional set-shift, IDS intradimensional set-shift

PHARMACOLOGICAL MANIPULATIONS IN ANIMALS

The use of pharmacological imaging in human subjects provides insight into the role of DA in cognitive flexibility, but the use of animals permits direct (and invasive) manipulations and measurements and can extend and specify findings obtained in human subjects. Here, we
will discuss animal studies that have used pharmacological manipulations of the DA system or DA depletion to investigate in what way DA in prefrontal and striatal regions contributes to cognitive flexibility.

DA depletion studies
In rodents, lesioning DAergic projections in the nucleus accumbens core (though DA in the medial PFC was similarly affected) impairs both spatial discrimination and reversal learning on a T-maze (Taghzouti et al., 1985). Selective depletion of DA neurotransmission in the dorsomedial striatum impairs odor guided reversal learning, without affecting initial discrimination learning (O’Neill and Brown, 2007). A selective deficit in reversal learning following DA depletion in the dorsomedial striatum was observed in primates as well (Clarke et al., 2011). The deficit in reversal learning following DA depletion is not perseverative, suggesting that DA may be particularly important for the learning phase after reversal, rather than mediating response inhibition to the previously rewarded side. The effect was not only shown in the first, but also in subsequent reversals. Importantly, the deficit is neurochemically specific, as depletion of 5-HT neurotransmission in the mediate caudate nucleus does not affect behavioral performance during reversal learning (Clarke et al., 2011). A previous study also found decreased performance on reversal learning (although this did not reach significance) (Collins et al., 2000). Subsequently, Crofts et al (2001) showed that although acquisition, maintenance and initial shifting of an attentional set are intact, monkeys with DA depletion in the caudate are impaired when they have to make an attentional shift to a stimulus dimension that was learned to be irrelevant in a previous extra dimensional shift (Collins et al., 2000); (Crofts et al., 2001). Therefore, DA in the caudate nucleus appears to be involved in situations that require a shift of established cognitive sets (Collins et al., 2000).

In contrast to DA depletion in striatal regions, selective DA depletion in frontal regions is complicated by the accompanied depletion of noradrenaline (Crofts et al., 2001) (Roberts et al., 1994). Although (Roberts et al., 1994) observed a specific improvement in performance on extra-dimensional set-shifts after prefrontal catecholamine depletion in non-human primates, a later study suggests that this may actually result from an inability to maintain an attentional set (Crofts et al., 2001). Prefrontal catecholamine depletion is associated with long lasting enhancement of striatal DA release, suggesting that it may be the balance between DA levels in prefrontal and striatal regions rather than DA levels in either region that affects behavior (Roberts et al., 1994).

DA versus 5-HT
Based on data from depletion studies, a neurochemical dissociation between prefrontal and striatal regions in the control of cognitive flexibility during reversal learning has been suggested. In the caudate nucleus, DA, but not 5-HT depletion impairs performance during reversal learning. Previously, it was reported that 5-HT, but not DA neurotransmission in the OFC is required for successful behavioral adaptation in a spatial reversal learning task (Clarke et al., 2004; Clarke et al., 2007). Depletion of 5-HT in the OFC specifically impairs reversal learning by increasing perseverative responding, but does not affect attentional set-shifting (Clarke et al., 2005). OFC DA depletion, however, leads to impaired extinction, albeit not in a perseverative manner (Walker et al., 2009). The contributions of 5-HT and DA neurotransmission to cognitive flexibility therefore appear to be confined to separate functions related to regions of the cortico-striatal circuit. Recently, Groman et al (2013) suggested that the
balance between 5-HT levels in the OFC and DA levels in the dorsal striatum contributes to individual differences in cognitive flexibility. Reduced performance on a reversal learning task is associated with low levels of 5HT in the OFC when DA levels in the putamen are low, but not when DA levels in the putamen are high (Groman et al., 2013). These findings indicate that cognitive flexibility is under control of DA and 5-HT, while other data show involvement of noradrenaline, as well (Lapiz and Morilak, 2006; Seu et al., 2009; Bouret and Sara, 2004).

**Effects of psychostimulants**

Psychostimulants such as methylphenidate, (meth)amphetamine and cocaine increase release of DA and other monoamines by blocking catecholamine re-uptake or promoting DA release (Sulzer et al., 2005). Administration of methylphenidate in rodents does not affect reversal learning (Cheng and Li, 2013; Seu and Jentsch, 2009), although the latter authors observed beneficial effects in animals with reversal learning impairments (spontaneously hypertensive rats). Effects of amphetamine and methamphetamine on reversal learning have been variable, but possibly dose-dependent: high doses (5 mg/kg) impair reversal learning (Arushanian and Baturin, 1982; Cheng et al., 2007; Idris et al., 2005; Izquierdo et al., 2010; Koshel' et al., 2012; Ridley et al., 1981; Talpos et al., 2012; White et al., 2009), while intermediate doses 1-2 mg/kg show no effect or improved learning (Daberkow et al., 2008; Wilpizeski and Hamilton, 1964; Mead, 1974; Pastuzyn et al., 2012; Soto et al., 2012; Kulig and Calhoun, 1972; Weiner et al., 1986; Weiner and Feldon, 1986) and low doses again impair reversal performance (Idris et al., 2005; Ridley et al., 1981). These results are compatible with the general idea that cognitive function depends on DA activity in an inverse U-shaped fashion (Cools and D’Esposito, 2011; Arnsten et al., 2012). However, given the multiple and differential effects of psychostimulants on monoamine release in prefrontal and striatal regions it is often difficult to conclude whether these effects depend on increased DA release. Yet, for methylphenidate (Cheng and Li, 2013) showed that the beneficial effect were blocked by local injections with haloperidol in the OFC.

**Systemic effects of DA (ant)agonists**

While selective depletion studies indicate specific brain areas where DA modulates flexible behavior, administration of pharmacological agents that are selective for a specific receptor subtype indicate how D1 and D2 receptor subtypes are involved. In primates, both stimulation and inhibition of D2/D3 receptor function results in difficulties in adapting behavior following changing task demands, but not during acquisition of the original discrimination (Lee et al., 2007; Smith et al., 1999). Administration of the D2/D3 antagonist raclopride affects performance on reversal learning when administered alone, but only when the reversal is preceded by retention of the originally acquired discrimination (Lee et al., 2007). Performance is also reduced by the D2/D3 agonist 7-OH-DPAT (Smith et al., 1999) and this deficit is antagonized by co-administration with the D2/D3 antagonist raclopride, but not the D2-selective antagonist sulpiride, suggesting stimulation of D2 receptors impairs performance (Smith et al., 1999).

In rodents, like in primates, administration of a D2/D3 agonist (quinpirole) impaired spatial reversal learning in an operant chamber by increasing the number of perseverative errors. Administration of a D1/D2 antagonist (raclopride) or selective D2 antagonist (nafadotride) had no effect (Boulougouris et al., 2009). The quinpirole-induced deficit is attenuated when raclopride is co-administered, but worsens after co-administration with nafadotride. Selective stimulation of D1-receptors (co-administration of quinpirole and nafadotride) increased both
the number of discrimination errors and of perseverative and learning errors in the reversal phase (Boulougouris et al., 2009). Thus, stimulation of D₂ receptors may be important for the acquisition of altered response-reward contingencies during reversal learning whereas D₁-receptor activation may cause a more generalized impairment (Boulougouris et al., 2009). Systemic administration of a D₁/D₅ antagonist does not affect reversal learning in primates (Lee et al., 2007), though in rodents systemic administration of a D₄ agonist (SKF-812979) impairs early, but not late stages of reversal learning (Izquierdo et al., 2006). Extradyssynaptic set-shifting on the other hand improves following intermediate, but not high or low doses of a D₁ agonist (Nikiforuk, 2012).

These findings suggest that D₂-like receptors contribute to the regulation of cognitive flexibility, possibly in a dose-dependent manner. System administration of D₁-like receptors has received less attention and could affect cognitive flexibility depending on the species or behavioral task used.

Local effects in the striatum

Local manipulations of DA neurotransmission can elucidate in which way DA neurotransmission in specific subregions of the frontostriatal circuit can contribute to cognitive flexibility (although see (Arnt, 1985) for the limitations of this approach). Execution or suppression of actions leading to reward are controlled by two parallel corticostriato-thalamo-cortical pathways (Frank and Claus, 2006). From the striatum, output neurons in the direct pathway connect to cortical regions via connections to globus pallidus pars interna (GPi)/substantia nigra pars reticulata (SNr) and thalamus. Output neurons in the indirect pathway project via globus pallidus pars externa, subthalamic nucleus to GPi/SNr, thalamus and cortex. Activity in these pathways can be differentially modulated by activation of D₁ or D₂ receptors in the striatum (Frank and Claus, 2006). Yawata et al (2012) investigated pathway specific control of reward learning and cognitive flexibility. Blocked neurotransmission in the direct pathway, combined with D₁ blockade in the contralateral nucleus accumbens impaired the acquisition phases of the original discrimination as well as the discrimination presented after a reversal or a rule shift, while stimulation of D₂ receptors did not influence behavior (Yawata et al., 2012). Application of a D₂ agonist combined with contralateral blockade of the indirect pathway induced perseverative responding during reversal learning and also affected rule shifting, without affecting acquisition of the original discrimination problem (Yawata et al., 2012). These findings suggest that within the nucleus accumbens, stimulation of dopamine D₁ receptors (direct pathway) aids the acquisition and relearning of behavioral responses to a particular stimulus, whereas suppression (i.e. a phasic interruption) of D₂-mediated transmission (indirect pathway) may be required to allow reorganization of ongoing behavioral patterns. These results are in line with previous findings reporting impaired reversal learning after local stimulation of D₂ receptors, while during set-shifting blocking D₁ receptors impaired maintenance of the new strategy and stimulation of D₂ receptors induced perseverative responding (Haluk and Floresco, 2009).

Local effects in the prefrontal cortex

DA depletion in the OFC did not affect reversal learning (Clarke et al., 2007), but local manipulation of DA receptors in the OFC can influence aspects of cognitive flexibility. Blockage of D₁ or D₂ receptors in OFC prevents development of discriminative reaction times to high and low rewards under reversal conditions, without affecting accuracy (Calaminus and Hauber, 2008).
In a task that required rats to adapt behavior following a change in reward value, by manipulating the amount of lever presses required to obtain a food pellet, local inhibition of D1 but not D2 receptors in the OFC impaired performance (Winter et al., 2009). In the MPFC, local inhibition of both D1 and D2 receptors inhibits performance (Winter et al., 2009). Set-shifting ability in a maze-based shifting task is affected by manipulations of several DA receptors in the MPFC. Local blockade of D1 and D2 receptors as well as stimulation of D4 receptors results in perseverative responding, whereas blockade of the D4 receptor improves performance (Floresco et al., 2006b; Ragozzino, 2002). This contrasts with the findings of D1 blockade in the nucleus accumbens, which did not induce perseverative responding, but affected maintenance of the new strategy.

In vivo DA measurements related to cognitive flexibility

Only a few reports on the measurement of extracellular levels of DA in the brain (reflecting DA release) are available. In the nucleus accumbens, these levels are higher during acquisition of a rule shift compared to simple rule acquisition in a T-maze set-shift paradigm (Stefani and Moghaddam, 2006), clearly suggesting a role for DA in the nucleus accumbens in the regulation of cognitive flexibility, in particular strategy or set-shifting. In the mPFC, both rule acquisition and rule shifting in a T-maze are accompanied by increased DA levels and higher basal mPFC DA levels were associated with rapid shifting between discrimination rules (Stefani and Moghaddam, 2006). After inhibition of COMT, animals also show increased task-related, but not basal extracellular DA levels in the medial prefrontal cortex, suggesting that task-induced increases in PFC DA release may contribute to set-shifting performance (Tunbridge et al., 2004).

DA (but not noradrenaline) release in the MPFC is elevated and prolonged during performance of a spatial reversal session in a skinnerbox, compared to release in a discrimination session preceding reversal (van der Meulen et al., 2007). Within the reversal session, the DA elevation was most pronounced during the phase in which rats improved performance. These findings suggest elevated DA release in both striatal and prefrontal regions during execution of cognitive flexibility tasks.

Summary and conclusion

Taken together (see Table 2), DA appears to be actively involved in the performance of tasks requiring cognitive flexibility: DA release is increased, local DA depletion impairs performance and pharmacological interference alters task execution. Whereas DA depletion studies indicated ventral and dorsomedial striatum as the primary location where DA influences cognitive flexibility, specific DA receptor stimulation/blockade studies and in vivo release measurements implicate prefrontal regions as well. A complicating factor is that manipulation of prefrontal DA also affects striatal DA transmission (Roberts et al., 1994).

It is important to note that impairment of reward-related learning and cognitive flexibility following perturbations in DA signaling is almost always of transient nature: subjects eventually do make the switch when sufficient trials are presented, suggesting that DA may facilitate these behaviors, but is not indispensable.

Interestingly, most pharmacological studies investigating the involvement of DA-subtype selective receptors have indicated that striatal blockade of D1 receptors and overactivation of D2 receptors impairs performance. This was most elegantly shown in the study of Yawata et al (2012): DA signaling through D1 receptors in the nucleus accumbens and the direct
basal ganglia pathway contributes to the acquisition of a new reward-directed behavior in a four-armed maze once switching has occurred (i.e. $D_1$ stimulation could contribute to new learning following a behavioral switch), whereas suppression of $D_2$-mediated transmission in the accumbens and the indirect pathway is required for the reorganization of behavioral patterns. A transient elevation in DA potentiates connections in the direct pathway to initiate movement towards reward, whereas a transient dip in DA potentiates connections in the indirect pathway to suppress movements that are no longer rewarded (Hong and Hikosaka, 2011). The findings from animal studies do indicate a role for the DA in the nucleus accumbens mediating cognitive flexibility, both reversal and strategy or set-shifting, whereas less research has focused on local manipulation of $D_1$ or $D_2$ receptors in dorsomedial or dorsolateral striatal regions. However, a role for dorsal striatal regions has been indicated by selective DA depletion studies as well as a significant amount of human data. Moreover, in the primate dorsal striatum (caudate and putamen), availability of $D_2$-receptors can be related to performance during reversal but not discrimination learning (Groman et al., 2011). This warrants further investigation of the effects of manipulating $D_1$ or $D_2$ signaling in striatal regions other than the nucleus accumbens.

In general, these conclusions are similar to those based on human data, as discussed in the previous section. However, unlike what was reported in humans, $D_2$-based manipulations seem to affect lower order (cue reversal) and higher order (rule or task switch) processes in a similar way. It is unclear if $D_2$-mediated effects in animals depend on DA synthesis capacity.

**CONTRIBUTIONS OF DA GENOTYPE TO COGNITIVE FLEXIBILITY IN HUMANS**

Individual variability in executive functioning may be subserved by a strong genetic component (Friedman et al., 2008). The expression of complex traits such as cognitive flexibility is likely regulated by multiple genes that each contribute a small effect. Several polymorphisms in genes affecting DA functioning have been investigated to explain individual variability in cognitive flexibility.

**DA receptors and intracellular signaling**

$D1$

DARPP-32 (DA and cAMP regulated phosphoprotein of 32kDa) is strongly expressed in medial spiny neurons in the striatum, where it is stimulated by $D_1$ and inhibited by $D_2$ receptor activation and mediates post-receptor effects of DA (Nishi et al., 1997; Svenningsson et al., 2004). Enhanced performance on several cognitive tasks, including the WCST, was observed for a frequent haplotype in the DARPP-32 gene that is associated with increased post-mortem DARPP-32 expression and affects structural and functional connectivity between PFC and striatum (Meyer-Lindenberg et al., 2007). The polymorphism was also associated with better learning from positive feedback (Frank et al., 2007). This suggests $D_1$ receptors in the striatum could contribute to learning after positive feedback, supporting successful switching of behavior in cognitive flexibility tasks by maintaining responses to the newly rewarded site.
Table 2 Summary of effects of pharmacological manipulations to the dopamine system on cognitive flexibility in animals. = no effect, ↑ increased performance, ↓ decreased performance, EDS extra dimensional set-shift, IDS intradimensional set-shift, MPFC medial prefrontal cortex, OFC orbitofrontal cortex

<table>
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<tr>
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<th>Manipulation</th>
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<td>Set-shift</td>
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<tr>
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<td>Antagonist</td>
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<td>Antagonist</td>
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<td>(Ragozzino, 2002)</td>
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<tr>
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<td>Agonist</td>
<td>Antagonist</td>
<td>↓ more trials/errors to criterion. Perseverative</td>
<td>(Floresco et al., 2006b)</td>
</tr>
<tr>
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<td>Agonist</td>
<td>Antagonist</td>
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<td>(Roberts et al., 1994)</td>
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<tr>
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<td>Antagonist</td>
<td>↓ perseveration</td>
<td>(Boulougouris et al., 2009)</td>
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<tr>
<td></td>
<td>Systemic (primate)</td>
<td>D2/ D3 Antagonist</td>
<td>↓ more trials/errors to criterion</td>
<td>(Lee et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>Systemic (rodent)</td>
<td></td>
<td></td>
<td>(Boulougouris et al., 2009)</td>
</tr>
<tr>
<td></td>
<td>D3/ D2 Agonist</td>
<td>Antagonist</td>
<td>↓ more trials/errors to criterion</td>
<td>(Smith et al., 1999)</td>
</tr>
<tr>
<td>Nucleus Accumbens</td>
<td>D1 Agonist</td>
<td>Antagonist</td>
<td>=</td>
<td>(Haluk and Floresco, 2009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Calaminus and Hauber, 2007)</td>
</tr>
<tr>
<td>D2</td>
<td>Agonist</td>
<td>Antagonist</td>
<td>↓ trials to criterion/ errors, but not perseveration</td>
<td>(Haluk and Floresco, 2009)</td>
</tr>
<tr>
<td></td>
<td>Depletion</td>
<td></td>
<td>↓</td>
<td>(Taghzouti et al., 1983)</td>
</tr>
</tbody>
</table>
DAergic control of cognitive flexibility

<table>
<thead>
<tr>
<th>Paradigm Region</th>
<th>Manipulation</th>
<th>Performance</th>
<th>Reference</th>
</tr>
</thead>
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<tr>
<td>Dorsomedial striatum</td>
<td>Depletion</td>
<td>↓ more trials to criterion</td>
<td>(O’Neill and Brown, 2007)</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>(Clarke et al., 2011a)</td>
</tr>
<tr>
<td>OFC</td>
<td>D1 Antagonist</td>
<td>↓ absence discriminative reaction times (high/low reward)</td>
<td>(Calaminus and Hauber, 2008)</td>
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<td></td>
<td></td>
<td>↓ impaired maintenance</td>
<td>(Winter et al., 2009)</td>
</tr>
<tr>
<td></td>
<td>D2 Antagonist</td>
<td>↓ absence discriminative reaction times (high/low reward)</td>
<td>(Calaminus and Hauber, 2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>= reversal required effort not affected</td>
<td>(Winter et al., 2009)</td>
</tr>
<tr>
<td>MPFC</td>
<td>D1 Antagonist</td>
<td>↓ impaired maintenance</td>
<td>(Winter et al., 2009)</td>
</tr>
<tr>
<td></td>
<td>D2 Antagonist</td>
<td>↓ impaired maintenance</td>
<td>(Winter et al., 2009)</td>
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</table>

The DRD2-TAQ1 polymorphism is located close to the exon coding for the D2 receptor. A1-allele carriers show a reduced number of available D2 receptors (Thompson et al., 1997; Pohjalainen et al., 1998), but see (Lucht and Rosskopf, 2008) and the A1-allele is associated with increased DA synthesis in the striatum (indicating reduced autoreceptor-mediated feedback regulation) (Laakso et al., 2005). In a probabilistic learning task, carriers of the A1-allele showed reduced ability to learn from errors accompanied by functional changes in the frontostriatal circuitry (Klein et al., 2007). A1-carriers showed blunted reward-related activity in the NAC, reduced activity in the posterior medial frontal cortex during negative feedback and reduced interactions between the medial frontal cortex and hippocampus (Klein et al., 2007). The use of feedback is required to adapt responding during reversal learning and, not surprisingly, A1-carriers perform worse (Jocham et al., 2009). Following presentation of a reversal, they were less likely to maintain the newly rewarded response, but kept alternating responses and showed diminished activation of orbitofrontal and ventral striatal regions during reversals (Jocham et al., 2009). Task-switching performance on the other hand is improved in A1-carriers, who show reduced switch costs associated with decreased activity in the lateral prefrontal cortex and decreased connectivity between PFC and dorsal striatal regions (Stelzel et al., 2010). Switching tasks does not depend on the use of feedback and is supported by different circuits/areas than switching responses based on the use of feedback (Stelzel et al, 2010). This illustrates how impaired DA transmission could have different effects depending on the operationalization of the cognitive flexibility task that is used, i.e. whether on-line feedback-induced response adaptation (“learning”) is essential or not.
A second polymorphism affecting availability of striatal D2 receptors is the C957T polymorphism of the DRD2 gene (Hirvonen et al., 2004; Hirvonen et al., 2005). CC-allele carriers show reduced binding potential to striatal D2 receptors (Hirvonen et al., 2004; Hirvonen et al., 2005) and impaired responding in the WCST (Rodriguez-Jimenez et al., 2006). In addition, CC-allele carriers are reduced in their ability to use negative feedback in a probabilistic reinforcement learning task (Frank et al., 2007). These concurrent findings suggest that reduced availability of D2 receptors is associated with impaired cognitive flexibility, resulting from an inability to use negative feedback to adapt behavior.

**DA transporter and metabolizing enzymes**

The DA transporter (DAT) regulates re-uptake of DA from the synaptic cleft in striatal regions, whereas its influence in the PFC is less pronounced (Sesack et al., 1998). Using a task-switching protocol based on the WCST, Garcia-Garcia et al (2010) observed impaired performance and electrophysiological differences in 9-repeat allele carriers compared to 10-repeat allele carriers of the DAT gene. During task-switching, manipulation of reward anticipation affects performance and striatal activity depending on DAT genotype, suggesting striatal DA levels mediate the influence of motivational effects on cognitive flexibility (Aarts et al., 2010). However, considering that it is unclear how this polymorphism relates to DAT expression in vivo (Heinz et al., 2000; van de Giessen et al., 2009; van Dyck et al., 2005; Martinez et al., 2001); meta-analysis by (Costa et al., 2011)), these results should be interpreted with caution.

The polymorphism that has received most attention relating DAergic gene function to executive functioning is the Valine (Val)/Methionine (Met) polymorphism at codon 158 of the Catechol-O-methyltransferase (COMT) gene (Lotta et al., 1995). Activity of COMT is thought to be lower in homozygote Met allele carriers compared to homozygote Val carriers, presumably resulting in higher prefrontal DA levels in Met homozygotes (Chen et al., 2004; Lotta et al., 1995; Meyer-Lindenberg et al., 2005), although striatal DA levels may also be altered (Akil et al., 2003). Most studies investigating the association between the COMT Val/Met polymorphism and cognitive flexibility used perseverative responding or perseverative errors in the WCST as a measure of flexible behavior. Results have not been consistent: although an initial meta-analysis (Barnett et al., 2007) reported a small effect of COMT genotype on performance in the WCST, with reduced perseverative errors for the Met homozygotes, a second meta-analysis could not confirm an association between COMT genotype and perseverative responding on the WCST and several other cognitive measures, suggesting that the COMT polymorphism does not consistently relate to cognitive functioning (Barnett et al., 2008). It has been suggested that the variety of cognitive functions contributing to WCST performance complicate attribution of impaired performance to deficits in cognitive flexibility or deficits in cognitive stability (Bilder et al., 2004). Other test measures of cognitive flexibility might be more sensitive and more selective indicators of alterations in this function.

Despite the inconsistent effects of COMT genotype on perseverative errors in the WCST, the COMT Val/Met genotype is associated with differential activation patterns in the PFC during other cognitive paradigms (Mier et al., 2010). Therefore, it is interesting to relate COMT genotype to neural activation during other tasks that measure separate aspects of cognitive flexibility more specifically, to see whether this genotype influences neural activation in these tasks. Indeed, when Krugel et al (2009) studied the influence of COMT gene polymorphisms on performance and neural activity during probabilistic reversal learning, Val homozygotes performed better than Met homozygotes and showed increased striatal...
BOLD responses during prediction errors. In addition, higher connectivity between frontal and ventral striatal regions could be related to learning rate in Val homozygotes (Krugel et al., 2009). Interestingly, these findings suggest that striatal activity reflecting prediction errors might be modulated by DA levels in the prefrontal cortex. However, during acquisition of probabilistic reinforcement learning, Val homozygotes show reduced switching of responses following negative outcomes on a trial-by-trial basis (Frank et al., 2007). This suggests that striatal DA function may be differentially regulated by DA levels in the prefrontal cortex during response acquisition or adaptation of an existing response. In addition to a behavioral advantage during reversal learning, Val homozygotes also have smaller switch costs on a task switching paradigm when trials have short intervals (Colzato et al., 2010). Together these findings indicate a behavioral advantage on both reversal learning and task switching paradigms for Val homozygotes, suggesting that lower baseline levels of prefrontal DA may benefit cognitive flexibility in humans.

Summary and conclusion
A substantial amount of studies investigating the influence of genes mediating DA function on cognitive flexibility have limited analysis to a task that likely measures several complex cognitive functions, i.e. the WCST (Friedman et al., 2008). A more promising approach may be to study the effect of DA related genes on well-defined operationalizations of cognitive flexibility, such as initial discrimination learning, reversal learning, attentional set-shifting or task switching. A confound in the study of cognitive effects of genetic polymorphisms is that the effect of a polymorphism on DA transmission or even on gene expression is often not known. This hampers translational approaches, in which effects of increased or decreased expression and/or DA transmission might be studied in a controlled and reproducible manner.

To summarize, the studies reviewed above suggest an association between polymorphisms regulating DA function and cognitive flexibility. Reduced availability of D2 receptors, presumably affecting striatal DA activity, impairs the use of negative feedback and the maintenance of a new response during reversal learning and set-shifting (in the WCST), whereas increased availability of D2 receptors impairs task switching, suggesting different involvement of D2 receptors in these tasks. Striatal D1 signaling, mediated by DARPP-32 function, also contributes to cognitive functioning, although this has not yet been verified using specific measures of cognitive flexibility. Presumed lower levels of prefrontal DA, mediated by COMT-genotype appear to facilitate behavioral adaptation in both reversal learning and task-switching paradigms (see Table 3).

To conclude, considering that the genetic underpinnings of complex cognitive functions are likely to be polygenic and not limited to DA, studying additive genetic effects of DA related genes on cognitive flexibility as well as the study of interactions between DA related genes and other genes regulating frontostriatal function could provide a better understanding of the genetic basis of cognitive flexibility (Frank and Fossella, 2011).
PART I – DAergic control of cognitive flexibility

<table>
<thead>
<tr>
<th>Paradigm</th>
<th>Gene</th>
<th>Presumed DA effect</th>
<th>Performance</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reversal</td>
<td>D2</td>
<td>↓ D2 binding striatum</td>
<td>↓ reversal learning</td>
<td>(Jocham et al., 2009)</td>
</tr>
<tr>
<td></td>
<td>COMT</td>
<td>Val/Val</td>
<td>↑ reversal learning</td>
<td>(Krugel et al., 2009)</td>
</tr>
<tr>
<td>Task Switch</td>
<td>D2</td>
<td>Non-A1</td>
<td>↑ D2 binding striatum</td>
<td>↓ increased switch cost</td>
</tr>
<tr>
<td></td>
<td>DAT</td>
<td>9-repeat</td>
<td>↑ D2 binding striatum</td>
<td>↓ increased RT cue switch/ task switch</td>
</tr>
<tr>
<td></td>
<td>COMT</td>
<td>Val/Val</td>
<td>↓ COMT activity PFC</td>
<td>↑ reduced switch cost</td>
</tr>
<tr>
<td>WCST</td>
<td>D2</td>
<td>C957T – CC</td>
<td>↓ D2 binding striatum</td>
<td>↓ WCST categories completed, perseveration</td>
</tr>
<tr>
<td></td>
<td>COMT</td>
<td>Val/Val</td>
<td>↓ COMT activity PFC</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td>DARPP-32</td>
<td>Haplotype</td>
<td>↑ WCST performance</td>
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EFFECTS OF GENETIC MANIPULATIONS IN DA RELATED GENES ON COGNITIVE FLEXIBILITY IN ANIMALS

The use of genetically modified animals provides an invaluable tool to study the role of DA related genes in cognitive flexibility. Selectively targeted mutations on a known genetic background can elucidate the genetic and neurobiological basis of complex behavior.

DA deficiency
An example of an advanced genetic approach is selective reinstatement of DA signaling in ventral or dorsal striatum of DA-deficient mice (Darvas and Palmiter, 2011). Restoring DA signaling specifically to either dorsal or ventral striatum supports acquisition and reversal of a turn-based escape strategy in a water maze (Darvas and Palmiter, 2011). However, the ability to switch from one escape strategy to another (strategy set-shift) is impaired when DA signaling is limited to the ventral striatum, suggesting DA neurotransmission in the dorsal striatum is required for strategy set-shifting, whereas DA in either ventral or dorsal striatum is sufficient to support reversal learning (Darvas and Palmiter, 2011). It should be noted, however, that the translational value of the tasks used is not established.
DAergic control of cognitive flexibility

**D1**

Mice lacking functional D1 receptors show attenuated operant responding for reward (El-Ghundi et al., 2003). They show a general deficit in reinforcement learning, impaired motivation to work for a reward, are slow to discriminate between a reinforced and non-reinforced lever and are impaired in reversal learning, during which they maintain responding to both levers. Heterozygote mice are also impaired on reversals, although not as severely (El-Ghundi et al., 2003). The observed general deficits in motivation and reinforcement learning in D1-knockout mice, however, prevent the drawing of conclusions about the contribution of D1 receptors to cognitive flexibility.

Activation of D1 receptors modulates striatal function through phosphorylation of DARPP (Walaas and Greengard, 1984). Next to a minor reduction in performance during discrimination learning, DARPP-32 knockout mice show a pronounced deficit in reversal learning. Although knockout mice eventually were able to switch responding to the newly rewarded side, it took them significantly more sessions to do so (Heyser et al., 2000). This is indirect evidence that D1-receptor activation is needed for reversal learning.

**D2**

Genetic manipulations of D2 receptors also affect performance on cognitive flexibility tasks. Female mice with a complete knock-out of functional D2 receptors make more errors during odor discrimination and reversal learning whereas male D2-knockout are impaired during reversal learning only; both sexes show perseveration to the previously rewarded side (Kruzich and Grandy, 2004; Kruzich et al., 2006). This was confirmed by De Steno and Schmauss (2009), who also showed a similar impairment with chronic treatment with the D2 antagonist haloperidol. Glickstein et al (2005) observed a deficit of male D2-knockouts during compound discrimination, but not reversal, whereas D2 receptor knockouts showed increased performance during the reversal. The differences in behavioral performance were paralleled by opposite prefrontal activation patterns following the task sequence: activity dependent gene expression in the MPFC is increased for D3 mutants and decreased for D2 mutants (Glickstein et al., 2005; De Steno and Schmauss, 2009). Interestingly, knockout of neither D2 nor D3 receptors affects performance on intra- or extradimensional set-shifts (De Steno and Schmauss, 2009), suggesting differential contribution of D2/D3 receptors to the regulation of reversal learning or set-shifting.

Selective overexpression of D2 receptors in the striatum does not affect learning of a discrimination, a reversal or an intra- or extradimensional set-shift. Response latencies were longer during reversal trials only, suggesting the animals had some difficulties adapting established responses (Kellendonk et al., 2006). Interestingly, these mice also show physiological changes in the medial prefrontal cortex where DA turnover was decreased and activation of D1 receptors increased (Kellendonk et al., 2006).

**Metabolizing enzymes**

Overexpression of the human COMT-Val polymorphism in mice increases COMT enzyme activity (suggesting lower prefrontal extracellular DA) and induces specific deficits in cognitive flexibility. Although discrimination and reversal learning are not affected, these mice make more errors and need more time to complete an extra-dimensional set-shift (Papaleo et al., 2008). In contrast to behavioral impairments observed after increased COMT enzyme activity, pharmacological inhibition of COMT can improve performance (Tunbridge et al., 2004).
Summary and conclusion
The studies using selective DA-reinstatement in DA-deficient mice show that higher order flexibility (strategy shifting (Wise et al., 1996)) is associated with dorsal striatal DA, whereas lower order flexibility (reversal learning) may be supported by DA in all striatal areas. Similarly, human studies suggest influence of DA genotype on activity in ventral striatal regions or increased connectivity between PFC and ventral striatum during reversal learning and in dorsal striatal regions during task switching.

The D_{1} receptor is involved in cognitive flexibility, although this is overshadowed by a general impairment in goal-directed behavior in full knock-outs. DARPP-32 expression (reflecting D_{1} activity) is associated with cognitive performance in both humans and animals.

The findings described above, and the observation that performance of reversal learning in mice covaries with D_{2} receptor levels in the ventral midbrain (Laughlin et al., 2011), indicate the importance of D_{2} receptors for flexible behavior, specifically in a situation where response-reward contingencies are reversed (see Table 4). This compares to the influence of polymorphisms in the D_{2} receptor gene on the ability to learn from negative feedback in human subjects.

Expressing the human COMT-Val polymorphism (increasing COMT-activity and presumably decreasing extracellular prefrontal DA) in mice impairs extra dimensional set-shift. This concurs with the improved set-shifting performance after COMT-inhibition in rats. However, presence of the Val-polymorphism in humans has been associated with a behavioral advantage during reversal learning and task-switching suggesting that confirmation of these studies is needed before we can draw conclusions.

Caution should be exerted when interpreting results from animals in which a receptor is completely knocked out as compensatory mechanisms (such as increased neurotransmitter levels) during development may contribute to the observed deficits. Also, in the case of complete knock-outs it is not possible to locate the neurobiological substrate of the impairment as the knock-out is present throughout the brain. Finally, mice with intermediate expression of specific receptors (heterozygotes) are useful for studying gene-dosage effects on behavior, which could be particularly relevant when compared to differences in receptor expression levels observed in humans.

OCD
OCD is a psychiatric disorder that is characterized by recurrent intrusive, unwanted thoughts (obsessions) that are often accompanied by repetitive ritualistic behaviors (compulsions). Although the precise neurobiological substrates underlying OCD symptoms are not known, structural and functional imaging studies show alterations in frontal and orbitofrontal cortices and basal ganglia in OCD patients (Rotge et al., 2010; Pujol et al., 2004; van den Heuvel et al., 2009; Menzies et al., 2008b; Menzies et al., 2008a). Symptom severity correlates with increased functional connectivity between OFC and striatal regions (Harrison et al., 2009), which normalizes after treatment (Figue et al., 2013).

The repeated performance of ritual-like action sequences has led to the hypothesis that decreased cognitive flexibility or increased habitual behavior (Gillan et al., 2011) is a major underlying factor of OCD and could be a potential endophenotype for the disorder (Robbins et al., 2012). This might be an attractive suggestion considering that associated circuits and
neurotransmitters related to these processes are (partly) known. Indications for abnormal flexibility have been described in OCD patients (Chamberlain et al., 2006; Gu et al., 2008) and there is evidence for altered DA signaling (Denys et al., 2004a; Denys et al., 2004b; Moresco et al., 2007; Perani et al., 2008). Therefore, an important question is how DA contributes to this disorder. In the next sections, we will describe studies reporting alterations in the DA system in OCD patients as well as studies investigating cognitive flexibility in OCD.

**DA alterations in OCD**

Although there is strong evidence that serotonin plays a role in the treatment of OCD (van Dijk et al., 2010), it is clear that OCD pathophysiology also involves alterations in fronto-striatal circuitry and its neuromodulation by DA. Indirect evidence comes from clinical observations that administration of DA antagonists can improve symptoms in OCD-patients that do not respond to SSRIs alone ([Dougherty et al., 2004; McDougle et al., 2000]; see [Denys et al., 2004b] for review). In animals, administration of drugs acting on DAergic receptors and genetic manipulations of DA receptors induces compulsive, stereotypic behaviors similar to the repetitive behaviors of OCD patients ([Campbell et al., 1999; Joel and Doljansky, 2003; Denys et al., 2004b; Perani et al., 2008]; [Sesia et al., 2013; Szechtman et al., 1998]).

Importantly, direct evidence indicating altered DA signaling in OCD patients is also available. Kim et al. (2003) observed a higher density of the DA transporter (DAT) in the right basal ganglia of OCD patients compared to healthy controls.

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**Table 4** Effects of genetic manipulations to dopamine related genes on cognitive flexibility in animals.

<table>
<thead>
<tr>
<th>Paradigm</th>
<th>Gene</th>
<th>Performance</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discrimination</td>
<td>D1 KO</td>
<td>↓ more errors</td>
<td>(El-Ghundi et al., 2003)</td>
</tr>
<tr>
<td></td>
<td>D2 KO Female</td>
<td>↓ more errors</td>
<td>(Kruzich and Grandy, 2004)</td>
</tr>
<tr>
<td></td>
<td>D2 KO Male</td>
<td>=</td>
<td>(Kruzich et al., 2006)</td>
</tr>
<tr>
<td></td>
<td>COMT-Val overexpression</td>
<td>=</td>
<td>(Papaleo et al., 2008)</td>
</tr>
<tr>
<td>Reversal</td>
<td>D1 KO</td>
<td>↓ more errors</td>
<td>(El-Ghundi et al., 2003)</td>
</tr>
<tr>
<td></td>
<td>D2 KO Male + female</td>
<td>↓ more errors, ↓ increased RT reversal phase set-shift = reversal phase set-shift</td>
<td>(Kruzich and Grandy, 2004; Kruzich et al., 2006)</td>
</tr>
<tr>
<td></td>
<td>DARPP-32 KO</td>
<td>↓ more errors</td>
<td>(Heyser et al., 2000)</td>
</tr>
<tr>
<td>Attentional set-shift</td>
<td>D2 KO</td>
<td>=</td>
<td>(De Steno and Schmauss, 2009; Glickstein et al., 2005)</td>
</tr>
<tr>
<td></td>
<td>D2 overexpression Striatum only</td>
<td>=</td>
<td>(Kellendonk et al., 2006)</td>
</tr>
<tr>
<td></td>
<td>COMT-Val overexpression</td>
<td>↓ impaired EDS</td>
<td>(Papaleo et al., 2008)</td>
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</table>
ganglia that normalized after SSRI treatment (Kim et al, 2007). However, these findings were not consistently replicated (Nikolaus et al., 2010; van der Wee et al (2004) also showed higher binding ratios using OCD patients without co-morbid disorders, but Hesse et al (2005) observed reduced striatal DAT binding and Pogarell et al (2003) did not observe differences in DAT availability between OCD patients and healthy controls. The latter authors also reported increased instead of decreased DAT binding after SSRI’s.

OCD-patients show reduced binding to D1 receptors in caudate nucleus and putamen (Olver et al., 2009) and in anterior cingulate cortex (Olver et al., 2010), although reduced binding does not correlate with symptom severity.

Studies investigating binding to striatal D2 receptors in OCD patients present a more consistent picture. The original finding by Denys et al (2004a) of reduced binding to D2 receptors in the caudate nucleus of OCD patients was replicated by others (Perani et al., 2008; Schneier et al., 2008; Denys et al., 2013). In medication-naive OCD patients, repeated administration of an SSRI increased binding to striatal D2 receptors, illustrating that regulation of DA release can be modulated by 5-HT (Moresco et al., 2007).

Taken together, the studies mentioned here described reduced binding to DA receptors in OCD patients, mainly in, but not limited to striatal regions. The most replicated finding is reduced availability of D2 receptors in striatal regions. It has been hypothesized that reduced availability of DA receptors in OCD patients could be the result of increased DA release in the striatum (Denys et al., 2004a). However, the observed changes in the DA system do not correlate with symptom severity or duration of illness and it is possible that the DAergic alterations are secondary to diminished serotonergic tone.

**Cognitive flexibility in OCD**

Although the repetitive execution of behavioral patterns that is often observed in OCD patients could be defined as inflexible or perseverative behavior, the question is whether this translates to impaired performance on measurements of cognitive flexibility that are currently used in tests of executive functioning.

Findings using the Wisconsin Card Sorting Test (WCST) have been contradictory, with some studies observing impaired performance in OCD patients (Bohne et al., 2005; Bucci et al., 2007; Cavedini et al., 2010; de Geus et al., 2007; Lacerda et al., 2003; Lawrence et al., 2006; Lucey et al., 1997), whilst others do not (Abbruzzese et al., 1995; Abbruzzese et al., 1997; Cavedini et al., 1998; Fenger et al., 2005; Gambini et al., 1993; Henry, 2006; Moritz et al., 2002). The former studies often describe an increase in the number of perseverative errors. The observation that deficits in flexibility may persist after remission or use of medication and that unaffected family members also show reduced flexibility, suggests that these deficits are trait-like and independent of OCD-symptomatology (Cavedini et al., 2010; Bannon et al., 2006), supporting the hypothesis that inflexible, rigid and habit-like behavior is an endophenotype in OCD.

**Reversal learning**

Alterations in recruitment of fronto-striatal circuitry in the absence of behavioral impairments have been observed in both OCD patients and their unaffected first-degree relatives during reversal learning (Chamberlain et al., 2008). Remijnse et al (2006) observed attenuated responsiveness of OFC and striatal regions during reward and affective switching in OCD patients with and without comorbidities. In these studies, as well as in others (Ersche et al., 2011;
Valerius et al., 2008) no clear evidence for behavioral impairments during task performance was obtained, although OCD patients do show a somewhat slowed response pattern, suggesting they may require more processing time when faced with altered response-reward contingencies. Altered recruitment of fronto-striatal circuitry during these tests suggests that even though overt behavioral performance (i.e. reaction times, number of errors, number of trials required to reach criterion) may not be impaired, the processing of cognitive information is altered in OCD patients during reversal learning.

**Attentional set-shifting**

Performance on tasks that require shifting between different stimulus dimensions does appear to be affected in OCD patients. Behavioral impairments have been observed in OCD patients and unaffected first-degree relatives in an attentional set-shifting task (Fenger et al., 2005; Watkins et al., 2005; Chamberlain et al., 2006; Chamberlain et al., 2007; Veale et al., 1996) but see (Purcell et al., 1998a; Purcell et al., 1998b), with some reporting reduced performance on extra-dimensional set-shifts (Chamberlain et al., 2007; Watkins et al., 2005; Chamberlain et al., 2006; Veale et al., 1996) and others on intra-dimensional set-shifts (Fenger et al., 2005; Veale et al., 1996). Response to SSRI-treatment was found to be related to set-shifting ability (Fontenelle et al., 2001).

**Task switching**

Increased switch costs (decreased accuracy or increased response times) have been observed in OCD patients during performance of task switching paradigms (Moritz et al., 2004; Gu et al., 2008; Page et al., 2009). Gu et al (2008) found an increase in the number of errors made during task-switching trials in OCD patients, but others report slowed responding (Remijnse et al., 2013; Moritz et al., 2004) or no effect (Page et al., 2009). However, when task switching is combined with functional imaging, activity in the dorsal fronto-striatal circuit is consistently found to differ between OCD patients and healthy controls. Whereas activation of the dorsal fronto-striatal circuit is observed in healthy controls during task-switching trials, this is not the case in OCD patients (Gu et al., 2008; Remijnse et al., 2013; Page et al., 2009).

**Summary and conclusion**

Several problems arise when interpreting the deficits of OCD patients on cognitive flexibility and the mixed outcomes of the studies investigating these deficits. Next to the influence of medication and the need for careful matching of patient and control groups, the high comorbidity with other psychiatric disorders, in particular depression is an important confounding factor. Although the use of subject groups with OCD as the only clinical diagnosis could be thought of as misrepresentative for the population of OCD patients because comorbidity is so common (Olley et al., 2007), the use of well defined clinical populations in studies combining neuropsychological testing with measurements of brain activity in particular, could contribute to the knowledge about distorted recruitment of fronto-striatal circuitry in cognitive flexibility.

As far as we know, studies directly linking measurements of cognitive flexibility to alterations in DA signaling have not been performed in OCD patients. The most consistent alteration in the DA system is changed DA receptor binding, mostly in striatal regions. Replication of these findings, especially of both D1 and D2 receptor binding, in different OCD samples would enhance our understanding of the contribution of DA to OCD. For performance on
cognitive flexibility tasks, behavioral performance on lower order cognitive flexibility (reversal learning) is not altered, whilst OCD patients may be impaired on higher order flexibility tasks (attentional set-shift and task switching). Irrespective of the presence of behavioral impairments, activity and connectivity in neural circuits regulating flexible behavior (OFC-ventral striatum for reversal learning, PFC-dorsal striatum for task-switching) are altered in OCD patients during task execution. Considering the modulatory effect of DA in these neural circuits, it is possible that altered striatal DA contributes to different activity in these circuits during task performance.

OCD Animal Models: Dopamine and Cognitive Flexibility

Animal models of psychiatric disorders cannot reflect all aspects of the disease (Nestler and Hyman, 2010). In line with this, OCD models that show a combination of the critical face, predictive and construct validities (Wang et al., 2009; Korff and Harvey, 2006; Fineberg et al., 2011; Albelda and Joel, 2012b) predominantly mirror the compulsive acts of OCD patients. This applies for models based on spontaneous behavior (ethological models, e.g. compulsive dogs, (Vermeire et al., 2012)), behavioral models (e.g. compulsive lever-pressing during signal attenuation in rats (Joel, 2006)), pharmacological models (e.g. quinpirole-induced checking in rats (Szechtman et al., 1998), and transgenic models (e.g. compulsive grooming in Sapap3-mutant mice, (Welch et al., 2007)). Compulsive acts are behaviorally and conceptually not always clearly differentiated from simple repetitive behaviors. Repetitive, stereotyped, perseverative, rigid and habitual behavior have been grouped together into (overlapping) clusters of compulsive-like behavior ((Ting and Feng, 2011; Langen et al., 2011; Robbins et al., 2012b); for a critical discussion of the distinction between stereotypies and compulsions, see (Lewis et al., 2007)). These clusters are relevant not only for OCD, but also for other psychiatric disorders and may share a relative DAergic hyperactivity in the basal ganglia (Pitman, 1989). Two recent studies highlight the direct involvement of specific projections from OFC to ventromedial striatum in the regulation of compulsive-like, repetitive behavior in normal mice (Ahmari et al., 2013) and compulsively grooming Sapap-3 mice (Burguiere et al., 2013).

Stereotyped repetitive behavior, in particular, is strongly linked to DA mechanisms (Randrup and Munkvad, 1975; Ridley, 1994). Next to the quinpirole-model (repeated administration of a D2/3-selective agonist), the DAT-knockdown mouse that shows stronger and more rigid grooming behavior, has been proposed as an OCD-model based on DA hyperactivation (Berridge et al., 2005). Another model of increased DA-related neuronal activity is the D1CT transgenic mouse, showing repetition of all normal behaviors (Campbell et al., 1999). Most other validated OCD-models also show involvement of DA mechanisms in their compulsive behavior (Vermeire et al., 2012; Joel and Doljansky, 2003; Moreno and Flores, 2012; Presti et al., 2003; Albelda and Joel, 2012a; Sesia et al., 2013), although DA mechanisms were not tested in compulsively grooming transgenic mouse models (Welch et al., 2007; Shmelkov et al., 2010).

The relationship between repetitive behavior and cognitive flexibility as probed in tasks using translationally valid constructs of reversal learning, attentional set-shifting or task switching has received only limited attention. In deer mice, stereotyped jumping was correlated with the number of incorrect responses in a reversal of escape-learning in a water-filled T-maze (Taninemura et al., 2008). BTBR T+ tf.J mice, showing compulsive grooming and increased marble burying, show impaired probabilistic reversal learning (Amodeo et al.,
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2012). A task probing recurrent perseveration (two-choice task where continuous switching provides the optimal strategy) showed a correlation between stereotyped behavior and recurrent perseveration in farmed minks, but not in ICR CD-1 mice (Gross et al., 2011). Finally, rats compulsively drinking in the schedule-induced polydipsia model displayed increased perseveration during extinction of the 5-choice serial reaction time task and perseveration during extinction of other operant procedures was reported in bank voles (Garner and Mason, 2002) and caged bears (Vickery and Mason, 2005).

However, if we focus on reversal learning, attentional set-shifting or task switching there are no studies available that show task impairments in OCD animal models, let alone impairments related to DA mechanisms. The only possible exception is stereotyped behavior in deer mice, which correlated to the number of incorrect responses during reversal learning and decreased after striatal administration of a D₁-selective antagonist (Tanimura et al., 2008; Presti et al., 2003), though the relation between reversal learning and DA was not directly investigated.

In conclusion, a possible relation between compulsive behavior and cognitive flexibility, including the possibility that DA mechanisms might play a role in this, did not receive much attention up to now. One can understand that the introduction of translational valid paradigms for cognitive flexibility in exotic species such as bank voles, mink or bears is not an easy task. But using behavioral testing in reversal learning, attentional set-shifting or task switching in rodent OCD-models should be a priority for researchers who want to study the neurobiological underpinnings of OCD.

CONCLUSION

Evidence for a role of DA in the control of cognitive flexibility comes from a range of human and animal studies that have been reviewed above. This overview indicates that DA is involved in different facets of cognitive flexibility, including reversal learning, set-shifting and task-switching. Moreover, DA in both cortical and subcortical parts of the corticostriatal circuits seem to be involved in the regulation of these different aspects of cognitive flexibility. The idea that DA facilitates flexibility or switching behavior can be traced back to older studies that used different behavioral paradigms than the studies reviewed here. For example, a role for DA in switching strategies in a swim test was suggested by Cools (Cools, 1980; van den Bos and Cools, 1989), while the importance of DA in switching (increasing the probability that another behavioral output is chosen) was advocated by Oades (1985). However, the general picture arises that although DA may facilitate cognitive flexibility, it is not required. Following a variety of manipulations to the DA system the ability to successfully shift behavior following changes in reinforcer contingencies is impaired but not completely absent (in rodents, non-human primates and humans). How does the supportive role of DA in cognitive flexibility (i.e. behavioral adaptation to a change in conditions) compare to its role in initial learning about rewards? The question whether DA is necessary for learning has been addressed by studying acquisition of learning in DA deficient mice – the conclusion was that loss of DA may impair, but does not inhibit reward learning (Robinson et al., 2005; Berridge, 2005; Darvas and Palmiter, 2010; Palmiter, 2008). Animals may become less motivated, but were still able to learn cue-reward associations. Disruption of phasic DA activity by deletion of NMDA-receptors from DA neurons again showed that learning may be retarded, but not
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inhibited (Zweifel et al., 2009). A recent study using an optogenetics approach showed that phasic DA stimulation may drive associative learning or impair extinction learning, suggesting a causal role for DA (Steinberg et al., 2013). However, DA stimulation could not maintain the original behavior, so that other processes are probably involved as well. During performance of cognitive flexibility tasks, a number of cognitive processes act simultaneously and DA may be especially important to switch behavior rapidly. The contribution of DA to new learning therefore appears to be facilitatory rather than a prerequisite and the supportive role of DA appears to be present both in initial learning and adaptation of learning.

Both pharmacological and genetic studies in human subjects and animals point to a role for D₂ receptors in the regulation of cognitive flexibility. However, the regulation is not limited to D₂ receptor activity: D₁ and D₂ receptors both contribute and appear to be cooperatively involved in discrimination learning and the flexible adaptation of behavior. One could argue that successful behavioral switching requires three processes that may partly occur in parallel: extinction of the response that is no longer rewarded, behavioral switch to the newly rewarded side and response maintenance. A complication in delineating the contribution of DA to either process is that these processes occur simultaneously during behavioral adaptation. DA signaling through D₁ receptors may not be essential for switching behavior per se, but animal studies suggest that activation of D₁ receptors contributes to the acquisition and maintenance of a new response, also when acquisition follows a reversal. In contrast, inactivation of D₂ receptors may allow switching of behavior patterns. The contributions of D₁ versus D₂ receptors in the regulation of reward learning and behavior switching has been related to involvement of the direct and indirect pathway of the basal ganglia in these processes, and several models have been put forward to describe the possible components involved in regulating this behavior (Hong and Hikosaka, 2011; Frank and Claus, 2006). In general these models assume the presence of D₁ receptors in the direct pathway (direct projections from striatal medium spiny neurons (MSN) to the substantia nigra) and expression of D₂ receptors on MSN's of the indirect pathway (projections from MSN to substantia nigra via the globus pallidus) (Deng et al., 2006). Because binding affinity differs for D₁ and D₂ receptors (Richfield et al., 1989), fluctuations in DA levels during different stages of discrimination and reversal learning may result in different activation of D₁ (direct pathway) or D₂ (indirect pathway) expressing neurons. When a reward is presented unexpectedly, or when a stimulus that predicts reward is presented, a transient increase in DA release occupies low affinity D₁ receptors and activates the direct pathway, allowing facilitation of response execution and prompting reward-related learning. Switching of behavioral patterns on the other hand might require reduced occupancy of high affinity D₁ receptors. Omission of an expected reward following altered reinforcer contingencies results in transient reductions in striatal DA levels and diminished inhibition of the indirect pathway by D₂ receptors, resulting in inhibition of the previously successful response. Both facilitation of behavioral adaptation by deactivation of striatal D₁ receptors and facilitation of the acquisition of the ‘new’ behavioral response by striatal D₂ receptors suggests the importance of phasic fluctuations in striatal dopamine levels during execution of cognitive flexibility. This may be illustrated for the D₂-mediated response: both continuously higher and lower tonic D₂ activation could impair detection of the transient reduction of DA. As tonic DA may be related to general synaptic factors such as synthesis capacity, uptake activity and metabolic efficiency, all these factors may influence flexible responding through D₂-receptor dependent transmission. However,
it is difficult to separate tonic from phasic DA signaling with most manipulations used. Tonic prefrontal DA (Seamans and Yang, 2004) probably contributes as well. In addition, activation of D₁/D₂ receptors in prefrontal regions may differ from the activation in striatal regions. It has been suggested, for example, that D₂ stimulation in prefrontal regions may facilitate flexible behavior (Durstewitz and Seamans, 2008) whereas in striatal regions, deactivation of D₂ receptors is suggested to facilitate cognitive flexibility (Yawata et al., 2012). The combined study of genetic effects on behavioral performance and patterns of neural activation also suggests that although DA genotype may primarily affect expression of DA related genes in either striatal or prefrontal areas, functional effects of DA genotype are not limited to either region but are observed throughout the frontostriatal circuit. Genetic and imaging studies suggest that DA in ventral regions of the striatum (or connections between PFC and ventral striatum) contributes to reversal learning (lower order complexity), whereas DA in dorsal regions may be more important for attentional set-shifting and task switching (higher order complexity). However, animal studies have also described effects of DA in the NAC on attentional set-shifts and animals that only have DA signaling in dorsal striatal regions are able to learn a reversal. In addition, in human imaging studies it is not always clear if activation is limited to either ventral or dorsal striatum because analysis was limited to that particular striatal region or because the other striatal region was not activated. Therefore, it appears to be more likely that the relative activation of D₁/D₂ in prefrontal and striatal regions as well as the interaction with other neuromodulators (5-HT, NA) determines the control of cognitive flexibility. Considering the complexity of DA modulation in frontostriatal circuitry (Seamans and Yang, 2004), it may not be surprising that it is DA modulation in neither frontal nor striatal regions that exclusively determines behavioral performance on tasks of cognitive flexibility.

So how do these findings related to altered cognitive flexibility in OCD patients? If cognitive flexibility can indeed be used as an endophenotype for OCD, do the alterations in DA signaling that have been observed in OCD patients comply with the proposed role for DA in cognitive flexibility? The most replicated alteration in the DA system of OCD patients is reduced binding to D₂ receptors in the striatum. A question remains, how reduced D₂ receptor binding relates to DAergic activity in vivo. A reduction in binding potential to D₂ receptors may result from increased striatal DA levels or altered availability of D₂ receptors. In both cases, reduced flexibility could be expected. However, behavioral performance (i.e. accuracy) on reversal learning tasks is not impaired in OCD patients. On reversal learning tasks, if any behavioral effect is found, it is a slowing of response times rather than an effect on the amount of errors that are made. Differences in accuracy have been observed in attentional set-shifting and task switching paradigms. It is possible that reversal learning may be a paradigm that is too simple for gross behavioral abnormalities to be observed in OCD patients. Increased reaction times on flexibility tasks, however, do suggest altered cognitive processing in OCD patients during cognitive flexibility and the measurement of reaction times should therefore be included in studies investigating differences in cognitive flexibility between healthy controls and OCD patients. The altered recruitment of frontostriatal circuitry during the execution of reversal learning as well as task switching is another indication for altered cognitive processing in OCD patients. Altered DA signaling is a potential contributor to changes in frontostriatal activity when performing cognitive tasks. Altered activity in the frontostriatal circuit (OFC-ventral striatum) during reversal learning, as observed in OCD patients, is also found in subjects with polymorphisms in the D₂ gene that result in reduced
binding to D$_2$ receptors. Most likely, however, abnormalities in prefrontal regions and 5-HT modulation in OCD patients also contribute.

An important step in investigating the possibility of altered cognitive processing in cognitive flexibility tasks as an endophenotype for OCD would be the replication of studies using cognitive flexibility tasks in OCD patients with the use of strictly defined patient and control groups. Considering that altered neural correlates of OCD could be symptom dimension-specific (van den Heuvel et al., 2009), separate study of the different symptom dimensions contributes to the identification of possible endophenotypes. Preferably, these studies combine behavioral testing with measurements of brain activity and/or DA activity to further investigate the neurobiological basis of altered cognitive processing during cognitive flexibility tests in OCD patients.
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Phasic dopamine release induced by positive feedback predicts individual differences in reversal learning

Marianne Klanker, Tessa Sandberg, Ruud Joosten, Ingo Willuhn, Matthijs Feenstra, Damiaan Denys

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ABSTRACT

Striatal dopamine (DA) is central to reward-based learning. Less is known about the contribution of DA to the ability to adapt previously learned behavior in response to changes in the environment, such as a reversal of response-reward contingencies. We hypothesized that DA is involved in the rapid updating of response-reward information essential for successful reversal learning. We trained rats to discriminate between two levers, where lever availability was signaled by a non-discriminative cue. Pressing one lever was always rewarded, whereas the other lever was never rewarded. After reaching stable discrimination performance, a reversal was presented, so that the previously non-rewarded lever was now rewarded and vice versa. We used fast-scan cyclic voltammetry to monitor DA release in the ventromedial striatum. During discrimination performance (pre-reversal), cue presentation induced phasic DA release, whereas reward delivery did not. The opposite pattern was observed post-reversal: Striatal DA release emerged after reward delivery, while cue-induced release diminished. Trial-by-trial analysis showed rapid reinstatement of cue-induced DA release on trials immediately following initial correct responses. This effect of positive feedback was observed in animals that learned the reversal, but not in ‘non-learners’. In contrast, neither pre-reversal responding and DA signaling, nor post-reversal DA signaling in response to negative feedback differed between learners and non-learners. Together, we show that phasic DA dynamics in the ventromedial striatum encoding reward-predicting cues are associated with positive feedback during reversal learning. Furthermore, these signals predict individual differences in learning that are not present prior to reversal, suggesting a distinct role for dopamine in the adaptation of previously learned behavior.
INTRODUCTION

Throughout the day, we perform numerous behavioral actions to pursue things we desire or to prevent adverse events. In a constantly changing environment, this behavior has to be adaptive and flexible. One form of adaptive behavior is reversal learning, which requires the ability to use negative feedback to inhibit a learned response that was previously rewarded and at the same time to use positive feedback to switch to a response that was previously unrewarded. The ability to adapt goal-directed behavior to changes in the environment depends on frontal and striatal regions, as demonstrated in rodents (Castane et al., 2010; McAlonan and Brown, 2003), non-human primates (Dias et al., 1996; Clarke et al., 2008) and humans (Bellebaum et al., 2008). Indeed, compromised frontostriatal integrity results in deficient adaptive behavior and cognitive dysfunctions in various neurological and psychiatric disorders, such as obsessive-compulsive disorder (OCD), drug addiction and Parkinson’s disease (Chamberlain et al., 2006; Cools et al., 2001; Ceaser et al., 2008; Yerys et al., 2009; Verdejo-Garcia et al., 2006; Millan et al., 2012).

Dopamine (DA) is an important neuromodulator in frontostriatal networks. Striatal DA release facilitates reward learning and mediates approach behavior towards rewards. Specifically, DA neurons increase firing in response to unexpected rewards and reward-predicting stimuli and decrease firing when an expected reward is omitted (Schultz et al., 1997; Pan et al., 2005). These and other findings suggest that DA neurons encode a ‘reward-prediction error’ that serves as a teaching signal to guide behavior (Montague et al., 1996; Schultz et al., 1997; Waelti et al., 2001; Steinberg et al., 2013). It is known that burst firing of DA neurons facilitates initial learning of response-reward associations (Zweifel et al., 2009). Consistently, selective optogenetic activation of DA neurons affects operant responding in a similar manner as positive feedback does (Kim et al., 2012; Witten et al., 2011), and pharmacological and genetic manipulations demonstrate DA-mediated regulation of adaptive behavior (Clarke et al., 2011; Haluk and Floresco, 2009; Laughlin et al., 2011; Klanker et al., 2013). DA may not only play such role during initial learning, but also in adaptation of established operant responding. Indeed, optogenetic activation of DA neurons (simulating positive feedback) can also mediate reversal of reward-seeking behavior (Adamantidis et al., 2011). However, theories of reinforcement learning and models of DA-mediated prediction errors suggest that fluctuations in striatal DA concentration mediate the effects of both positive and negative feedback on learning (Hong and Hikosaka, 2011; Frank and Claus, 2006). It remains to be shown whether DA mediates the effects of negative feedback during behavioral adaption following an unexpected switch in reward contingencies and whether striatal DA reflects the receipt of feedback on a trial-by-trial basis. Therefore, we used fast-scan cyclic voltammetry (FSCV) in behaving animals (Millar et al., 1985) to monitor rapid changes in DA release in the ventromedial striatum during an operant spatial reversal learning task, in which rats had to adapt goal-directed behavior following a reversal of response-reward contingencies. Our results show that phasic cue-evoked DA signals were promptly updated following positive, but not negative feedback. Furthermore, we observed individual differences in the extent to which the receipt of positive feedback updated the reward-predicting DA signal on subsequent trials, where DA signaling predicted successful reversal learning. Thus, our findings suggest that phasic DA in the ventromedial striatum provides a positive feedback signal to facilitate adaptation of previously learned behavior following a behavioral switch.
MATERIALS AND METHODS

Animals
Male Wistar rats (Charles River) were housed in a controlled environment under a reversed day-night schedule (white lights: 7 p.m.-7 a.m.). Rats were food-restricted (16 grams animal/day), with unlimited access to water during behavioral training. All experiments were approved by the Animal Experimentation Committee of the Royal Netherlands Academy of Arts and Sciences and were carried out in agreement with Dutch laws (Wet op de Dierproeven, 1996) and European regulations (Guideline 86/609/EEC).

Surgery
Rats (weighing ~300 grams) were anesthetized with Isoflurane (induction: 3%, maintenance: 1.8-2.5 %) and placed in a stereotactic frame. Subcutaneous Metacam® (Meloxicam, 1 mg/kg, Boehringer-Ingelheim, Germany) was given as analgesic. A guide cannula (custom made, NIN mechanical workshop) was implanted above the nucleus accumbens (anterioposterior +1.3 mm, lateral ±1.3 mm relative to bregma (Paxinos and Watson, 2007). An Ag/AgCl reference electrode was implanted in the contralateral hemisphere. A bipolar stimulation electrode (Plastics one, Roanoke, VA, USA) was lowered into the ventral tegmental area (AP -5.2mm, ML -1.0 mm, DV – 8-9 mm from skull). The guide cannula, reference electrode and stimulating electrode were fixed to the skull with screws and dental cement. A removable stylet was used to close the cannula after surgery. Unlimited access to food and water was provided the day before surgery and during post-operative recovery. Rats were individually housed following surgery.

Behavioral training
All behavioral testing (see Table 1 for training phases) was performed in a custom made operant chamber (40 x 40 x 40 cm, NIN mechanical workshop) with MED Associates parts (Med Associates, Sandown Scientific, Hampton, UK). Two retractable levers were placed left and right from a food dispenser. Cue lights were positioned above both levers. Nose-pokes in the food dispenser to retrieve sucrose pellets (Dustless precision pellets®, 45 mg, Bio-Serv) were detected by an infrared sensor. During shaping sessions, rats were trained to press a lever for a food reward. Rats were randomly presented with the right or left cue light and corresponding lever (extended after a 2 sec delay). After a lever press, the lever was retracted, the cue light switched off and a sucrose pellet was delivered in the food dispenser. In case of an omission, cue and lever presentation ended after 30 seconds. Shaping sessions consisted of 32 trials, with a 10 or 20 second interval. Rats received up to three shaping sessions per day with an inter-session interval of 2-3 hours. After reaching a 90% correct response criterion (measured over the complete session), rats continued in a 64-trial shaping session. Rats then progressed to spatial discrimination learning (120 trials per session, variable inter-trial intervals (15/25/35/45 seconds)). On every trial both cue lights were illuminated and two seconds later both levers were extended into the operant chamber. Thus, cue lights did not signal which side was rewarded, but indicated that reward was available provided the correct choice was made. Responding to the lever on one side was rewarded, while responding to the other lever on the other side was never rewarded. The rewarded side was counterbalanced between rats. If rats did not make a lever-press within 10 seconds, levers were retracted and the trial was scored as omission. Rats received one session per day. If rats did not reach a 90% correct
response criterion during the second discrimination session, a third, shorter discrimination session (max 64 trials) was presented to allow them to get to the 90% response criterion without overtraining them. Reversal sessions consisted of 120 trials, with variable inter-trial intervals (15/25/35/45 seconds). The reversal was presented randomly between the 16th and 32nd trial in the session, so that a response to the previously non-rewarded lever was now rewarded and vice versa. The reversal was not cued to the animals; instead animals had to use the change in feedback to adapt responding.

Table 1 Overview procedure for behavioral training

<table>
<thead>
<tr>
<th>Stimuli</th>
<th>Lever-press training (shaping)</th>
<th>Discrimination</th>
<th>Reversal</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Randomly 1 lever + cue light</td>
<td>Both levers + cue lights; spatial contingency</td>
<td>Both levers + cue lights; contingency switch after 16/32 trials</td>
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<tr>
<td>Trials</td>
<td>32</td>
<td>64</td>
<td>120</td>
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<td>10/20 sec</td>
<td>15/25/35/45 sec</td>
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<td>Sessions</td>
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<td>2*</td>
<td>1</td>
</tr>
<tr>
<td>Criterion for next stage</td>
<td>&gt;90% response &gt;90% response</td>
<td>&gt;90% response &gt;90% correct responses</td>
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*additional session (max 65 tr) if criterion not reached

**Fast-scan cyclic voltammetry**

FSCV was used to record DA changes during discrimination and reversal learning. For FSCV recordings (Phillips et al., 2003), a micromanipulator holding a glass-enclosed carbon fiber (electrode tip: diameter 7μm, length 100-150 μm) and attached to a head-mounted amplifier was used to record DA concentration in the ventromedial striatum. As a clear distinction between nucleus accumbens core and the ventromedial part of the caudate putamen cannot be made based on structural, functional, or connectivity differences (Zahm and Brog, 1992; Voorn et al., 2004a), we recorded from both regions. A potential of -0.4 V was applied to the carbon fiber electrode (vs an Ag/AgCl reference electrode). Resting potential changed to 1.3 V and back to resting potential in a triangular waveform (8.5 msec) every 100 msec. Redox reactions of DA molecules in vicinity of the electrode at specific applied potentials (0.6V oxidation, -0.2 V reduction) in the waveform result in the release of electrons that can be measured as current at the carbon fiber electrode. A fresh carbon fiber electrode was inserted before each recording session. Placement of the electrode in a DA-rich region was verified by presence of spontaneous DA release events (‘transients’ (Wightman and Robinson, 2002)) and/or a time-locked DA response to the presentation of unexpected food pellets. After the behavioral session, VTA Stimulation (8, 12, 16 pulses, 30 Hz frequency, 125 μA intensity, 4 msec pulse width) was performed to construct a training set for chemometric analysis from electrically stimulated DA release and pH changes. Chemometric analysis was used to identify DA from other electro-active species (Heien et al., 2004; Keithley et al., 2009). Use of acutely
inserted glass electrodes before each recording session limited the amount of recordings possible in the same animals. Therefore, most animals only contributed voltammetry data to either one of the two discrimination sessions or the reversal learning session making it impossible to correlate changes in DA release during discrimination sessions with DA release of the same animals during reversal learning. After the last behavioral session, a stainless steel lesion electrode was inserted to the recording location and a 100 µA direct current was passed through the electrode to mark the final placement of the electrode. Rats were deeply anesthetized using an overdose of pentobarbital (Erasmus MC pharmacy, Rotterdam, the Netherlands) and decapitated. Brains were removed and frozen. For histological verification of electrode placement, 40 µm coronal slices were cut on a cryostat and stained with cresyl violet.

**Data analysis and statistics**

The following behavioral measures were analyzed: number/percentage of responses to the rewarded and non-rewarded lever and latency to lever press. For the reversal session, a cumulative response record was plotted to visualize learning over the course of the session. Based on performance in the reversal session rats were divided into groups of ‘learners’ (>10 correct responses after presentation of reversal) and ‘non-learners’ (<10 correct responses after presentation of reversal). For the reversal session, a change point in behavior was defined as the point where the learning curve (cumulative number of correct responses) deviates maximally from a straight line drawn from the origin to the last point in the cumulative line (Gallistel et al, 2004). One change point was defined for each learning curve. Behavioral data was analyzed with independent t-tests (discrimination learning) or repeated measures ANOVA (reversal session; reversal stage (before/after) as repeated measure).

For analysis of the DA recordings, trials were averaged over a behavioral session for each rat, then averaged over rats. For cue-evoked responses, baseline value was an average of the measurements during 5 seconds before cue onset. Peak values were the maximal value in a 2 second window following cue presentation. For DA responses to reward delivery, DA following 4 seconds after lever press was averaged (for reward delivery, an average measure rather than peak value was chosen as less data points were available in period following lever press due to noise). For a more detailed analysis of DA changes related to reward delivery after reversal presentation, we took one-sec bins of the first four seconds following lever press for trials after reversal presentation. Here, average DA response in consecutive one-sec bins was compared to average DA during baseline period (first four seconds in trial, to exclude DA changes following cue presentation). Repeated measures ANOVAs were used to analyze changes in DA release during discrimination and reversal learning. Bonferroni-corrections were applied for post-hoc t-tests when appropriate. When assumption of sphericity was violated, Greenhouse-Geisser or Huyn-Feldt corrections were applied as appropriate. Independent t-tests were used to compare group differences (Welch t statistic reported when assumption of homogeneity of variances was violated). Statistical analyses were performed with SPSS Statistics 21 (IBM, Armonk, NY). Statistical significance was set to p<0.05.
RESULTS

Histology

Fig. 1 illustrates the recording sites in the ventromedial striatum (dorsomedial accumbens core and ventromedial regions caudate putamen in right hemisphere). Final group sizes for animals included in FSCV data: first discrimination session n=11, second discrimination n=8, reversal session n=21. A statistical comparison of DA responses at the different locations used in the reversal session revealed no significant differences.

Phasic DA changes in the ventromedial striatum during spatial discrimination learning

After learning to lever-press for reward in several shaping sessions, rats were trained on a spatial discrimination paradigm: a lever-press on one lever was always rewarded, whereas lever-press on the other lever was never rewarded. Number of rewarded responses increased from first (D1) to second (D2) discrimination session (t(17)=-5.228, p<0.001), whereas the number of non-rewarded responses decreased (t(17)=6.089, p<0.001). Response times did not differ between rewarded or non-rewarded responses. DA increased relative to baseline at time of cue onset during both discrimination sessions (D1: F(1,20)=71.907, p<0.001; D2: F(1,14)=46.355, p<0.001), but was not different between rewarded and non-rewarded trials, therefore did not predict whether animals made a correct or incorrect response. Thus, in our task cue-evoked DA did not encode the chosen response, but signaled the availability of reward (Roesch et al., 2007; Sugam et al., 2012). Cue-evoked DA was not different between trials that followed a correct response and trials following an incorrect response. No significant DA response to reward delivery was found during discrimination sessions. During shaping sessions, prior to discrimination learning, rats were trained to press a lever for reward. As (repeatedly) shown by others (Wassum et al., 2012; Roitman et al., 2004), the shift of DA release from time of reward delivery to time of reward-predicting cue likely already takes place during acquisition of instrumental behavior, prior to discrimination learning.

Figure 1 Histological verification of electrode placement in ventromedial striatum. Recordings were made in nucleus accumbens core and ventromedial part of caudate nucleus right above the nucleus accumbens. Electrode placement is shown for animals, in which a post-experimental lesion was made. Each circle represents one animal.
PART I – DAergic control of cognitive flexibility

Dynamic changes in DA release during reversal learning

During the reversal session, the rewarded lever was switched at a random time point in the session. Rats then had to use feedback (i.e. previously rewarded response no longer rewarded; previously non-rewarded response now rewarded) to adapt their responding because the reversal was not cued. Before reversal, rats had a clear preference to press the rewarded lever. After reversal presentation, the number of rewarded responses decreased, whereas non-rewarded responses increased (reversal*reward interaction (F(1,40)=299.238, p<0.001; Fig. 2A). In addition, response latencies were significantly longer after reversal than before (F(1,33)=7.836, p=0.008), but did not differ between rewarded and non-rewarded responses. Fig. 2A shows the gradual adaptation of response behavior during reversal learning (session divided into blocks of 8 trials). Rewarded responses gradually increased (main effect for block (F(3.527,70.545)=7.979, p<0.001, simple contrasts vs block 1: differences from block 7 onwards), whereas non-rewarded responses gradually decreased (F(10,200)=18.145, p<0.001, simple contrasts vs block 1: differences from block 5 onwards).

Phasic DA responses in the ventromedial striatum quickly adapted to reversed response-reward contingencies (Fig. 2B,C). Fig. 2B shows examples of DA release in single trials with correct responses at different stages of reversal learning. Before reversal, when discrimination is well learned, DA increases to cue onset only (Fig 2B, left panel). After reversal of response-reward contingencies, cue-evoked DA signal is decreased and reward delivery now induces an increase in DA (Fig 2B, middle panel). Cue-evoked DA response is reinstated when animals have made several correct responses (Fig 2B, right panel).

Cue-evoked DA release was higher before than after reversal of response-reward contingencies (main effect reversal, F(1,33)=28.714, p<0.001; Fig. 2D, left), but did not differ between rewarded or non-rewarded trials (reversal*reward, F(1,33)=3.789, p=0.06; main effect reward, F(1,33)=0.475, p=0.495). Before reversal, reward delivery did not evoke DA release. After reversal, DA differed in rewarded and non-rewarded trials (reversal*reward interaction F(1,33)=25.574, p<0.001; reversal F(1,33)=6.557, p=0.015, reward F(1,33)=3.415, p=0.074; Fig. 2D, right). Post-hoc analysis showed that DA release at the time of lever-press increased in rewarded trials (paired t-test: t(18)=-4.771, p<0.001) but not in non-rewarded trials (t(15)=2.334, p=0.034).

For a more detailed analysis of the DA response after lever-press, we plotted changes in DA during four consecutive one-sec time bins following lever-press (Fig. 2E). We observed a decrease in DA below baseline on non-rewarded trials that was masked when analyzing the average over the four second period (time*reward interaction (F(14,152)=9.408, p<0.001); main effect time (F(2.118,80.476)=7.201, p=0.001) and reward F(1,38)=23.435, p<0.001; post-hoc analysis revealed significant effect of time for both rewarded (F(1.091, 34.487)=7.455, p=0.002) and non-rewarded trials (F(2.212,44.243)=9.430, p<0.001). The decrease in DA does not immediately follow the lever-press response, but occurs in the third and fourth second after, suggesting that the decrease in DA at a time when expected rewards are omitted occurs later than the peak increase in DA following receipt of an unexpected reward.

Trial-by-trial analysis of DA changes during reversal learning reveals rapid updating of the cue-evoked DA signal

Averaging DA traces over a complete session may obscure rapid changes in DA release patterns occurring on a trial-by-trial basis during acquisition of reversal learning (Fig. 3 lower panel; upper panel shows similar trials prior to reversal). To study the effects of positive feedback on DA release patterns, we separately analyzed the first ten trials on which the animals made a correct
Figure 2 Phasic DA release in the ventromedial striatum during reversal learning (n=21).
A. Behavioral performance across reversal learning session. Lines show percent response during rewarded (blue) and non-rewarded (red) trials across consecutive blocks of trials before and after reversal (block 3). Numbers in grey circles correspond to examples of individual trials in panel B. B. Examples of individual trials. Red bar indicates presentation of cue lights, grey bar the presentation of levers and black triangle time of reward collection. Left – before reversal, cue presentation evokes DA release in ventromedial striatum, middle – after reversal, cue-evoked DA is diminished and reward delivery evokes DA release, right – after several correct trials, cue-evoked DA is reinstated. C. Fluctuations in striatal DA averaged over trials. Left – before presentation of reversal, cue presentation evokes DA response in ventromedial striatum in both rewarded and non-rewarded trials. Right – after presentation of reversal, cue-evoked DA release is still apparent, but followed by an additional, gradual increase in DA release, in rewarded, but not in non-rewarded trials. Blue lines – mean rewarded trials, red lines – mean non-rewarded trials, shaded regions – SEM. D. Quantification of DA release to cue presentation and reward delivery. Left – cue-evoked DA release is lower after presentation of reversal. Right – reward delivery evokes DA release after reversal presentation. E. Bidirectional DA signal on rewarded and non-rewarded trials. After reversal, increased striatal DA is observed following reward delivery. In non-rewarded trials, DA decreases below baseline.
response after reversal (n.b. first ten correct trials, not always consecutive trials) and the trials that immediately followed (correct+1 trials). Our data demonstrate rapid reinstatement of cue-evoked DA release on trials that follow positive feedback. For analysis, average cue-evoked DA release on trials 1-3 was compared to average cue-evoked DA release on trials 8-10. On trials 1-3, cue-evoked DA release was higher on trials that followed a correct response (correct+1) than on trials on which the correct response was made (correct), whereas on trials 8-10 cue-evoked DA release was similar for correct trials and trials following a correct response (feedback*trial interaction (F(1,9)=14.757, p=0.004, main effect trial F(1,9)=0.093, p=0.77, main effect feedback F(1,9)=7.795, p=0.021), suggesting the effect of positive feedback on cue-evoked DA release may be most pronounced in the initial correct responses after reversal.

**Learners and Non-learners differ in the extent to which reward-predicting DA signal is updated**

Based on performance following reversal of response-reward contingencies, animals were divided into groups of ‘learners’ (>10 total correct responses following reversal, n=11) and ‘non-learners’ (<10 correct responses following reversal, n=10; see Fig. 4A). The cut-off criteria of 10 correct responses was based on the cumulative response curves of the animals. Our intention was to make a distinction between animals that do not learn to reverse responding at all during the reversal session and animals that are able to adapt responding to the newly rewarded side. Learners and non-learners did not differ in the amount of shaping sessions needed for lever-press training or the number of correct responses during discrimination learning. Moreover, learners and non-learners needed a similar number of trials to reach the 90% correct criterion in the discrimination phase (learners: 264.8±11.7, non-learners: 269.5±12.5 trials to criterion), suggesting the distinction was not based on a general learning defect in non-learners, but instead was specific to the reversal phase. Before reversal, response latencies on rewarded and non-rewarded trials and number of omissions were similar for learners and non-learners (Fig. 4C) suggesting motivation to respond did not differ between groups. In addition, cue-evoked DA release (Fig. 4B) was similar for learners and non-learners before reversal. After reversal, cue-evoked DA further decreased in non-learners, reflecting extinction of the conditioned response, whereas it reinstated in learners (Fig. 4B). After reversal, learners made at least 6 correct responses in a block of 10 trials. On average learners made 33.9±5.58 rewarded responses (range 16-69), whereas non-learners made 1.9±0.57 rewarded responses (range 0-6) after reversal. The number of non-rewarded responses made after reversal is similar for learners (59.1±5.5) and non-learners (55.1±7.5), but non-learners make more omissions (38.1±8.4; learners 4±2.7).

To analyze differences between learners and non-learners following reversal, we looked at the DA response for the first two correct responses (in case non-learners only made one correct response (n=3) then that value was used; excluding these animals from analysis did not affect the results) and the first two trials on which positive feedback could be used (correct+1 trials; Fig. 4E). After reversal, learners and non-learners showed similar DA release to reward delivery (t(16)=-0.993, p=0.336; Fig. 4D, Fig. 4E, left panel), but differed in cue-evoked DA release following the first correct responses (Fig. 4E, center panel). In correct+1 trials (Fig. 4E right panel), cue-evoked DA increased in learners, but not in non-learners. For cue-evoked DA release, we compared difference scores (peak DA value correct+1 – peak DA value correct) for the first two correct responses after reversal. The difference score was significantly higher in learners compared to non-learners (t(11.575)=3.851, p=0.002, Fig. 4D right panel), suggesting that cue-evoked DA is updated in learners exclusively. Latency (amount of trials be-
Positive feedback-induced DA predicts differences in reversal learning. Importantly, in learners, cue-evoked DA increased following trials with a correct response irrespective of latency until correct response, whereas in non-learners, cue-evoked DA was not increased after positive feedback irrespective of whether it took them longer to make the first correct response. Across all animals, we found a significant positive correlation between the percentage of correct responses after reversal and the cue-evoked peak DA response (normalized to last 10 trials before reversal to control for individual differences; \( r=0.626, p=0.002, \) Fig. 4F).

Regarding negative feedback, we compared DA release on the first two incorrect responses after reversal (error) and the trials on which this negative feedback could be used (error+1). In the first two incorrect trials, DA release after lever-press (i.e. around the time that reward was expected; Fig. 4G, left panel) did not differ between learners and non-learners.

Figure 3: Rapid updating of reward-predictive DA signal after positive feedback. Heat plots show average DA values per trial for the first 10 correct trials (correct) and the trials immediately following correct trials (correct+1) made before (top panel) and after (lower panel) reversal (shown here for animals that learned reversal, \( n=11 \)). Striatal DA shows a leftward shift from time of reward delivery to time of cue presentation in first 10 trials after reversal. Receipt of unexpected reward on first correct trials after reversal induces updating of cue-evoked DA signal on trials that immediately follow the correct response. Blue boxes indicate trials used for statistical analysis. Onset of cue presentation and approximate time of lever press for each trial indicated by vertical white lines.
(t(19)=-1.475, p=0.157). Difference scores were calculated for cue-evoked DA release: Peak DA response on error trials was subtracted from peak DA response on error+1 trials and averaged across animals. No effect of negative feedback on cue-evoked DA release on consecutive trials was found (t(19)=-0.484, p=0.634; Fig. 4G, right panel). This indicates that the cue-evoked DA signal is rapidly updated following the receipt of positive feedback, but that negative feedback is not immediately reflected in the cue-evoked DA signal during reversal learning.

DA changes surrounding the time point at which learners acquire the reversal
Changes in the slope of a cumulative response record correspond to changes in performance level of the behavioral task performed (Gallistel et al., 2004). For learners, a change point was defined (trial number where a straight line drawn from origin until end of cumulative response record deviates maximally from the cumulative response curve, see Fig. 5A). The average trial for the change point was 54.9 ± 4.2 trials after presentation of the reversal. When comparing DA release on all correct responses made before and after the change point, cue-evoked DA release did not differ (paired t-test t(9)=0.420, p=0.684, Fig. 5B, left panel). However, reward delivery evoked higher DA release before the change point than after (paired t-test t(9)=3.620, p=0.006, Fig. 5B, right panel), suggesting that the switch from reward- to cue-induced phasic DA release coincided with the behavioral change point. Fig. 5C shows a quantification of the cue-evoked DA signal on trials that followed a correct response (correct+1) and trials on which the correct response was made (correct) before and after the change point. Updating of the cue-evoked DA signal after positive feedback differed before and after the change point (repeated measures ANOVA feedback*change point interaction (F(1,9)=5.278, p=0.047; main effect feedback (F(1,9)=8.790, p=0.016, main effect change point F(1,9)=0.100, p=0.759). Post-hoc analysis showed that before the change point, cue-evoked DA release was higher on trials that followed a correct response (correct+1) compared to trials on which the correct response was made (correct) (t(9)=-3.278, p=0.010), suggesting that higher DA responses to reward presentation and stronger effects of positive feedback on cue-induced DA release are associated with the initial learning phase, before the behavioral change point.

Figure 4 Individual differences in DA signaling and performance of reversal learning. A. Cumulative response curves for learners (black, n=11) and non-learners (red, n=10). B. Peak values of cue-evoked DA release are similar for learners and non-learners preceding reversal presentation. After reversal, cue-evoke DA continues to decrease for non-learners, but stabilizes for learners. Learners – black, non-learners – red. Blue line shows average peak value during first session of discrimination learning for comparison. C. Learners and non-learners show similar motivation to lever press as indicated by similar response latencies before reversal. Learners – black bars, non-learners – open bars. D. Learners and non-learners show similar DA release to unexpected reward delivery but DA release to cues differs. Left – response to reward delivery averaged across the first two correct responses after reversal. Right – cue-evoked DA is updated after positive feedback in learners, but not in non-learners. Bar graph shows mean difference scores (cue-evoked DA on correct+1 trials – cue evoked DA on correct trials) for the first two correct responses after reversal. E. Changes in DA during first two correct responses after reversal. Heat plots show average DA values per trial following reward delivery (left panel) and cue presentation (center and right panels) for the first two correct trials (correct) and for the trial immediately following correct trials (correct +1). Upper panels show results for learners, lower panels show results for non-learners. Left panel – response to reward delivery, middle panel – response to cue presentation on correct trials, right – response to cue presentation on correct+1 trials. F. Positive correlation between percentage correct and cue-evoked DA release after reversal across all animals. Cue-evoked DA after reversal was normalized to cue-evoked DA release on last 10 trials before reversal to control for individual differences. Red dots indicate non-learners, black dots indicate learners. G. Changes in DA during first two incorrect responses after reversal. Left – response to lever press averaged for first two incorrect responses after reversal. Right – For cue-evoked responses difference scores between the first incorrect trials (error trials; negative feedback received) and the first trials on which this negative feedback could be used (error+1 trials) were not different between learners and non-learners.
Positive feedback-induced DA predicts differences in reversal learning

A Peak DA (nA) to cue onset

B Peak DA (nA) to cue onset

C Response Latency (sec)

D Mean DA (nA) after reversal

E Correct Reward Cue

F Correct Reward Cue

G Correct Reward Cue

H Correct Reward Cue

I Correct Reward Cue

J Correct Reward Cue

K Correct Reward Cue

L Correct Reward Cue

M Correct Reward Cue

N Correct Reward Cue

O Correct Reward Cue

P Correct Reward Cue

Q Correct Reward Cue

R Correct Reward Cue

S Correct Reward Cue

T Correct Reward Cue

U Correct Reward Cue

V Correct Reward Cue

W Correct Reward Cue

X Correct Reward Cue

Y Correct Reward Cue

Z Correct Reward Cue
PART I – DAergic control of cognitive flexibility

DISCUSSION

Successful adaptation of behavior following reversal requires the ability to use a change in reinforcing feedback. To investigate whether phasic DA release in the ventromedial striatum contributes to such an adaptation, we recorded DA release in rats during a spatial discrimination and reversal task in which a non-discriminative cue signaled trial onset. During successful responding in the discrimination phase (prior to reversal), DA was evoked by the cue, but not the reward. However, during adaptation of choice behavior following reversal of response-reward contingencies, reward delivery evoked DA release, paralleled by temporal decrease in cue-induced DA. Trial-by-trial analysis revealed rapid reinstatement of DA release to cue presentation on trials following correct responses, but no changes in DA following incorrect responses. Reinstatement of the cue-evoked DA signal was observed only in animals that learned the reversal, time-locked to their behavioral “change point”. Together, this suggests that the modification of established behavior is facilitated by updating cue-evoked DA release as a consequence of positive feedback.

Phasic DA rapidly adapts to reversal of contingencies

Encountering unexpected rewards evokes a brief increase in striatal DA. After repeated pairing with a cue, the DA signal shifts from the time of reward delivery to the time of cue presentation (Day et al., 2007; Schultz et al., 1997; Pan et al., 2005), consistent with the idea that DA signaling codes a quantitative ‘reward prediction error’ (RPE) that serves as a teaching signal guiding behavior (Montague et al., 1996; Schultz et al., 1997; Waelti et al., 2001; Steinberg et al., 2013). Elevated DA in response to cue stimulus presentation may represent motivational properties of the stimulus and promote the initiation of reward-seeking actions (Flagel et al., 2011; Berridge et al., 2009; Wise, 2004).

Figure 5 In animals that learned the reversal (n=11), a change point in behavioral performance is reflected in DA signal. A. Change point in behavior was defined as the point where the cumulative correct response curve deviates maximally from a straight line drawn from the origin to the maximum of the cumulative line. B. Quantification of DA signal to cue presentation (left: cue) and reward delivery (right: reward) for all correct responses made before and after the change point (rewarded trials only). For rewarded trials, cue-evoked DA is similar before and after change point. DA release to reward delivery is higher before than after change point. C. Quantification of cue-evoked DA signal for trials on which positive feedback was received (correct) and the trials in which animals could use this feedback (correct+1) before and after the change point. Before the change point, cue-evoked DA is higher on trials that immediately follow a correct response compared to trials in which the correct response is made.
According to RPE theory, a reward that is fully anticipated no longer induces DA release. Consistently, we observed phasic DA release following cue onset, but not following lever-press or reward delivery during discrimination learning. During lever-press training, prior to discrimination learning, our rats learned that specific operant actions lead to reward delivery. Therefore, the shift of DA release from time of reward delivery to time of cue presentation presumably already occurred during acquisition of instrumental behavior, as shown by others (Wassum et al., 2012; Roitman et al., 2004). During discrimination learning, cue-evoked DA release did not differ on trials that followed positive feedback (correct+1 trials) and trials that followed negative feedback (error+1 trials), suggesting that when reward receipt does not differ consistently from what is expected (i.e., after lever press training), the reward-predicting DA signal is not updated on subsequent trials. Also, cue-evoked DA release was similar on trials where subjects made a rewarded response and trials where subjects made a non-rewarded response. Thus, in our task cue-evoked DA was not predictive of the subsequent choice of subject, but reflected the best available or preferred option (Roesch et al., 2007; Sugam et al., 2012). Moreover, this signal might induce incentive motivation and promote behavioral actions irrespective of trial outcome (Flagel et al., 2011; Berridge et al., 2009; Wise, 2004).

Studies using long-term manipulations of the DA system (Darvas and Palmiter, 2011; Clarke et al., 2011; O’Neill and Brown, 2007) suggest that striatal DA contributes to the regulation of adaptive behavior. Modeling studies propose that 1) reduced DA levels after omission of an expected reward and 2) increased DA following unexpected reward or reward-predicting stimuli, may facilitate altered response execution via different basal ganglia output pathways (Hong and Hikosaka, 2011; Frank and Claus, 2006). Similarly, reorganization of established behavioral patterns requires suppression of DA D2-receptor mediated transmission in the nucleus accumbens, whereas acquisition and relearning of behavioral responses after a reversal or rule shift requires stimulation of accumbal D1 receptors (Yawata et al., 2012).

Together, these findings suggest the importance of bidirectional phasic fluctuations in striatal DA levels when adapting behavior to changes in the environment. Although mimicking positive feedback by optogenetic stimulation of DA neurons supports reversal learning (Adamanitis et al., 2011), it is unknown whether the receipt of positive and negative feedback during behavioral adaptation are reflected by bidirectional changes in striatal DA release on a trial-by-trial basis.

Following successful spatial discrimination, we tested the ability to modify an established response pattern after a reversal of reinforcement contingencies. Reward delivery following the initial lever presses on the newly rewarded side now rapidly increased striatal DA, as predicted by RPE theory. If DA functions as a teaching signal (Hart et al., 2014; Schultz et al., 1997) during reversal learning, the receipt of positive feedback should update the reward prediction signal in trials following correct responses. Indeed, on trials immediately following the first correct responses after reversal, cue-evoked DA increased. Thus, the receipt of positive feedback was rapidly reflected in the cue-evoked DA signal on subsequent trials, in accordance with RPE theory.

In non-rewarded trials, DA decreased below baseline after the lever press, suggesting that DA could act as a bidirectional teaching signal during reversal learning. However, this decrease was only detected across the entire reversal session (in a sec-by-sec analysis), but not in the initial incorrect responses after reversal, suggesting that this effect develops more slowly or that the decrease in DA after reward omission is relatively small, requiring a larg-
er number of trials to be detected. This result is consistent with previously published data on extinction learning (Stuber et al., 2005; Sunsay and Rebec, 2014; Owesson-White et al., 2008), but differs from the results presented by Hart et al (2014). However, the latter study did not investigate the first reversal of reward contingencies, but tested extensively trained animals under frequently changing contingencies. Moreover, in our study, unexpected reward omission did not influence cue-evoked DA release on trials that immediately followed a non-rewarded response, suggesting that the receipt of negative feedback did not induce rapid updating of DA responses in our rats. However, we cannot exclude that non-reward was not sufficiently aversive to show a decrease in DA release or prolonged experience with non-reward may be needed to decrease the cue-evoked DA signal.

We show that with the completion of operant reversal learning, phasic DA in the ventro-medial striatum shifts from time of reward delivery to time of cue presentation, similar to the shift of DA during initial learning of Pavlovian associations (Schultz et al., 1997; Stuber et al., 2008a; Pan et al., 2005; Day et al., 2007). The effect of positive feedback on cue-evoked DA release was restricted to the reversal phase (and was not observed during the discrimination phase), corroborating previous data showing that striatal DA may be less important for learning to discriminate between two rewarded responses than for learning the reversal of such associations (Clarke et al., 2011; O’Neill and Brown, 2007; Groman et al., 2011). Together, our results indicate that during the modification of established behavior, the receipt of positive feedback induces immediate updating of cue-evoked DA release, whereas the receipt of negative feedback does not. This suggests that phasic DA release during reversal learning shows an asymmetric RPE-signal (Bayer and Glimcher, 2005).

Individual differences in DA signaling predict performance of reversal learning
Successful adaptation of behavior following a change in response-reward contingencies requires several processes: i) extinguish response that is no longer rewarded, ii) switch responding to the alternative (side), iii) consolidate alternative (side) responding. These processes are thought to entail learning from both positive and negative feedback. Individual differences in sensitivity to positive feedback during reversal learning in animals have been related to D2-receptor availability (Groman et al., 2011). Similarly, in humans, learning from trial-by-trial feedback has been associated with striatal DA function (Cools et al., 2009; Frank et al., 2004; Wilkinson et al., 2014). As learning curves during reversal learning varied greatly between individuals in our study, we hypothesized that updating of cue-evoked DA release after positive feedback relates to the rate of reversal learning. Indeed, we found that animals that were slow to reverse their behavior, did not update the cue-evoked DA signal in trials following correct responses. This result has several interesting aspects. First, these non-learners were indistinguishable from learners based on behavioral and DA parameters during discrimination learning, prior to reversal.

Second, although negative feedback can robustly drive adaptation of behavior (Porter-Stransky et al., 2013), we found no difference in cue-evoked DA release to negative feedback in learners and non-learners in the initial trials following reversal, suggesting that performance differences in our reversal learning paradigm are not driven by the DA response to negative feedback.

Finally, most non-learners (8/10) sampled the reversed response-reward contingency (behavioral switch) and experienced subsequent reward delivery, but they did not sustain responding on the newly rewarded side. Instead, they returned to press the non-rewarded
lever and eventually ceased lever-pressing in this session. Although we have no indication that motivation at the onset of the session was different between learners and non-learners, the increased number of omissions after reversal may indicate that non-learners differ in their motivation to respond to the newly rewarded side once the initial switch has been made.

Reward-induced DA release following the first couple of responses on the newly reward-ed side could drive learning about the newly reinforced response. Moreover, the subsequent feedback-induced increase in cue-evoked DA may help to sustain motivation to regularly sample and consolidate responding to the newly rewarded side, as this was observed in learners, but not in non-learners. However, it is our experience that ‘non-learners’ generally are able to persistently switch behavior when given more time (i.e., in additional retention sessions) and that was confirmed a subset of non-learners that were exposed to more reversal sessions. This suggests that DA signaling supports a more rapid adaptation of established behavior (Klanker et al., 2013). This is similar to the presumed facilitatory, but not essential role for DA in the acquisition of reward-related learning (Robinson et al., 2005; Palmiter, 2008; Darvas and Palmiter, 2010; Zweifel et al., 2009).

To conclude, we showed that DA dynamics in the ventromedial striatum during reversal learning predict individual differences in adaptive behavior: Increased striatal DA following positive feedback may support the stabilization of adaptive behavior. This interpretation is further substantiated by our finding of DA changes in temporal proximity of a change point in behavior in animals that learned the reversal. Additionally, our finding that individual differences in reversal learning and associated DA release are not related to a previous learning phase, supports the notion that these two processes are regulated by distinct mechanisms.

CONCLUSION

Impaired behavioral adaptation to environmental changes can result in behavioral rigidity and maladaptive behavior as observed in various neurological and psychiatric disorders, such as Parkinson’s disease, drug addiction and OCD (Chamberlain et al., 2006; Cools et al., 2001; Ceaser et al., 2008; Yerys et al., 2009; Verdejo-Garcia et al., 2006). Our study suggests that individual differences in reversal learning could be related to differences in DA dynamics following positive feedback. Thus, compromised DA transmission during feedback learning could contribute to the inability to correct maladaptive behavior and the development of cognitive dysfunctions observed in psychiatric disorders such as OCD and drug addiction.

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Regionally distinct patterns of phasic dopamine release in the striatum during reversal learning

Marianne Klanker, Lisanne Fellinger, Matthijs Feenstra, Ingo Willuhn, Damiaan Denys

In preparation
ABSTRACT

Striatal dopamine (DA) plays a central role in reward-related learning and behavioral adaptation to changing environments. Recent studies suggest that rather than being broadcast as a uniform signal throughout the entire region, striatal DA shows noticeably different release dynamics in different functional units of the striatum. In a previous study, we showed that phasic DA release patterns in the ventromedial striatum (VMS) rapidly adapt to a reversal of response-reward contingencies. However, it is unknown how DA dynamics in the dorsolateral striatum (DLS) are modulated during such adaptive behavior. Here, we used fast-scan cyclic voltammetry to measure phasic DA release in the DLS during spatial reversal learning. In the DLS, we observed minor DA release to a cue signaling reward availability, but pronounced DA release around the time of the lever press, both in rewarded and non-rewarded trials. These release dynamics (minor DA to the predictive visual stimulus, prominent DA during the operant response) did not change following a reversal of response-reward contingencies. Notably, the DA increase to the lever press did not reflect a general signal related to the initiation of a motor response, as we did not observe DA release when rats initiated nose pokes during inter-trial intervals. This suggests that DA release in the DLS occurs selectively during a learned operant response initiated to obtain a reward.

Together with our previous results obtained in the VMS, these findings reveal distinct phasic DA release patterns during adaptation of established behavior in DLS and VMS. The VMS DA signal, which is highly sensitive to reversal of response-reward contingencies, may provide a teaching signal to guide reward-related learning and facilitate behavioral adaptation, whereas DLS DA reflects a ‘performance signal’ independent of outcome, that may enable initiation and execution of previously learned operant responses or the motivation to perform them.
INTRODUCTION

Corticostriatal circuits are important for the regulation of motivated behavior and cognitive functioning. These circuits consist of converging input from different cortical areas and midbrain dopamine (DA) neurons to projection neurons in the striatum. The projections from cortex to the striatum are organized topographically, where sensory and motor cortical areas dominate inputs to the dorsolateral striatum (DLS) and limbic areas constitute the majority of the cortical projection to the ventromedial striatum (VMS) (Webster, 1961; McGeorge and Faul, 1989). DA is an essential neuromodulator that regulates activity in corticostriatal circuits. Loss of the modulatory role of DA in these circuits leads to impairments in motor performance, motivated behavior and executive functioning (Cools et al., 2001; Sawamoto et al., 2008). One of the executive functions that is affected by compromised DA function is cognitive flexibility, the ability to adapt behavior in response to changes in the environment (Klanker et al., 2013).

Successful adaptation of behavior requires the use of negative feedback to switch behavior and sensitivity to positive feedback to acquire a new response. Previously, we showed that DA signaling in the VMS is associated with positive feedback during reversal learning (Klanker et al., 2015), consistent with the idea of a quantitative reward prediction error (Schultz et al., 1997; Steinberg et al., 2013). However, several studies suggest that DA in the dorsal striatum might also be important for the regulation of this type of behavior. For example, dorsal DA is not just involved in the acquisition and performance of operant actions and habit formation (Faure et al., 2005; Amalric and Koob, 1987; Beninger and Ranaldi, 1993; Robbins et al., 1990; Robinson et al., 2007), but also in cognitive flexibility (Cools et al., 2001; Sawamoto et al., 2008; Clarke et al., 2011). In other words, dorsal striatal DA may invigorate both flexible and inflexible behavior.

Although classic electrophysiological studies suggest that all midbrain DA neurons behave similarly during conditioning tasks (Schultz et al., 1997), more recent findings reported differences between the characteristics of the VMS and DLS DA systems (Bromberg-Martin et al., 2010; Lerner et al., 2015). However, not only are VMS and DLS innervated by separate populations of DA neurons (Beckstead et al., 1979; Bjorklund and Dunnett, 2007), that are under excitatory influence of somewhat distinct afferent projections (Lerner et al., 2015; Watabe-Uchida et al., 2012), these regions also differ in DA receptor density (Dubois and Scatton, 1985; Savasta et al., 1986), DA uptake kinetics (Garris et al., 1994; Missale et al., 1985; Stamford et al., 1988) and local mechanisms influencing DA release (Rice et al., 2011). Together, these factors induce regionally distinct release patterns in striatal subregions during the execution of goal-directed behavior (Brown et al., 2011; Cacciapaglia et al., 2012; Shnitko and Robinson, 2015; Willuhn et al., 2012). However, while a large body of work investigated VMS DA during reward-related learning, it is unclear how phasic DA release in the DLS is modulated during performance of instrumental actions, as previous work produced inconsistent findings (Brown et al., 2011; Shnitko and Robinson, 2015). It is also unknown how DLS DA dynamics are modulated during adaptation of response behavior to a reversal of response-reward contingencies. Our previous results showed that DA signaling in the VMS rapidly adapts to reversed response-reward contingencies and that updating of cue-evoked DA following positive feedback predicts performance of reversal learning (Klanker et al., 2015). Here, we extend these findings by describing DA release dynamics in the DLS during reversal learning.
MATERIALS AND METHODS

All experiments were approved by the Animal Experimentation Committee of the Royal Netherlands Academy of Arts and Sciences and were carried out in agreement with Dutch laws (Wet op de Dierproeven, 1996) and European regulations (Guideline 86/609/EEC).

Animals
Male Wister rats (Charles River, n=13) were housed socially under a reversed day-night schedule (lights on from 7 p.m. – 7 a.m.). During behavioral training, rats were food-restricted (16 grams/animals/day) with water available ad libitum. The day before and 3-4 days after surgery, unlimited access to food and water was provided. Rats were individually housed after surgery.

Surgery
Rats (weighing ~300 grams) were anaesthetized with Isoflurane (induction: 3%, maintenance:1.8-2.5%) and received subcutaneous Metacam® (Meloxicam, 1 mg/kg, Boehringer-Ingelheim, Germany) as analgesic. Body temperature was maintained by a temperature controller and heating pad. Rats were placed in a stereotactic frame and implanted with a guide cannula (custom made, NIN mechanical workshop) above the DLS (anterioposterior +1.2 mm, lateral ±3.3 mm relative to bregma (Paxinos and Watson, 2007) and an Ag/AgCl reference electrode in the contra-lateral hemisphere. A bipolar stimulation electrode (Plastics one, Roanoke, VA, USA) was placed into the medial forebrain bundle (MFB; AP -3.6 mm, ML ± 1.8 mm, DV -8.5 mm from skull). The guide cannula and electrodes were fixed to the skull with screws and dental cement. The cannula was closed with a removable stylet after surgery.

Apparatus
All experiments were performed in a custom-made operant chamber (40x40x40 cm, NIN mechanical workshop) with Med Associates components (Med Associates, Sandown Scientific, Hampton, UK). The operant chamber was connected to a Med-PC interface and controlled by Med-PC software (Med Associates, Sandown Scientific, Hampton, UK). One wall of the operant chamber contained two retractable levers placed left and right from a food dispenser, with a cue light above each lever. An infrared sensor detected nose-pokes made to retrieve sucrose pellets (dustless precision pellets®, 45 mg, Bio-Serv) from the food dispenser.

Behavioral training
Rats were habituated to the operant chamber and sucrose pellets before behavioral training (also see methods in Klanker et al, 2015). During lever-press training, rats were randomly presented with either the left or the right cue light. After a 2 sec delay, the corresponding lever was presented. If a lever-press was made within 30 sec, the lever was retracted, the cue light switched off and a sucrose pellet was delivered in the food dispenser. Failure to respond within 30 sec ended cue light illumination and lever presentation and the trial was scored as omission. Sessions consisted of 32 trials, with variable inter-trial intervals (10/20 sec). Rats received up to 3 training sessions per day with 2-3 hours between the sessions.

During discrimination and reversal sessions, rats were presented with both cue lights simultaneously, and 2 sec later both levers were presented. During discrimination session, lever presses on one side were always rewarded, whereas lever presses on the other side were never rewarded. Thus, the task was spatial: cue lights did not signal which side was rewarded,
but indicated the potential availability of a reward. The rewarded side was counterbalanced between rats. Discrimination and reversal sessions consisted of 120 trials, with a variable inter-trial interval (15/25/35/45 sec). In case of an omission, cue light and lever presentation ended after 10 sec.

During the reversal session, response-reward contingencies reversed between the 16th and 32nd trial in the session, so that a response to the previously rewarded lever was no longer rewarded and a response to the previously unrewarded lever was now rewarded. The reversal of response-reward contingencies was unexpected for the animals; they had to use the change in feedback to adapt responding.

Fast-scan cyclic voltammetry
Glass-enclosed carbon fibers (sensing tip of the electrode: diameter 7 μm, length ~150 μm) were inserted into a custom-made micromanipulator (NIN mechanical workshop). The micromanipulator was then inserted in the guide cannula and attached to a head-mounted amplifier. The electrode was gradually lowered into the DLS (DV ~4-5 mm from skull). A resting potential of -0.4V was applied to the carbon fiber electrode (vs the reference electrode). Every 100 msec (sampling rate 10 Hz), the resting potential changed to 1.3 V and back to resting potential in a triangular waveform (duration 8.5 msec). At specific applied potentials in the waveform, changes in current can be measured due to redox reactions of DA molecules (~0.6 V oxidation, ~-0.2 V reduction) close to the electrode surface. After the experiment, MFB stimulation (6-30 pulses, 30 Hz frequency, 125 μA intensity, biphasic, 2 msec per phase) was performed to evoke DA release in the DLS. Chemometric analysis was used to identify DA and distinguish it from other electro-active species (Heien et al., 2004; Keithley et al., 2009).

After the final experiment, rats were deeply anaesthetized with an overdose of pentobarbital (Erasmus MC pharmacy, Rotterdam, The Netherlands) and the final recording location was marked by passing a 100 μA direct current through a stainless steel lesion electrode inserted to the depth of the last recording. Then, rats were decapitated and brains were removed and frozen. Coronal sections (40 μm) sections were cut on a cryostat and stained with cresyl violet for verification of electrode placement.

Data analysis and statistics
We analyzed the following behavioral measures: number/percentage of responses to the rewarded and non-rewarded lever as well as latency to lever press. Repeated measures ANOVAs were used to analyze behavioral data.

All FSCV data was smoothed with a 0.5 s moving average. To analyze changes in DA signaling during reversal learning, trials were averaged across a behavioral session for each rat and then averaged across rats. For cue-evoked responses, we averaged the 2 sec following cue presentation (in this time window the cue light is illuminated, and the levers are not yet presented) and compared to baseline (average of 2 sec immediately preceding cue presentation). For lever press DA release, we averaged the 2 sec following lever press and compared that to baseline (average of 2 sec before cue presentation). In addition, we analyzed separate 1-sec bins following lever press and compared these to baseline. For nose poke DA release, we compared the 2 sec following a nose poke to baseline (average 2 sec immediately preceding the nose poke). Similarly, for response to unexpected food reward, the average of the two seconds following reward presentation was compared to baseline (average 2 sec before reward presentation).
Changes in DA release compared to baseline were analyzed with paired t-tests. Changes in DA release during discrimination and reversal learning were analyzed with repeated measures ANOVA’s. Bonferroni-corrections were applied for post-hoc t-tests when appropriate. Greenhouse-Geisser corrections were applied when the assumption of sphericity was violated.

Comparison DA release DLS and VMS
In a previous paper (Klanker et al., 2015), we showed phasic DA release patterns in the VMS during reversal learning. To be able to directly compare our current findings in the DLS to the release patterns in the VMS, we re-analyzed the VMS in the same way as described here for the DLS (see above).

Data was analyzed with repeated measures ANOVA’s. Bonferroni-corrections were applied for post-hoc t-tests when appropriate. Greenhouse-Geisser corrections were applied when the assumption of sphericity was violated.

RESULTS
Subjects
Four animals were excluded from analysis because they did not show spontaneous DA release, no release to food rewards or stimuli presented, and no release to electrical stimulation of the MFB (no voltammograms indicating DA release before or during recordings). Of the animals included in the analysis, two did not show DA release after electrical stimulation of the MFB. However, this was likely due to stimulation electrode misplacement because these animals did show DA release during the session. Thus, they were included in the analysis. Recording sites in the DLS of all animals included in the analysis are depicted in Figure 1. Final group size was n=9.

Behavioral performance
Behavioral performance during reversal learning is shown in Fig 2A. The rewarded lever was switched at an unexpected time point in the reversal session (between trial 16-32). The reversal was not cued to the animals: rats had to use the change in reinforcing feedback (previously rewarded lever no longer rewarded and vice versa; grey bar in Fig 2A represents time of reversal) to adjust responding.

To better visualize the development of behavior after reversal, we divided the session in 12 blocks of 8 trials.
Regionally distinct DA patterns during reversal learning

(grey bar indicates time of reversal, Fig 2A). In the pre-reversal phase of the session (Fig 2A, block 1 & 2), rats showed a clear preference for the rewarded lever. After reversal, response to the rewarded lever declined, whereas responses to the non-rewarded lever increased (reversal*reward interaction F(1,16)=171.746, p<0.001). After reversal, lever press behavior to the rewarded or non-rewarded lever was different over time (block*reward interaction F(2.74; 43.88)=8.453, p<0.001). The number of rewarded responses differed between blocks (main effect of block F(10,60)=2.949, p=0.004; but no significant simple contrasts compared to first block after reversal). Non-rewarded responses steadily decreased (main effect of block F(9,72)=6.192, p<0.001, simple contrasts vs first block after reversal: significant differences last 5 blocks). On average, rats made 16.5 ± 6.8 % rewarded responses after reversal. However, as we showed previously (Klanker et al., 2015), behavioral performance during reversal learning can vary substantially between animals: one group of animals (‘learners’, n=4) made 34.3 ± 7.9 % responses (range 22.0-58.3%), whereas ‘non-learners’ (n=5) made 1.6 ± 0.9 % rewarded responses (range 0-4.4%) on all trials after reversal.

Phasic DA changes in the DLS during pre-reversal discrimination

Figs 2B&C show DA fluctuations in the DLS during reversal learning. Before reversal, cue stimulus presentation induced a small increase in DA (0.09 ± 0.03 nA) in the DLS. Cue-evoked DA release was significantly higher than baseline (t(13)=-3.278, p=0.006). A subgroup of animals (n=5) was trained longer on the spatial discrimination task (data not shown). Cue-evoked DA release did not differ between animals that were trained longer (750 ± 30 trials, receiving 658 ± 45 food pellets) or shorter (327 ± 56 trials, receiving 242 ± 32 food pellets) on the spatial discrimination task (data not shown), suggesting that the cue-evoked DA signal is not influenced by extended training.

Two seconds after cue presentation, two levers were presented. A lever press on the rewarded lever resulted in retraction of the levers and immediate delivery of a food reward, whereas a lever press on the non-rewarded lever resulted in lever retraction only. In contrast to the small cue-evoked signal, we observed more pronounced DA release around the time of lever-press (rewarded trials: 0.23 ± 0.05 nA; non-rewarded trials: 0.17 ± 0.07 nA). DA to lever-press was significantly higher than baseline for rewarded trials (t(8)=-4.142, p=0.003), but not for non-rewarded trials (t(4)=-2.123, p=0.101). Thus, when performing a well-learned discrimination, DA release in the DLS is centered around the lever press; cue-evoked DA release is minor.

DA release in DLS does not change after reversal

At an unexpected moment in the session, the rewarded lever was switched and rats had to reverse responding in order to get food reward. As in the pre-reversal phase, cue-evoked DA release after reversal was small (0.09 ± 0.03 nA), but increased relative to baseline (t(14)=4.144, p=0.001, Fig 2C). In trials where rats made an omission, no cue-evoked DA was observed (t(4)=1.063, p=0.348, Fig 2E).

Similar to the DA release pattern before reversal presentation, the most prominent DA release was observed around the time of lever press (rewarded trials: 0.29 ± 0.05 nA; non-rewarded trials: 0.17 ± 0.03 nA, Fig 2C) – DA release to lever press was significantly higher than baseline for rewarded (t(5)=-4.985, p=0.004) and non-rewarded trials (t(8)=-4.457, p=0.002) after reversal. DA release to lever press is slightly higher on rewarded compared to non-rewarded trials. Unexpected food reward presentation (before the behavioral session) induced DA release in the DLS (0.31±0.06 nA, Fig 2D) that was significantly higher than baseline (t(8)=
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Figure 2 Phasic dopamine (DA) release in the DLS during spatial reversal learning (n=9).

A. Behavioral performance during reversal learning session. Blue lines represent percentage rewarded responses per block of 8 trials, red lines represent percentage non-rewarded responses. The grey bar indicates the time point of the reversal of response-reward contingencies. B. Individual example trials of DA release in the DLS during reversal learning. The left panel shows DA release on a rewarded trial, right panel shows DA release during a non-rewarded trial. DA release was observed both in rewarded and non-rewarded trials around the time of lever press. Red bar indicates presentation of the cue light, gray bar indicates lever extension and black triangles indicate nose pokes into the food receptacle in order to collect the reward. Lever press is made when gray and red bars end. C. DA release is predominantly observed when rats perform an operant response. Changes in DA release averaged per trial across all trials of the reversal session. Left – before reversal presentation, similar DA release is observed around the time of lever press on both rewarded and non-rewarded trials. Right – after presentation of reversal, DA release is still observed on both rewarded and non-rewarded trials around the time of lever press. Blue lines represent rewarded trials, red lines represent non-rewarded trials, red bar indicates average duration of cue light presentation, black arrow indicates time of lever press. D. Unexpected delivery of a food reward (before the reversal session) evokes DA release in the DLS. Black arrow indicates time of reward delivery. E. During omission trials, cue and lever presentation do not evoke DLS DA release. Black arrow indicates time of cue presentation. F. Nose pokes into the food receptacle made in the absence of food reward, and in the absence of the cue or levers, are not associated with DLS DA release. Black arrow indicates time of nose poke.
Figure 3 Comparison of phasic DA release in DLS (panels A,C,E,G) and VMS (panels B,D,F,H) during reversal learning. Black (DLS) and grey (VMS) bars - quantification of DA release on rewarded trials, white bars – quantification of DA release on non-rewarded trials. A. Cue-evoked DA release in DLS is small and does not change after reversal presentation. B. In VMS, cue-evoked DA release is more prominent, and decreases after reversal presentation. C. DLS DA release significantly increases around the time of lever press in both rewarded and non-rewarded trials. D. In VMS, DA release around the time of lever press is not significant before reversal presentation. After reversal, reward delivery evokes strong DA release (significantly higher than baseline), whereas no release was observed on non-rewarded trials as expected. E. Development of DA release during lever press/reward delivery over time (4 consecutive secs following lever press, asterisks indicate significant difference from baseline), shows DA release to rewarded and non-rewarded lever presses in DLS (not significantly different), whereas in VMS (F), there is a bidirectional DA signal on rewarded and non-rewarded trials (significantly different). DA increases above baseline on rewarded trials, and decreases below baseline on non-rewarded trials (asterisks indicate significant difference from baseline). G. Unexpected reward delivery evokes DA in DLS. H. Unexpected reward delivery evokes DA in VMS.
5.234, \( p = 0.001 \), suggesting that the trend towards higher DA release on rewarded trials may be explained by an additive effect of DA release to food pellet release.

We separately analyzed DA release to nose pokes that were not made in temporal proximity to cue presentation or lever press (no cue or lever press present in the 10 seconds before or after the nose poke; first nose poke after lever press always excluded). One animal was excluded from analysis because no nose pokes meeting these criteria were registered. Such nose pokes made in the food receptacle were not associated with DA release (\( t(7) = 0.204, p = 0.844 \), Fig 2F). This indicates that nose pokes that are made without a food reward being present in the food receptacle do not induce DA release in the DLS.

**Different DA release patterns during reversal learning in VMS and DLS**

Previously, we characterized DA release patterns in the VMS during reversal learning (Klanker et al., 2015). In the following section, we compare our current findings to the results described previously (see Fig 3). In the DLS, cue-evoked DA was similar before and after reversal (reversal*reward interaction, \( F(1,9) = 0.592, p = 0.461 \); main effect of reversal, \( F(1,9) = 0.981, p = 0.348 \); main effect of reward, \( F(1,9) = 0.101, p = 0.758 \); Fig 3A). In the VMS, cue-evoked DA was higher before than after reversal (main effect of reversal, \( F(1,32) = 18.823, p < 0.001 \); Fig 3B, left panel), but did not differ between trials with rewarded or non-rewarded responses (reversal*reward interaction \( F(1,32) = 0.095, p = 0.760 \); main effect of reward, \( F(1,32) = 0.505, p = 0.482 \)).

In the DLS, DA release following lever press was similar before and after reversal, and was not different for rewarded and non-rewarded responses (reversal*reward interaction, \( F(1,9) = 0.257, p = 0.624 \); main effect of reversal, \( F(1,9) = 0.012, p = 0.914 \); main effect of reward, \( F(1,90 = 3.826, p = 0.082 \); Fig 3C). Also, DA release on rewarded and non-rewarded trials showed a similar development in the seconds after lever press, in both types of trials DA increased above baseline (main effect of time, \( F(2.107,27.386) = 17.622, p < 0.001 \); main effect of reward, \( F(1,13) = 4.572, p = 0.052 \); time*reward interaction, \( F(2.107,27.386) = 1.934, p = 0.162 \); Fig 3E). DA release was slightly, but not significantly, higher on rewarded than on non-rewarded trials. In contrast, in the VMS, DA release differed depending on trial-type and reversal phase: before reversal, DA release was not observed to reward delivery, whereas reward delivery did induce DA release after reversal (reversal*reward interaction \( F(1,33) = 17.998, p < 0.001 \); main effect of reversal \( F(1,33) = 3.254, p = 0.08 \); main effect of reward \( F(1,33) = 2.648, p = 0.113 \); Fig 3D). Analysis of consecutive time bins after lever press showed a bidirectional VMS DA release on rewarded and non-rewarded trials (time*reward interaction, \( F(2.058,76.135) = 10.313, p < 0.001 \); main effect of time, \( F(2.288,45.762) = 9.046, p < 0.001 \); simple contrast vs baseline: significant for sec 3 and sec 4 after lever press).

Unexpected presentation of a food reward induced DA release that was significantly higher than baseline in both DLS (\( t(8) = -5.234, p = 0.001 \)) and in VMS (\( t(18) = -5.951, p < 0.001 \)) (Fig 3G,H). Together, these findings show dynamic changes in DA release patterns in the VMS during reversal learning, whereas in the DLS, DA release appears to be constant over the session. A visual representation of the differences in DA release patterns in VMS and DLS is shown in Fig 4. These heat plots show DA release averaged across animals per trial. Data is synchronized to presentation of the cue stimulus. The approximate latency of the lever press response is indicated by white dots. These heat plots show different release patterns in DLS.
and VMS across the session of reversal learning: DA release in DLS is mostly associated with the lever-press response, whereas DA release in VMS is evoked by cue presentation. In both DLS and VMS, DA release can be observed throughout the session in learners, whereas DA signaling gradually extinguishes when animals cease responding (non-learners). In the DLS, release patterns are constant throughout the session of reversal learning and presentation of the reversal does not change the DA release patterns. In the VMS cue-evoked DA temporarily decreases, but is rapidly reinstated once animals adapt their response behavior. Together, these findings show distinct release patterns in DLS and VMS during adaptation of established response behavior.

**Figure 4** Comparison of phasic DA release in DLS and VMS over the course of the reversal session. In the DLS (top two heat plots), DA release is predominantly associated with the performance of an operant response. The white dots indicate the approximate latency of lever press in each trial. In animals that learn to adapt behavior to a reversal of response-reward contingencies (learners: top left panel), DA release that peaks during the lever press is observed throughout all trials of the session. In DLS non-learners (top right panel), DA release extinguishes when rats cease to press the levers. In the VMS (bottom two heat plots), DA release is predominantly associated with presentation of the cue light. In VMS learners (bottom left panel), DA release patterns adapt to reversed response-reward contingencies: Before reversal, only cue presentation evoked DA release, whereas after reversal, DA release is also observed following reward delivery (which in the group average is most visible in towards the end of the reversal session). In VMS non-learners (bottom right panel), cue-evoked DA gradually diminishes when rats extinguish lever press responding. For each trial, data was averaged over animals. Black arrow indicates presents presentation of cue stimulus. White dots indicate approximate latency of lever press (in case rats did not make a lever press, 10 sec was taken as latency value). White horizontal line indicates reversal trial.
DISCUSSION

We recorded DA release in the DLS in rats performing a spatial discrimination and reversal learning task. In a well-learned discrimination between two operant levers, a phasic increase of extracellular DA was observed during the performance of the lever press, whereas only a small increase was seen following presentation of the non-discriminative cue light signaling trial onset. Reversing the response-reward contingencies of the levers did not alter the pattern of DA release in the DLS: Cue-evoked DA release remained small whereas greater DA release was evoked around the time of lever press. DA release around the time of lever press was observed in both rewarded and non-rewarded trials, demonstrating a dissociation of DA dynamics and trial outcome. These results contrast our previous observations on DA release in the VMS where cue-induced release predominates during (well-learned) discrimination, and temporarily switches to the time of reward delivery during early reversal learning. Together, these findings demonstrate that phasic DA release in the DLS is predominantly associated with the performance of an operant response, and that DLS DA release does not rapidly adapt to changes in reinforcing feedback, as reported for the VMS.

Functional differences in DA release in the striatum

Rapid processing of information about response-reward contingencies is essential to acquire goal-directed behavior and adapt that behavior to changes in the environment. DA neuron activity provides a neural substrate for reinforcement learning: DA neurons show burst firing to unexpected reward and reward-associated stimuli, and are inhibited when an expected outcome is omitted (Schultz et al., 1997; Pan et al., 2005). However, the relation between changes in firing rate of DA neurons and extracellular DA concentration in projection regions of these neurons is not always proportional. Thus, to learn more about regional DA function, measurements of DA release directly at the terminal level (rather than recording from the cell body) are required in relation to reward-oriented behavior. Consistent with reports of DA firing activity, DA release in the ventral striatum increases in response to natural rewards and reward-predicting stimuli (Roltman et al., 2004) and these release patterns change during learning (Stuber et al., 2008; Day et al., 2007). However, recent findings indicate that these release patterns may not be uniformly present throughout the striatum (Brown et al., 2011; Cacciapaglia et al., 2012) and it is less clear how phasic DA in the dorsal striatum is modulated during learning and behavioral adaptation (Brown et al., 2011; Shnitko and Robinson, 2015; Willuhn et al., 2012). Previously, we characterized phasic DA release in the VMS during reversal learning, and showed that these signals rapidly adapt to changing response-reward contingencies (Klanker et al., 2015), consistent with a reward-prediction error signal (Schultz et al., 1997). Here, we demonstrate that phasic release patterns in the DLS, a region that receives input predominantly from sensory and motor cortices and is strongly implicated in habit formation, are fundamentally different from those observed in the VMS during reversal learning.

DLS DA is mainly associated with the performance of operant behavior

In our experiment, non-discriminative cues signaled trial onset. Thus, whenever the cues were presented the animals had an opportunity to gain rewards, provided they made a correct lever press. Cues were presented prior to lever extension/lever press in order to separate DA release signals related to these different task components. We found that during performance of the well-learned discrimination, cue presentation evoked only a small DA
Regionally distinct DA patterns during reversal learning

signal in the DLS. Previously, Brown et al (2011) did not find such phasic release to a cue that specifically predicted reward in an operant box, whereas Howe et al (2013) showed transient DA signals in the DLS to a discriminative cue indicating trial onset (often superimposed on a slower ramping signal) during a maze running task, yet it only occurred infrequently. Thus, cue-related DA signals are often absent or small in the DLS, although this may also depend on the specific task demands.

It has been hypothesized that the relative contribution of dorsal striatal subregions to the control of motivated behavior varies with how well the behavior is established, whereby activity in the DLS dominates during the execution of more established behavior, when stimulus-response associations drive responding (Yin et al., 2009; Segovia et al., 2012; Hernandez et al., 2006; Kimchi et al., 2009). Therefore, we hypothesized that the cue-evoked signal in the DLS might develop later on in the training of our task, during strengthening of stimulus-response associations. We trained a sub-group of animals for a longer period of time in the discrimination task, but observed no difference in cue-evoked signaling. Together, this suggests that conditioned cues do not acquire a strong propensity to induce DA release in the DLS.

In contrast to the small cue-evoked signal, we observed pronounced DLS DA release when animals executed the lever press to obtain food reward, consistent with previous findings (Brown et al., 2011). In an instrumental discriminative learning task, a rise that occurred several seconds after presentation of a discriminative cue was reported previously, but this rise was not significantly different from baseline (Brown et al., 2011). However, DA measurements in that study were not specifically time-locked to the lever press, but used a general epoch following cue presentation, which may explain the differences with our results.

In trials where rats did not press the lever (omission trials), we detected no DA release around the time of cue presentation or lever extension, strongly suggesting that DA release relates to performance of an action, and not to presentation of conditioned stimuli. The question arises whether this increase is associated with the execution of the lever press or the subsequent reward delivery, as those two task components occurred in close temporal proximity. Although we observed DA release in the DLS to delivery of an unexpected food reward, it is extremely unlikely that reward delivery during the behavioral session is unexpected, as the animals induce pellet delivery themselves. The idea that DA release to reward delivery cannot fully explain the DA release to lever press, is further supported by the fact that DA was also present in non-rewarded trials (see below). Importantly, the increase in DA around the time of lever press most likely does not represent a general signal for the initiation of motor responses per se, as nose pokes made in the food receptacle during inter-trial intervals (no food reward present) were not associated with DA release in DLS. Howe et al (2013) concluded that gradually increasing DA concentrations in the DLS may be important to maintain and direct motivation during goal-directed actions, for example during reward uncertainty (Howe et al., 2013). Although partially consistent, the non-discriminative character of the DLS DA signal (present irrespective of receipt of reward) that we now report (see below) suggests that DLS DA release is partially outcome-independent. This suggests a role for DLS DA in motivation for, but also in initiation of, an operant response that is intended to produce a reward.

DLS DA release dynamics do not adapt during reversal learning

At an unexpected moment in the session, response-reward contingencies were reversed, and rats had to modify their established response pattern in order to receive rewards. Remarkably, DA release patterns in the DLS did not adapt to this reversal of response-reward
contingencies. Instead, cue-evoked release was similar before and after reversal and was not influenced by the receipt of positive feedback. DA release increased around the time of lever press on rewarded as well as on non-rewarded trials. Similarly, Shnitko and Robinson (2015) also reported DA release to lever press on rewarded as well as non-rewarded trials during sucrose self-administration. However, release on rewarded trials was higher in their task, most likely due to combined presentation of a reward-predicting cue and reward delivery immediately following the lever press. After reversal, we found a trend towards higher DA release around the time of lever press on rewarded compared to non-rewarded trials. This may be explained by reward-induced DA because when rats adapt their response pattern after reversal, the initial reward deliveries following a lever press on the newly rewarded side may be unexpected. Previous reports on reward-induced DA release in the DLS were inconsistent (Brown et al., 2011; Natori et al., 2009; Shnitko and Robinson, 2015). In our rats, presentation of an unexpected food reward before the behavioral session did evoke DA release (albeit considerably smaller than in the VMS), suggesting that DA release to reward delivery after reversal may have an additive effect on the release to lever press. However, the observed DA release around the time of lever press on non-rewarded trials, and the absence of DA release related to nose pokes, suggests that DLS DA release is not solely related to reward delivery or motor performance, but might instead be related to the cross section of the two: The performance of a previously learned operant response to obtain a reward.

To conclude, DA release in the DLS is predominantly associated with operant performance and less with specific task-related events such as presentation of a reward-predicting cue or reward delivery. These findings extend and corroborate previous data suggesting that DA in the DLS is primarily associated with behavioral responses rather than with the presentation of reward-related cues (Young et al., 1998; Ito et al., 2002; White and Rebec, 1993; Fanelli et al., 2013). Moreover, when animals adapt their response behavior during reversal learning, DLS DA release patterns do not adapt to reversed contingencies but remain stable. Together, these findings suggest that DLS DA may reflect a ‘performance signal’, enabling the initiation of operant responses or the motivation to perform them. This signal, which is selectively present in the DLS and relatively insensitive to the outcome of the response, may underlie the important role of this area in the formation and execution of habits, operant responses that are performed irrespective of the outcome.

**Different DA release patterns in DLS and VMS during reversal learning**

Several lines of evidence suggest that there is no uniform DA signal that is broadcasted throughout the brain. Indeed, we observed remarkably distinct phasic DA release patterns in VMS and DLS during performance of spatial discrimination and when rats adapt responding to reversed response-contingencies. DLS DA is predominantly associated with the performance of an operant response that is initiated to obtain a reinforcer, whereas DA release to presentation of a cue stimulus is almost absent and occurs infrequently. These release dynamics do not change when animals adapt behavior to a reversal of response-reward contingencies. Such dynamics are in stark contrasts to the highly adaptable DA release patterns in the VMS, where DA release switches from the time of cue presentation to the time of reward delivery and back to the cue when animals modify their response behavior.

Several factors that influence DA release in striatal subregions may be responsible for regional differences in DA release. First, there is separation in the DA cell groups that project to either DLS (projections originating in substantia nigra) or VMS (projections originating in
ventral tegmental area; (Bjorklund and Dunnett, 2007). These cell groups receive different proportions of afferent input from other brain regions (Watabe-Uchida et al., 2012), which may result in heterogeneous firing responses to reinforcers, reward-predicting stimuli and salience signals (Bromberg-Martin et al., 2010; Lerner et al., 2015). Second, sensitivity of DA release in response to tonic or phasic firing patterns of DA neurons may differ between projection regions (Cragg et al., 2002; Trout and Kruk, 1992; Zhang et al., 2009). Third, modulation of release by excitatory input to DA terminals as well as other mechanisms influencing DA release locally can induce distinct release patterns in striatal subregions (Rice et al., 2011). Finally, different uptake DA rates/kinetics in DLS and VMS influence the duration of the DA signal (Garris et al., 1994; Stamford et al., 1988; Missale et al., 1985). Together, these factors contribute to complex and heterogeneous DA release patterns to natural rewards, reward-related stimuli and during operant behavior throughout the striatum (Brown et al., 2011; Shnitko and Robinson, 2015; Zhang et al., 2009; Cacciapaglia et al., 2012). In line with this, we now show distinct DA release patterns in DLS and VMS that are differently modulated during reversal learning.

The observed differences between DLS and VMS DA dynamics during the performance of behavior are consistent with the suggested dissociation between the involvement of sensorimotor circuits (associated with the DLS) and limbic circuits (associated with VMS or medial striatum) in the control of behavior (Voorn et al., 2004; Yin and Knowlton, 2006). The DLS and its associated circuitry are essential to initiate an action that is driven by a reward-related stimulus, whereas limbic circuitry is associated with learning about the relation between expected and received rewards following a certain response and the stimuli predicting reward (Corbit and Janak, 2007; Yin et al., 2008).

CONCLUSION

Together, these findings show different release patterns in VMS and DMS during the performance of reversal learning: Stable release patterns in the DLS and highly adaptable DA release patterns in the VMS. The VMS DA signal, which rapidly adapts to reversal response-reward contingencies and is sensitive to positive feedback, may provide a teaching signal and represent motivational properties of the stimulus to promote reward seeking actions (Berridge et al., 2009; Flagel et al., 2011; Montague et al., 1996; Schultz et al., 1997; Steinberg et al., 2013; Waelti et al., 2001). In contrast, the stability of DLS DA patterns during adaptation of behavior suggests that DLS DA is not consistent with a teaching signal, but instead is associated with the execution of operant responses or the motivation to perform them (Howe et al., 2013).

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PART II

Deep brain stimulation in corticostriatal circuits – effects on dopamine and cognition
Deep brain stimulation of the medial forebrain bundle / ventral tegmental area elevates striatal dopamine concentration without affecting spontaneous or reward-induced phasic dopamine release

Marianne Klanker, Ingo Willuhn, Matthijs Feenstra, Damiaan Denys

In preparation
ABSTRACT

Deep brain stimulation (DBS) of the medial forebrain bundle (MFB)/ventral tegmental area (VTA) region induces a rapid improvement of depressive symptoms in patients suffering from treatment-refractory major depressive disorder. It has been suggested that activation of the dopamine (DA) system may contribute to this effect. Here, we investigated whether DBS in the MFB/VTA region affects phasic DA release dynamics in the rat striatum. To investigate phasic DA dynamics during MFB-DBS, we combined DBS with fast-scan cyclic voltammetry (FSCV) in awake, freely moving rats. Animals were implanted with a stimulating electrode in the MFB/VTA and a FSCV microelectrode in the ventromedial striatum to monitor dopamine release during DBS. We investigated the acute effect of DBS onset on DA release as well as the effect of continuous stimulation on spontaneous DA fluctuations (transients) and DA release related to the unexpected presentation of food reward. Onset of DBS induced a significant increase in extracellular concentration of DA in the ventromedial striatum that was sustained for at least 40 seconds. However, the amplitude and frequency of spontaneous DA transients or DA release in response to the delivery of unexpected food pellets were not influenced by ongoing stimulation. These findings suggest that onset of DBS in the MFB/VTA induces DA release, but that continuous DBS does not modify spontaneous or reward-induced phasic DA dynamics in the ventromedial striatum. Thus, DBS of MFB/VTA affects specific aspects of dopamine signaling that may be of mechanistic importance in DBS treatment of depressed patients.
INTRODUCTION

In the last decade, deep brain stimulation (DBS) emerged as an innovative treatment for refractory psychiatric disorders, such as major depressive disorder (MDD) and obsessive-compulsive disorder (OCD). With significant responses in approximately 50% of patients, DBS appears to offer a last resort treatment to treatment-resistant patients (Holtzheimer and Mayberg, 2011; Goodman and Alterman, 2012). Interestingly, successful remission can be achieved with stimulation in different target regions, the most widely used targets for MDD being the subcallosal cingulate (Mayberg et al., 2005; Puigdemont et al., 2012), nucleus accumbens (Schlaepfer et al., 2008; Bewernick et al., 2012) and the anterior branch of the ventral capsule (Malone, Jr. et al., 2009). The commonality between these areas is that they are part of cortical-striatal circuits. These circuits are important for motivation and the regulation of cognitive and motor functions, and show structural and functional disturbances in patients with MDD (Drevets et al., 2008; Furman et al., 2011). Although the precise working mechanism of DBS is unknown, the restoration of aberrant network activity in cortical-striatal circuits may be an underlying factor in the effective use of DBS in psychiatry (McIntyre et al., 2004; Figeé et al., 2013; Mayberg et al., 2005).

A recent study investigated DBS to the supero-lateral branch of the medial forebrain bundle (MFB) and showed promising effects in a small patient group with MDD (Schlaepfer et al., 2013). It has been hypothesized that DBS in the MFB region induces dopamine (DA) release in striatal and prefrontal regions through activation of ascending dopaminergic (DAergic) fibers that run through to the MFB (Schlaepfer et al., 2013; Schlaepfer et al., 2014). Dysregulation of DAergic pathways is thought to contribute to anhedonia and motivational dysfunction in depressive patients (Kapur and Mann, 1992; Russo and Nestler, 2013; Dunlop and Nemeroff, 2007) and has been linked to depression-like behavior in rodent models (Chaudhury et al., 2013; Tye et al., 2013; Friedman et al., 2012). Thus, by activating the DA system, DBS in the MFB may counteract these disturbances and exert its treatment effects.

Previous preclinical studies have shown that DBS in the MFB/VTA reduces depressive symptoms in animal models of depression (Bregman et al., 2015; Edemann-Callesen et al., 2015; Furlanetti et al., 2015a; Friedman et al., 2009), and that continuous DBS in this region does not have adverse effects (Furlanetti et al., 2015b). Moreover, preclinical studies have proven a useful tool for the investigation of DBS effects on neurotransmitter release in the brain (Hamani et al., 2010; Bruet et al., 2001; van Dijk et al., 2012; Bregman et al., 2015; Paek et al., 2013; Shon et al., 2010; Chang et al., 2013). However, these studies either used microdialysis measurements (Bregman et al., 2015; Bruet et al., 2001; Hamani et al., 2010; van Dijk et al., 2012), which detect slow changes in DA release, or used voltammetry in anesthetized preparations (Paek et al., 2013; Shon et al., 2010; Chang et al., 2013), measuring fast DA changes, but only electrically stimulated release. DBS effects on phasic DA release (e.g., spontaneously occurring transients or transients time-locked to reward delivery) have not been studied in awake, freely moving animals. Thus, we used fast-scan cyclic voltammetry (FSCV) to investigate if continuous DBS in the MFB/VTA area region affects phasic dopamine release patterns in the ventromedial striatum. We observed an increase in extracellular DA after onset of DBS that lasted at least 40 seconds. Continued stimulation did not affect characteristics of spontaneously occurring DA transients or DA release to food reward presentation. These findings suggest that continuous high frequency stimulation in the MFB/VTA...
may elevate DA concentrations in the ventromedial striatum, but without modifying phasic DA dynamics.

**MATERIAL AND METHODS**

All experiments were approved by the Animal Experimentation Committee of the Royal Netherlands Academy of Arts and Sciences and were carried out in agreement with Dutch law (Wet op de Dierproeven, 1996) and European regulations (Guideline 86/609/EEC).

**Animals**

Male Wistar rats (Charles River, DBS group n=16; control group n=8) were socially housed (per 2) under a reversed day-night schedule (lights on from 7 p.m. to 7 a.m.). Unlimited access to food and water was available the day before and days following surgery. During behavioral training and experiments, rats were food-restricted (16 grams/animal/day) with unlimited access to water. After surgery, rats were housed individually.

**Surgery**

Rats (weighing ~300 grams) were anesthetized with Isoflurane (induction: 3%, maintenance: 1.8-2.5%), received subcutaneous Metacam® (Meloxicam, 1 mg/kg, Boehringer-Ingelheim, Germany) as analgesic, and were then placed in a stereotactic frame. Body temperature was maintained by a temperature controller and heating pad. A custom made guide cannula (made in house) was implanted above the right nucleus accumbens (anterior posterior +1.3 mm, lateral ±1.3 mm relative to bregma (Paxinos and Watson, 2007)). An Ag/AgCl reference electrode was implanted in the contralateral hemisphere. For the DBS group, a bipolar stimulation electrode (Plastics one, Roanoke, VA, USA) was inserted above the MFB/VTA (targeted at AP -4.6 mm, ML 1.0mm, DV -7 mm from bregma (Paxinos and Watson, 2007)). A micro-manipulator holding a glass-enclosed carbon fiber electrode (electrode tip: diameter 7 μm, length 100-150 μm) was placed into the guide cannula and the electrode was lowered into the ventromedial striatum. A head-mounted amplifier for FSCV recordings was attached to the reference and working electrode. A resting potential of -0.4 V was applied to the carbon fiber electrode (vs the reference electrode). Every 100 msec, the potential was driven to 1.3 V and back in a triangular waveform (lasting 8.5 msec). At specific applied potentials in the waveform, changes in current can be measured due to redox reactions of DA molecules (~0.6 V oxidation, ~-0.2 V reduction) close to the electrode surface (Phillips et al., 2003). To ensure placement of the stimulating electrode in a region where DA could be evoked, we gradually lowered the stimulating electrode into the MFB/VTA with 0.2 mm increments. Whenever the stimulating electrode was lowered, a biphasic stimulation (24/60 pulses, 60 Hz frequency, 2 msec pulse width per phase, 125 μA intensity) was applied. The stimulating electrode was lowered until evoked DA release could be measured at the working electrode in the ventromedial striatum. There has been inconsistent targeting of specific subregions of the MFB in preclinical experiments, with some studies stimulating the MFB close to the lateral hypothalamus (Bregman et al., 2015; Edemann-Callesen et al., 2015) and others more posterior, close to the VTA (Furlanetti et al., 2015b; Furlanetti et al., 2015a; Furlanetti et al., 2016). Our placement correspond to those of Furnaletti et al (Furlanetti et al., 2015b; Furlanetti et al., 2015a).
Then, the working electrode was removed and the stimulating electrode was fixed with dental cement. Only animals that showed evoked DA release during surgery proceeded to the experiments described below. For the control group, a bipolar stimulating electrode was implanted in the VTA (AP -5.2 mm, ML 1.0 mm, DV ~8-9 mm from skull) and no stimulations were applied during surgery. A removable stylet was used to close the cannula after surgery.

**Apparatus**

Experiments were performed in a custom made operant chamber (40 x 40 x 40 cm, mechanical workshop of the institute) with MED Associates parts (Med Associates, Sandown Scientific, Hampton, UK). A house light was located in the middle of one of the walls close to the chamber ceiling. A food dispenser was placed in the middle of the opposite wall. Nose pokes in the food dispenser to retrieve sucrose pellets (Dustless precision pellets®, 45 mg, Bio-Serv) were detected by an infrared sensor.

**Experimental Procedure**

Before the start of the experiment, rats were habituated to the operant chamber and sucrose pellets. On the experimental day, rats were placed into the operant chamber and electrode implants attached to a head stage to allow DBS and FSCV recordings. For FSCV recordings (Phillips et al., 2003), a micromanipulator holding a fresh glass-enclosed carbon fiber electrode was placed into the cannula and connected to a head-mounted amplifier. The electrode was gradually lowered into the ventromedial striatum. The electrode was placed at a depth where spontaneous DA transients and DA release to unexpected food pellets were observed.

For DBS, the head stage was connected to an isolator (DLS 100, World Precision Instruments (WPI), Sarasota, FL, USA) and stimulator (DS 8000, WPI, Sarasota, FL, USA). Fig 1 shows the experimental timeline. During the first 90 minutes, the animals could habituate. In the subsequent 10 min (T1), spontaneous DA transients were recorded, but rats were not presented with any other stimulus. During the following 20 minutes, spontaneous transients were collected and rats received food pellets (10-16 per session) delivered with a variable inter-trial interval (30/60/90/120/150 sec; total session time: 20 min) throughout this period. After this period, DBS was applied (120Hz, 80 μsec pulse width, 200 μA) to animals in the DBS group. Control animals never received DBS. To prevent artefacts from the DBS obscuring FSCV measurements, DBS stimulation was only applied during the inter-scan intervals in between voltammetry scans. Thus, instead of continuous stimulation, 1 pulse that would overlap with the FSCV waveform was omitted and 11 pulses were applied during each inter-scan interval. When DBS was switched on, the same procedure was applied: first 10 minutes of spontaneous transients were recorded (T2), followed by 20 minutes in which the rats received sucrose pellets at unexpected time points. Then DBS was switched off and spontaneous transients were recorded for a final 10 minutes (T3). After the final experiment, a stainless steel electrode was lowered to the recording location and a direct current (100 μA) was passed to mark placement of recording and stimulating electrode. Rats were euthanized with an overdose of pentobarbital (Erasmus MC, Rotterdam, the Netherlands) and decapitated. Brains were removed and frozen. Histological sections were cut on a cryostat and stained with cresyl violet to reveal electrode positions.

**Data Analysis**

Chemometric analysis was performed on all FSCV recordings to separate DA-related currents...
from those of other electro-active species (Heien et al., 2004; Keithley et al., 2009). All data was smoothed using a 5-point moving average.

1. DBS onset

For the effect of DBS onset on DA release, we analyzed a 50-sec period during which DBS was switched ON. Data from this file was binned in three time bins: a 10-sec baseline bin and two 20-sec bins in which DBS was on. Using three bins allowed us to see whether any effect of DBS would sustain over this period. We compared the first 40 sec during which DBS was switched on to two control periods: the first 40 sec period of T1 (animals are their own controls) and the first 40 sec period of T2 recorded in a different cohort of animals that underwent the same procedures (see Fig 1), but were not implanted with DBS electrodes (separate control group). Data was analyzed statistically using repeated measures ANOVA.

2. Spontaneous transients

Spontaneous fluctuations in DA concentration commonly called ‘DA transients’ can be observed in striatal regions (Robinson et al., 2003). Only transients greater than 0.3 nA / 20nM were included in the analysis (Vander Weele et al., 2014). Transient amplitude and frequency were quantified using MiniAnalysis (Synaptosoft). Amplitude and frequency of spontaneous transients were analyzed during three blocks of 10 minutes (T1,T2,T3; repeated measures ANOVA) as well as during the two blocks in which animals received unexpected food rewards (paired t-test). General activity and number of nose pokes made in the food receptacle were recorded throughout the three “spontaneous transient”, 10-minute recording periods. Behavioral data was analyzed with a repeated measures ANOVA.

3. Unexpected food rewards

Rats were presented with unexpected food rewards during two blocks of 20 minutes (see Fig1). Throughout the 20-minute period, the following behavioral parameters were recorded: general activity, total number of nose pokes, and latency between pellet delivery and the first nose poke in the food receptacle to retrieve the pellet. Behavioral data were analyzed with
paired t-tests. For the DA response to unexpected food pellets, all DA traces were aligned to the time of pellet delivery in the food receptacle. The peak DA response to pellet delivery was obtained by taking the maximum value in a two-second window that followed pellet delivery. For each rat, peak DA values to pellet delivery were averaged across the entire session. Peak DA responses during DBS OFF and DBS periods were compared with paired t-tests.

All statistical analyses were performed with SPSS Statistics 21 (IBM, Amonk, NY). Statistical significance was set to $p<0.05$.

RESULTS

For the DBS group, eight animals were excluded from the experiment: $n=4$ rats did not show stimulated DA release during surgery, $n=1$ needed significantly higher stimulation parameters during surgery to achieve DA release and also did not show stimulated DA release (with standard parameters) after the experiment, $n=1$ died after surgery, and $n=2$ were not recorded from due to technical problems. Final group size was $n=8$.

Histology

Fig 2 shows the placement of recording (left) and stimulating electrodes (right) in the ventromedial striatum and MFB/VTA region, respectively. In three animals, histological verification of stimulating electrode was not possible due to errors with histological processing. However, these three animals did show good electrically stimulated dopamine release during surgery, and thus were included in the analysis. Depth of recording electrodes was estimated based on depth of cannula during surgery and how deep the electrode was lowered during the experiment. For the control group, $n=3$ animals were excluded from analysis because recordings contained too much noise (final group size $n=5$).

Onset of MFB DBS induces DA release in the striatum

For this experiment, one animal was excluded from data analysis as DBS was switched on from the beginning of the first recording file; therefore, we were not able to collect a baseline measure without DBS. Fig 3 shows the effect of DBS onset on striatal DA release. Data was separated into three time bins: a 10-sec baseline bin and two 20-sec DBS bins. We compared the DA trace during the first 40 sec of transient recording period T1 (NO DBS) and T2 (DBS) to see if onset of DBS would influence DA release (Fig 3B,C). A repeated measures ANOVA showed a DBS*time-bin interaction effect $F(2,12)=5.676$, $p=0.018$ (main effect DBS $F(1,6)=2.541$, $p=0.162$; main effect time-bin $F(1.103,6.616)=3.656$, $p=0.098$), indicating that the DA trace over three bins differed depending on stimulation condition (NO DBS vs DBS). Separate post-hoc ANOVA’s for DBS and NO DBS showed that when DBS was switched on (T2 period), DA increased above baseline (significant effect time-bin $F(1.022,6.134)=14.489$, $p=0.008$, simple contrasts showed significant differences between time-bin1 and time-bin2 $F(1.6)=15.205$, $p=0.008$ and between time-bin1 and time-bin3 $F(1.6)=14.319$, $p=0.009$). During the control period recorded in these same animals (T1, NO DBS), DA was not different across bins ($F(1.144,6.865)=0.477$, $p=0.537$). In addition, we compared the DBS onset trace to a similar time period in a separate control group (first file T2, these animals also received unexpected food pellets before transient recording T2). Similar to the findings described above,
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DA release across the three time bins was different between the DBS group and control group (significant Bin*Group interaction effect $F(1.097,10.975)=7.924$, $p=0.015$ (main effect Bin $F(1.097,10.975)=8.483$, $p=0.013$; Main effect Group $F(1,10)=3.000$, $p=0.114$). In the separate control group, DA did not change from baseline in the time period analyzed (T2; Main effect Bin $F(2,8)=0.529$, $p=0.608$). Together, these findings indicate that MFB DBS induced a rapid increase of striatal DA above baseline, which persisted for (at least) 40 seconds, whereas in a control period, DA did not change from baseline.

Continuous MFB stimulation does not affect spontaneous fluctuations in striatal DA

We recorded spontaneous dopamine transients during three 10-minute periods: T1: NO DBS, T2: DBS and T3: DBS OFF (see Fig 1 for timeline experimental procedure). General activity and number of nose pokes made in the food receptacle were similar for all three 10-minute periods of transient recordings (data not shown), suggesting that general motor behavior was not affected by stimulation. DBS did not influence amplitude or frequency of spontaneously...
DBS of the medial forebrain bundle evokes striatal DA

Figure 3 DBS onset induces an increase in striatal dopamine (DA) release. A. Example traces of DA release following DBS onset from an individual animals. Right panel shows DA release following onset of DBS (first 40 sec T2), left panel shows DA release during a similar time period without DBS (first 40 sec T1). Color plots show current changes (z-axis, color) at the recording electrode plotted against applied voltage (y-axis) and time (x-axis). B. Average trace showing DA release to DBS onset across all animals. Left panel – DBS onset induces DA release in the striatum. Red line represents DBS, black line represents NO DBS. Right panel – DBS onset trace (red line) compared to a “NO DBS” time period (black line) in a separate group of animals. C. Quantification of DA response to DBS onset. Left panel compares first 50 sec of T2 to first 50 sec of T1 (within-animal control), right panel shows first 50 sec of T2 to first 50 sec of T2 of control group (between-animal control). When DBS is switched on (red bars), DA significantly increases above baseline. Without DBS (black bars), DA remains stable over time bins. Asterisks indicate significant difference from baseline (p<0.01).
occurring DA transients during the three 10-minute periods of spontaneous transient measurements (T1,T2,T3; amplitude F(2,14)=0.548, p=0.590; frequency F(2,14)=0.149, p=0.863, Fig 4A,B). We also quantified spontaneous transients during the two 20-minute periods in which rats received unexpected food rewards (excluding the DA responses to food pellet delivery), and found no effect of DBS (amplitude t(7)=-0.329, p=0.752, frequency t(7)=-1.133, p=0.295; data not shown). Together, this shows that continuous DBS in the MFB does not influence the occurrence or amplitude of spontaneous dopamine transients.

Reward-induced DA is not altered by MFB DBS
Rats were presented with unexpected food rewards during two 20-minute sessions. Rats received 11.5 ± 0.4 food pellets during the first session (NO DBS) and 13.1 ± 0.7 pellets during the second session (DBS). DBS did not influence general activity or nose poke behavior: latency until first nose poke after pellet delivery (session 1: 5.6 ±2.8 sec; session 2: 2.3 ± 0.2 sec) and number of nose pokes (session 1: 232.9±31.7, session 2: 279.6±29.8) made into the food dispenser were similar in both sessions. Fig 4C shows the DA response to reward delivery in presence (red line) and absence (black line) of DBS averaged across all animals. Reward-induced DA release was not influenced by DBS (peak DA to reward delivery –NO DBS: 0.93 ± 0.12 nA, DBS ON: 1.10 ± 0.16 nA; t(7)=-2.180, p=0.066, Fig 4D).

DISCUSSION
Here, we report the effects of MFB/VTA DBS on phasic dopamine release characteristics in the ventromedial striatum. To our knowledge, this is the first report on the effects of DBS on basal and reward-related measurements of phasic DA release (using FSCV) in awake, freely moving rodents. Onset of DBS in the MFB/VTA induced DA release in the ventromedial striatum that lasted for at least 40 seconds. Continuous stimulation did not affect the frequency and amplitude of spontaneously occurring DA fluctuations (‘transients’). DA release in response to unexpected food rewards was also not affected by continuous stimulation. Together, these findings show that onset of DBS induces striatal DA release, but that continuous stimulation does not change spontaneous or reward-induced phasic DA transients.

Onset of stimulation induces striatal DA release
For MDD, DBS in a selection of target regions within the cortical-striatal circuit has been shown to result in symptom reduction (Schlaepfer et al., 2014). The MFB is the most recent addition to this list of DBS targets (Schlaepfer et al., 2013). The effects of stimulation in this area were somewhat distinct from previous targets, because they occurred rapidly after onset of stimulation, and less current was required for the treatment effect (Schlaepfer et al., 2013). With connections to all previously described target sites, the MFB is well positioned to influence activity throughout cortical-striatal networks (Dobrossy et al., 2015; Coenen et al., 2011). However, it is not yet understood which specific physiological effects of DBS are responsible for the changes in network activity and result in successful treatment. One of the ways by which DBS may modulate network activity is by influencing the release of neurotransmitters/neuromodulators (Anderson et al., 2012; Hamani et al., 2010).

DA is an important neuromodulator that can affect activity in cortical-striatal networks. Altered DA transmission has often been hypothesized to contribute to MDD symptoms, in
particular anhedonia and reduced motivation (Dunlop and Nemeroff, 2007; Kapur and Mann, 1992; Russo and Nestler, 2013). Direct manipulation of DA neuron activity can induce or alleviate depressive symptoms, such as anhedonia, in naïve animals as well as in a rodent depression model; and this effect is specific for DA neurons projecting to the striatum (Chaudhury et al., 2013; Tye et al., 2013). Together, this suggests the involvement of DA in some of the core symptomatology of MDD. As it has previously been hypothesized that MFB DBS may influence DA neurotransmission (Schlaepfer et al., 2013; Schlaepfer et al., 2014), we set up an experiment to directly test the effect of MFB DBS on DA release in the striatum.

Here we show that onset of MFB DBS induced an increase in striatal DA release that persisted over a period of at least 40 seconds. The significant increase over baseline was immediately apparent upon DBS onset and sustained over a longer period. This may suggest that the

![Image of graph showing transient amplitude and frequency](image.png)

**Figure 4** Continuous DBS does not affect striatal DA dynamics. MFB/VTA DBS does not affect amplitude (A) or frequency (B) of spontaneously occurring DA transients. Grey bars indicate group average, colored dots correspond to individual animals. C. Unexpected food-reward presentation induces DA release in the ventromedial striatum. DBS does not affect the DA response to unexpected food reward. Black line – control period (NO DBS), Red line – DBS, lines reflect mean, shaded region reflects SEM. D. Quantification of peak DA response to reward delivery. DBS does not influence peak DA response to reward delivery. Grey bars indicate group average, each colored dot corresponds to an individual animal.
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The effect of DBS onset may not be limited to a single evoked release event immediately upon onset, but could persist over a period that extends beyond the measured time bins. Because of the differential nature of the technique (background subtraction; FSCV applicable to measure deviations from baseline concentration, not the baseline concentration itself), FSCV is not well suited to track changes in neurotransmitter concentration over prolonged time period (minutes/hours) (Robinson et al., 2003). Thus, in the current experiment, we were not able to determine if the initial increase in DA concentration to onset of stimulation would be sustained throughout the entire period that DBS was applied for. In contrast, we did not see an immediate effect on DA release when DBS was switched off. This might indicate that the DA elevation induced by DBS onset is not a continuous elevation, but that DA levels gradually return to baseline while stimulation is still running. Alternatively, if DA would be chronically elevated during DBS, this DA elevation may persist for some time even when stimulation has ended. Experimental techniques that can measure neurotransmitter fluctuations over minutes rather than subsecond fluctuations (microdialysis or fast-scan adsorption voltammetry, (Atcherley et al., 2015)) would be better suited to monitor such long-term changes and to observe change in baseline concentrations. Previous studies also showed acute effects of DBS in different target regions on neurotransmitter release (Hamani et al., 2010; van Dijk et al., 2012; Paek et al., 2013). Interestingly, clinical studies report acute effects of DBS onset on and mood anxiety (Denys et al., 2010; Greenberg et al., 2010; Malone, Jr. et al., 2009; Mayberg et al., 2005; Schlaepfer et al., 2014) that occur within seconds to minutes after onset of stimulation (Denys et al., 2010; Greenberg et al., 2010). Thus, one possibility is that acute elevation of neurotransmitters following DBS onset contributes to these acute effects of DBS onset on mood and anxiety reported in the clinic.

The effects of short bouts of electrical stimulation of the MFB on striatal DA release have been studied extensively, for example in intracranial self stimulation (ICSS) experiments or to study striatal DA overflow. Indeed, this work has shown that stimulation with various parameters induces DA release in the striatum (Gratton et al., 1988; Nakahara et al., 1989; Young and Michael, 1993; Owesson-White et al., 2008; Hernandez et al., 2006; Miliaressis et al., 1991). There is, however, quite some variation in the specific stimulus parameters used. Voltammetry experiments generally use isolated stimulus trains with stimulus parameters (50/60 Hz frequency, 2 msec pulse width) that are optimized to induce DA release, rather than DBS parameters (120/130 Hz frequency, ~0.1 msec pulse width) (Garris et al., 1997; Stamford et al., 1987). Stimulus parameters in ICSS experiments also vary, but stimulations with parameters similar to those used in DBS (high frequency, small pulse width) can successfully sustain administration behavior and induce striatal DA release (Beninger et al., 1977; Gratton et al., 1988; Miliaressis et al., 1991; Hernandez et al., 2006; Cossette et al., 2016; You et al., 2001). However, the most fundamental differences between stimulation in ICSS and DBS experiments are that ICSS is self-initiated and consists of a relatively short sequence of stimulation pulses (stimulus trains). In contrast, the onset of DBS is not determined by the subject and the stimulation is applied continuously after onset of stimulation. Thus, although it was known that brief stimulus trains with DBS-like parameters can induce striatal DA release, the effect of continuous stimulation with clinically relevant stimulation parameters on DA release dynamics in the striatum has not been described before. The only other studies that report effects of continuous high-frequency stimulation in the MFB on striatal DA release were carried out under full anesthesia (Bregman et al., 2015; Paek et al., 2013), which can affect both basal and induced DA release parameters (Stahle et al., 1990; Adachi et al., 2005; Adachi et al.,
DBS of the medial forebrain bundle evokes striatal DA release. Furthermore, using an anesthetized preparation to study effects on DA release with FSCV is limited to studying the effect of evoked DA release rather than spontaneous or stimulus-induced DA transients. Importantly, here we show that onset of MFB DBS with clinically relevant stimulation parameters induces DA release in the striatum in awake, freely moving animals. It remains to be seen if this evoked release sustains over a longer time period, or slowly reduces back to baseline even when stimulation continues.

With the stimulus parameters commonly used for DBS (high frequency, low pulse width, and a relatively large electrode surface), direct activation of DA axons is unlikely (Yeomans et al., 1988; Yeomans, 1989). Although DA axons are capable of following high-frequency stimulation for a limited time period (1-3 sec), sustained stimulation at high frequencies is not accompanied by steady DA release, probably due to adjustment of DA neurons (Stamford et al., 1987). This suggests that although the DBS onset effect on striatal DA release may result from a brief activation of DA neurons, any sustained effect on DA release may be indirect by recruitment of one of the many fibers present in the MFB (Nieuwenhuys et al., 1982; Veening et al., 1982). This has also been suggested by Schlaepfer et al (Schlaepfer et al., 2013; Schlaepfer et al., 2014), who hypothesized that MFB DBS recruits glutamatergic fibers that originate in the PFC and project to DA neurons in the VTA, thereby indirectly influencing the DA system. MFB DBS affects activity in prefrontic regions (Bregman et al., 2015) and it was recently shown that selective activation of prefrontal terminals in the VTA can induce striatal DA release (Kim et al., 2015). However, it is still unknown whether modulation of PFC activity during MFB DBS is indeed responsible for the increase in DA release to DBS onset.

Continuous DBS does not affect spontaneous or reward-induced DA release

Apart from a brief effect on baseline concentration, DBS might influence other DA release parameters such as spontaneously occurring transients or DA release that is associated with reward processing. A brief increase in the firing rate of DA neurons (burst firing) results in a short lasting increase in the extracellular DA concentration in projection regions (DA transient). These DA transients can occur spontaneously (or at least in the absence of any discernable external or environmental factor), or can be evoked by external salient events, such as the presentation of an unexpected food reward or alerting stimulus (Cheer et al., 2004; Robinson et al., 2002). We did not observe any changes in the number or amplitude of spontaneously occurring transients: the frequency or amplitude of these transients did not change either in the seconds/minutes that immediately followed DBS onset or over a longer time period (30 minutes – 10 minute transient recordings and 20 minutes in between unexpected rewards). These findings suggest that although striatal DA release can be induced by the onset of MFB DBS, DA release dynamics are not altered by continuous stimulation. This also suggests that the longer term striatal DA release we observed following onset of stimulation cannot be explained by an increase in the frequency of DA transients and that onset of DBS may increase basal DA levels in the striatum, without influencing the occurrence of spontaneous release events.

MDD patients show altered motivational responses to reward and punishment and show impairments in reward-related learning. These deficits are associated with aberrant activity in cortical-striatal circuits that may result from reduced DA transmission in these circuits (Kumar et al., 2008; Gradin et al., 2011; Vrieze et al., 2013; Pizzagalli et al., 2009). The MFB contains DAergic fibers originating in the VTA, an important structure for reward processing. Therefore, modulation of MFB activity during DBS might influence sensitivity to rewards or reward-related learning in MDD patients by modulating reward-related DA release. Apart from
the spontaneously occurring transients, we therefore specifically looked at DA transients that were induced by presentation of unexpected food rewards. We did not see an effect of MFB DBS in the latency to pick up the food rewards from the food receptacle. In addition, MFB DBS did not change phasic DA release following presentation of an unexpected food reward. A previous study also failed to find an effect of MFB DBS on reward-seeking behavior in a self-stimulation paradigm (Edemann-Callesen et al., 2015). Together, these findings suggest that sensitivity to rewards is not altered by MFB DBS.

The ability to study the effects of DBS on DA release is not just relevant for the mechanisms of action of DBS in depression. Obsessive-compulsive disorder (OCD) is also associated with dysfunctions of the DA system (Perani et al., 2008; Denys et al., 2004a; Denys et al., 2004b; Denys et al., 2013; Schneier et al., 2008). Moreover, OCD patients also show altered activity in the ventral striatum during reward-related learning (Figue et al., 2011; Jung et al., 2011; Marsh et al., 2015), which may be an indirect indication of altered DA reward-processing. A recent study reported changes in D2/3 receptor binding potential following DBS in the nucleus accumbens/anterior limb of the internal capsule, suggesting that DBS may increase striatal DA (Figue et al., 2014). The combination of techniques as we described here can be used to directly study the effects of DBS in different target regions on DA release may provide insight into potential different physiological effects of stimulation between regions relevant for treatment of both MDD and OCD. Future studies may use the combination of FSCV and DBS during reward-learning paradigms in freely moving animals to further investigate whether DBS can influence DA responses during reward-related learning.

Limitations
There are some limitations to our study. Whereas patient and preclinical studies report antidepressant effects with bilateral stimulation in the MFB, we studied the effects of unilateral stimulation. Coenen et al (2013) report that bilateral stimulation is essential for an antidepressant effect, after observing a lack of antidepressant effect following a unilateral lesion to the MFB. However, unilateral stimulation in an otherwise intact circuit most likely differs from unilateral stimulation in a system that is unilaterally damaged. Moreover, clinical and preclinical studies have shown antidepressant efficacy, changes in neurotransmitter release and altered network activity in cortical-striatal circuits following unilateral DBS in the nucleus accumbens and lateral habenula (McCracken and Grace, 2007; McCracken and Grace, 2009; Friedman et al., 2009; Meng et al., 2011; Falowski et al., 2011; Schmuckermair et al., 2013; Huff et al., 2010).

A second caveat is the use of healthy animals in the current experiment. Stimulation of a compromised system may induce different results that stimulation in a healthy subject. However, it is important to realize that the relation between clinical symptoms of MDD and their underlying neurobiological disturbances is not completely understood (Anderson et al., 2012; Lujan et al., 2008), which makes it difficult to model such symptoms in animals. Therefore investigating the effects of DBS in healthy rats is required as well, as it may provide insight into physiological and cognitive effects of DBS and can hint towards possible side effects of stimulation in new target areas (Feenstra and Denys, 2012). This study provides a first step in the characterization of DBS effects on DA release in freely moving animals. Future studies may use this combination of techniques to investigate the effects of DBS in different target regions on phasic DA release as well as combine DA measurements with DBS in animal models of depression.
CONCLUSION

Here we demonstrate the feasibility of combining DBS with measurements of phasic DA release awake, freely moving animals. Importantly, the stimulation parameters that were used in the current experiment (high frequency, low pulse width) are in line with DBS parameters that have been shown to successfully reduce depressive symptoms in clinical (Schlaepfer et al., 2013) and preclinical studies (Bregman et al., 2015; Edemann-Callesen et al., 2015; Furlanetti et al., 2015b; Furlanetti et al., 2015a; Furlanetti et al., 2016). Patients with MDD show dysregulation of DAergic pathways and impairments in reward-related learning (Dunlop and Nemeroff, 2007; Gradin et al., 2011; Kapur and Mann, 1992; Kumar et al., 2008; Pizzagalli et al., 2009; Russo and Nestler, 2013; Vrieze et al., 2013). Here we show that MFB/VTA DBS with clinically relevant stimulation parameters does not influence reward-induced DA release, but that DBS onset can elevate striatal dopamine concentration.

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Deep brain stimulation in the lateral orbitofrontal cortex impairs spatial reversal learning

Marianne Klanker, Ger Post, Ruud Joosten, Matthijs Feenstra, Damiaan Denys

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ABSTRACT

Deep Brain Stimulation (DBS) is a successful novel treatment for treatment-resistant obsessive-compulsive disorder and is currently under investigation for addiction and eating disorders. Clinical and preclinical studies have shown functional changes in the orbitofrontal cortex (OFC) following DBS in the ventral capsule/ventral striatum. These findings suggest that DBS can affect neural activity in distant regions that are connected to the site of electrode implantation. However, the behavioral consequences of direct OFC stimulation are not known. Here, we studied the effects of direct stimulation in the lateral OFC on spatial discrimination and reversal learning in rats. Rats were implanted with stimulating electrodes and were trained on a spatial discrimination and reversal learning task. DBS in the OFC did not affect acquisition of a spatial discrimination. Stimulated animals made more incorrect responses during the first reversal. Acquisition of the second reversal was not affected. These results suggest that DBS may inhibit activity in the OFC, or may disrupt output of the OFC to other cortical or subcortical areas, resulting in perseverative behavior or an inability to adapt behavior to altered response-reward contingencies.
INTRODUCTION

Deep Brain Stimulation (DBS) is a novel treatment for treatment-resistant obsessive-compulsive disorder (OCD) (Denys et al., 2010; Greenberg et al., 2010; Nuttin et al., 1999) and is under investigation for intractable addiction and eating disorders (Luigjes et al., 2012). Despite the apparent success of DBS in reducing clinical symptoms, the mechanism by which DBS reduces symptoms remains elusive. In addition, it is not clear which target area can best be stimulated to result in optimal clinical effects. Preclinical animal studies have been initiated to answer these questions. For example, preclinical studies have shown that DBS in several target regions can influence behavioral processes that are altered in OCD and other compulsive disorders. Compulsive behavior is reduced by DBS in the subthalamic nucleus (Klavir et al., 2009; Winter et al., 2008), nucleus accumbens core and shell (van Kuyck et al., 2008; Mundt et al., 2009), globus pallidus and entopeduncular nucleus (Klavir et al., 2011). In addition, DBS in the ventral striatum influences anxiety (Rodriguez-Romaguera et al., 2012), impulsivity (Sesia et al., 2008) and drug taking behavior (Vassoler et al., 2008) and can evoke changes in cognitive functioning (Baunez et al., 2007). Effects of DBS on cognition are important to study; not only because DBS in psychiatry intends to induce changes in cognitive functioning that may be impaired in the disorder, but also to investigate possible cognitive side effects of stimulation.

A possible mechanisms through which DBS in commonly used targets for compulsivity disorders could be successful, is by modulation of activity in the frontostriatal circuit, a network connecting prefrontal regions with the thalamus and striatum. Modulation of activity in the orbitofrontal cortex (OFC) could be particularly important. OCD patients show hyperactivity of the OFC in rest and during symptom provocation, which normalizes following successful pharmacological or behavioral therapy (Nakao et al., 2005; Saxena et al., 1999). Functional changes in the OFC of OCD patients have also been observed following DBS in the ventral capsule/ventral striatum (Rauch et al., 2006) and subthalamic nucleus (Le Jeune et al., 2010). Similarly, in rodent studies, DBS in the core of the nucleus accumbens induced altered neuronal activity in OFC neurons (McCracken and Grace, 2007; McCracken and Grace, 2009) and affects monoamine release in prefrontal areas (van Dijk et al., 2012). These findings suggest that modulation of OFC activity may be required for successful treatment of compulsive symptoms. It would therefore be interesting to investigate whether stimulation directly in the OFC can also modulate activity in the frontostriatal circuit and whether it can affect cognitive functioning. However, the effects of manipulating OFC activity on compulsive behavior are not well studied. Preliminary data suggests that DBS in the OFC can reduce compulsive behavior in mice (de Haas et al., 2012), whereas lesioning the OFC exacerbates ‘compulsive’ lever pressing in a rat model of OCD (Joel et al., 2005). The effects of manipulating OFC activity on cognitive functioning (e.g. reversal learning) have been studied extensively in many mammalian species with the reproducible outcome that lesioning the OFC results in behavioral inflexibility (Boulougouris et al., 2007; Brown and Bowman, 2002; McAlonan and Brown, 2003; Schoenbaum et al., 2002). It is therefore important to investigate the cognitive consequences of stimulation in this area before it can be suggested as a new target for DBS. Here, we used a test that depends on OFC integrity to study the behavioral consequences of direct OFC stimulation. For this, we used reversal learning, a test measuring cognitive flexibility. We implanted rats bilaterally with bipolar stimulation electrodes in the lateral OFC and trained
them in a spatial discrimination and reversal task, in which they were presented with two reversals during which response-reward contingencies reversed (van der Meulen et al., 2007).

**MATERIALS AND METHODS**

All experiments were approved by the Animal Experimentation Committee of the Royal Netherlands Academy of Arts and Sciences and were carried out in agreement with Dutch laws (Wet op de Dierproeven, 1996) and European regulations (Guideline 86/609/EEC).

**Animals**

Fifteen male Wistar rats (weighing 290g – 340g at time of surgery, Harlan, Horst, The Netherlands) were socially housed in groups of 2-4 in a controlled environment under a reversed day-night schedule (white lights on from 7 p.m. to 7 a.m.) throughout the experiment. Before surgery, rats had unlimited access to food and water. After surgery and throughout the experiment, rats were food-restricted (16 grams/animal/day), maintaining 90% of free-feeding body weight.

**Surgery**

Rats were anaesthetized with intramuscular Hypnorm (0.22mg/kg fentanyl citrate and 7 mg/kg fluanison, VetaPharma) and subcutaneous Dormic (1.5 mg/kg midazolam, Roche) and placed in a stereotactic frame. During surgery, body temperature was maintained by a temperature controller and a heating pad. Bipolar stimulation electrodes consisted of two twisted platinum/iridium wires (wire diameter 60 µm) with one pole 500 µm ventral to the other. Stimulation electrodes were implanted bilaterally in the lateral orbital prefrontal cortex (anterioposterior +3.0 mm, lateral ±3.5 mm, ventral –5.4 mm relative to bregma (Paxinos and Watson, 2007)). Electrodes were fixed to the skull with screws and dental cement. For post-operative pain reduction, subcutaneous Finadyne (5 mg/kg flunixine, Schering-Plough) was given.

**Apparatus**

Operant chambers (Med Associates, St. Albans, VT, USA) were equipped with two retractable levers placed left and right from a food dispenser. Cue lights were positioned above both levers and a house light was located on the opposite wall of the operant chamber. Nosepokes in the food dispenser were recorded by an infrared sensor. Sucrose pellet (Dustless precision pellets®, 45 mg, Bio-Serv) delivery in the food dispenser was signaled by illumination of a light in the food tray that switched off when the first nosepoke after pellet delivery was made. The operant chambers were connected to a Med-PC interface and controlled by Med-PC software.

**Experimental procedure**

Approximately 1.5 weeks after surgery, rats were transported to the experimental room in their homecages. During the experiment, rats were attached to a cable connected to an isolator (DLS 100, World Precision Instruments, Sarasota, FL, USA) and stimulator (DS 8000, World Precision Instruments, Sarasota, FL, USA) and placed in the operant chamber. The cable was attached to a commutator (4 channel commutator, Plastics One, Roanoke, VA, USA) to allow free movement within the operant chamber. Experimental rats (n=8) received deep brain stimulation (120 Hz, biphasic, 200 µA, pulse width 0.08 msec) 10 minutes prior...
DBS in the lateral orbitofrontal cortex impairs reversal learning and throughout all the behavioral sessions. Based on experimental data by McCreery et al (McCreery et al., 1990), Shannon et al (Shannon, 1992) proposed a model to determine safety limits for electrical stimulation: log (D) = k – log(Q), where D is the charge density per phase (uC/cm²/phase) and Q is the charge (uC/phase). For our electrode and stimulation settings, the charge density per phase is 565 µC/cm²/phase and the charge per phase is 0.016 µC/phase, resulting in a k-value of 0.96, which is below the k-value of 1.5 that was indicated as safety limit (Shannon, 1992). Control rats (n=7) were attached to the stimulator, but were not stimulated. After finishing the behavioral session, rats were disconnected, placed back in their homecages and returned to the animal facility. Testing took place between 8.30 a.m. and 5 p.m. After the last behavioral session, a 100 µA direct current was passed through the electrodes to mark the final placement of the electrodes. Rats were deeply anesthetized by inhalation of a CO2/O2 mixture and decapitated. Brains were removed, cut on a cryostat and stained with cresyl violet for verification of electrode placement.

Behavioral training
During shaping sessions, rats were trained to press a lever for a food reward. Rats were randomly presented with the right or left cue light and corresponding lever. After a lever press, the lever was retracted, the cue light switched off and a sucrose pellet was delivered in the food dispenser. A successful response was followed by a variable inter-trial interval (10/20/30 seconds). In case of an omission, cue and lever presentation ended after 60 seconds and was followed by a 10 second inter-trial interval. Shaping sessions consisted of 32 trials. Rats received up to three shaping sessions per day with an inter-session interval of 2-3 hours. When responding to cue and lever was stable (>90% correct responses in a session) rats received one session during which response-reward contingencies changed from an FR1 to an FR3 ratio, so that three lever presses had to be made on the rewarded side in order to obtain a sucrose pellet (lever presses did not have to be made consecutively), three lever presses to the unrewarded side was classified as an incorrect response. After that, rats progressed to 5 spatial discrimination learning sessions. During discrimination sessions, rats were presented with left and right cue lights and levers simultaneously. Only responding to the lever on one side was rewarded, whereas responding to the other lever was not rewarded. The rewarded side was counterbalanced between rats. Discrimination sessions consisted of 64 trials with a variable inter-trial interval (15/25/35/45 seconds). Rats received one session per day. The day following the last discrimination session, rats were presented a reversal session. During the reversal session, response-reward contingencies were changed, so that a response to the previously unrewarded lever was now rewarded and vice versa. Reversal sessions consisted of 64 trials. Additional sessions with the reversed response-reward contingency (retention sessions) were given until rats reached a 90% correct response criterion. Retention sessions were given on the same day as the first reversal session, with an interval of at least 2 hours between behavioral sessions. If rats needed more than 2 retention sessions to reach criterion, these sessions were presented on the next day. After reaching criterion, rats were presented with a second reversal on the following day.

Data Analysis
All values are mean ± SEM. The total number of correct responses (3 lever presses on the rewarded lever) was analyzed for five sessions of discrimination learning. For reversal sessions, the 64 trial session was divided into 8 blocks of 8 trials, to see changes in performance
throughout the behavioral sessions. We analyzed the number of correct responses per session. Incorrect responses made after the reversal were labeled as perseverative or learning errors according to criteria described in Boulougouris and Robbins (Boulougouris and Robbins, 2010). Errors were scored as perseverative errors if six or more errors were made consecutively; errors were scored as learning errors if less than six errors were made consecutively (see supplementary information in Boulougouris and Robbins (Boulougouris and Robbins, 2010)). We compared the number of perseverative and learning errors made by stimulated and control rats. All statistical analyses were performed using PASW statistics 18. A repeated measures ANOVA was used. When a group or interaction effect was found, an independent t-test was used to test for group differences. Statistical significance was set to p ≤ 0.05. Huynfeldt corrections were applied when assumptions of sphericity were violated.

RESULTS

Histology
After histological verification, two animals were excluded from data analysis. Figure 1 shows the placement of the electrode tips in experimental animals that were included in data analysis. Final group sizes were n=7 for the control group and n=6 for the stimulated group.

Discrimination Learning
Figure 2A shows the performance during five sessions of discrimination learning for stimulated and control rats. A main effect of time was found (F(1.837, 20.205)=137.691, p<0.001) indicating that learning took place over sessions. No significant interaction or group effects were observed, suggesting acquisition of a spatial discrimination was not influenced by DBS in the lateral OFC. Response latencies to the lever were not different between groups (data not shown).

Reversal Learning
Performance during first and second reversal is shown in Figure 2B. All rats needed at least one retention session after the 64 trial reversal sessions to reach 90% correct response criterion. If rats did not reach the 90% correct response criterion in the first retention session, they were given additional retention sessions until criterion was reached. Figure 2B only shows the first retention sessions following reversal, in which all rats participated. Following the first reversal, 5 (of 7) control
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Rats and all (n=6) stimulated rats needed more than one retention session. Following the second reversal, 3 (of 7) control rats and 1 (of 6) stimulated rats needed more than one retention session. Response latencies to the lever were not different between groups (data not shown). For the number of correct responses during the first reversal session, a repeated measures ANOVA showed a main effect for block (F(7,77)=18.851, p<0.001, a significant block*DBS interaction (F(7,77)=4.411, p<0.001 and a between subjects effect for DBS (F(1,11)=8.442, p=0.014. An independent t-test showed blocks 3, 6 and 8 different between groups, (t(11)=2.732, p=0.019), (t(11)=4.211, p=0.001) and (t(11)=2.487, p=0.030) respectively. A trend was found for block 5 (t(11)=2.111, p=0.058). For other reversal sessions, no significant differences were found.

We then analyzed the type of errors made by stimulated and control animals (see Figure 3). For perseverative errors, a repeated measures ANOVA showed a main effect for session for the first reversal (F(1,11)=56.482, p<0.001) and for group (F(1,11)=5.453, p=0.040). An independent t-test showed that the DBS group made more perseverative errors in the first reversal session (t(11)=-3.784, p=0.003) but not in the retention session following the first reversal. The amount of perseverative errors made after the second reversal and the amount of learning errors made after the first and second reversal did not differ between groups.

A

B

Figure 2 A. Performance during five sessions of discrimination learning. Bars indicate number of correct responses. Black bars indicate control animals (n=7), grey bars indicate animals stimulated in the OFC (n=6). DBS in the OFC did not affect acquisition of spatial discrimination learning. Data are represented as mean ± SEM. B. Number of correct responses during reversal learning. The 64 trial reversal sessions were divided into 8 blocks of 8 trials to see performance during reversal sessions. Control animals (n=7) are shown in black, stimulated animals (n=6) in grey. Data are represented as mean ± SEM. R1.1 – first reversal, R1.2 – retention session after first reversal, R2.1 – second reversal, R2.2 – retention session after second reversal. Only retention sessions in which all rats participated are shown. Asterisks indicate blocks where stimulated and control rats show a significant difference (p<0.05) in the number of correct responses.
Here, we report that DBS of the lateral OFC impairs cognitive flexibility in a spatial reversal-learning paradigm. In the current study, OFC stimulation did not affect acquisition of spatial discrimination learning, but during reversal learning, stimulated animals made an increased number of errors. They did not use negative feedback to switch responding to the newly rewarded side, but kept lever pressing on the side that was rewarded before the reversal. The increased number of errors made during reversal learning and the persistent response to the non-rewarding lever can be regarded as an expression of behavioral inflexibility. The behavior in itself (the lever press) is appropriate, but the changing task demands have rendered the behavior inappropriate or disadvantageous in the current situation (after the reversal), as the animal will obtain fewer rewards if it does not switch its behavior. The perseverative responding to the previously rewarded lever occurred during the first reversal that was presented, but not during a second reversal. DBS in the OFC did not affect response latencies during discrimination or reversal sessions.

Behavioral inflexibility has been described in rodents as well as in human and non-human primates with permanent OFC damage (Boullougouris et al., 2007; Dias et al., 1996; Hornak et al., 2004). In addition, a dissociation between involvement of the OFC in reversal learning and the medial prefrontal cortex in extra dimensional set-shifting is observed across species (Brown and Bowman, 2002), making reversal learning a valid translational paradigm. Our findings may suggest that direct stimulation in the OFC can influence functionality of the OFC, resulting in perseverative responding during reversal learning. The observed impairment during reversal learning in animals receiving high-frequency stimulation in the OFC resembles deficits in reversal learning described following lesions in the OFC. Permanent lesions in the OFC result in impairments in the ability to disengage from a previously reward-
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The response after altered response-reward contingencies during initial, but not subsequent reversals (Boulougouris et al., 2007; Dias et al., 1997; Schoenbaum et al., 2002), similar to our present results. These findings suggest that the OFC may be particularly important during initial stages of reversal learning, when behavior has to be adjusted to novel task demands. As later stages of reversal are not affected by OFC damage or stimulation in the OFC, other brain areas may be more important during subsequent reversals (Dias et al., 1997). This is supported by the observation that OFC lesions made after experience with reversal learning do not impair behavior, whereas OFC lesions made before reversal learning induce perseverative responding (Boulougouris et al., 2007; Boulougouris and Robbins, 2009). In this experiment, we stimulated throughout all stages of behavioral training. The current stimulation protocol shows that the behavioral deficit observed after stimulation is specific for the adaptation of behavior following a reversal and does not affect learning of a spatial discrimination. Moreover, the similar response latencies in stimulated and control animals suggest that motor responses to obtain the reward are not influenced by OFC stimulation.

In reversal learning, the response to the previously rewarded lever has to be withheld, while a response to the previously non-rewarded lever must be initiated. A defect in the former process is associated with perseverative errors, while learning errors signal a defect in the latter process (Tait and Brown, 2007). Perseverative responding to the previously rewarded lever during reversal learning after OFC stimulation or permanent lesions to the OFC is not likely to be the result of impaired response inhibition, considering the intact ability to withhold responding to a non-rewarded lever during spatial discrimination learning. Also, devaluation studies have demonstrated intact response inhibition in OFC-lesioned animals when the outcome that could be obtained by a lever press was devalued beforehand (Ostlund and Balleine, 2007). Behavioral inflexibility following permanent damage to the OFC or decreased OFC functionality may be the result of impaired control of the OFC over subcortical regions, such as the basolateral amygdala and striatum (Stalnaker et al., 2007; Schoenbaum et al., 2009). Although we cannot draw any conclusions on the effects of DBS in the OFC on neuronal activity from the current study, the similarity of behavioral impairments observed after stimulation compared to lesions suggests that high frequency DBS in the lateral OFC either reduces activity in this area, or disrupts output of the OFC to other cortical or subcortical areas, thereby impairing communication in OFC circuitry and inducing behavioral inflexibility.

The clinical efficacy of DBS in a target area does not necessarily result from direct changes in activity in the structure where the electrode is implanted. Rather, the therapeutic effect of DBS may be due to axonal stimulation resulting in alterations throughout a functional network (Deniau et al., 2010; McIntyre and Hahn, 2010). Stimulation in different nodes within a functional circuit apparently results in similar clinical efficacy by affecting the same projection fibers (Lehman et al., 2011). OFC projections could be hypothesized as a target area for DBS electrode implantation, provided stimulation of this area results in normalization of OFC hyperactivity without adverse effects. However, direct stimulation of the OFC increased the number of perseverative errors during reversal learning, suggesting that behavioral inflexibility transiently increases instead of decreases after OFC stimulation. The present results suggest that caution should be exercised before applying high frequency stimulation of the OFC in disorders with behavioral flexibility as a core symptom and that cognitive side effect should be monitored closely. It is relevant to note that it is not known yet if the DBS-induced
inflexibility is sustained upon continuous stimulation. Whether low frequency stimulation in the OFC may have opposite behavioral effects, resulting in increased cognitive flexibility remains to be seen.

There are some limitations to our study. First, it was performed in healthy rats that do not show a hyperactive OFC or alterations in frontostriatal circuitry. We do not know whether OFC stimulation would normalize OFC hyperactivity in resting state and in which way impaired functional activity during cognitive performance may be affected by DBS. This would require the use of animal models that show prefrontal hyperactivity. However, we are not aware of a currently available animal model of compulsions for which OFC hyperactivity has been directly demonstrated. However, the use of healthy animals does permit to study the effects of DBS in new target areas on physiological functioning and normal behavior. Another limitation in the present study is the duration of stimulation in this experiment. DBS started 10 minutes before and lasted throughout a behavioral session, but animals were not chronically stimulated in between behavioral sessions. Acute effects observed after the start of stimulation may differ from effects following long-term stimulation. Although we observed DBS-induced inflexibility in the first reversal presented, it is not known yet if continuous stimulation has a similar effect.

CONCLUSION

This study shows that DBS in the lateral OFC selectively impairs performance on an initial reversal in a spatial reversal learning paradigm. Stimulated rats continue responding to the previously rewarded lever. This perseveration is short lasting and discrimination learning is not affected. These findings demonstrate that during a cognitive task depending on intact frontostriatal circuitry, DBS in the OFC resulted in behavioral deficits that parallel deficits observed after loss of function in the OFC.
DBS in the lateral orbitofrontal cortex impairs reversal learning

REFERENCE LIST


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DBS in the lateral orbitofrontal cortex impairs reversal learning
Summary and general discussion
SUMMARY

Dopaminergic control of cognitive flexibility

Chapter 2 provides a general overview of the involvement of the dopamine (DA) system in cognitive flexibility. This chapter summarizes how the DAergic system may contribute to the regulation of cognitive flexibility in both humans and animals. A range of genetic and pharmacological studies in humans and animals indeed provide evidence for a role of DA in the control of different facets of cognitive flexibility, including reversal learning. These studies suggest that behavioral adaptation is facilitated by deactivation of striatal D2 receptors and that acquisition of the ‘new’ behavioral response is facilitated by striatal D1 activation. What also becomes apparent, however, is that although DA facilitates adaptation of behavior, it may not be essential. The ability to successfully shift behavior following changes in reinforcer contingencies is impaired, but not completely absent following manipulations to the DA system.

Although patients with obsessive-compulsive disorder (OCD) do not always show impaired performance (accuracy) on cognitive flexibility tasks, they show altered activity patterns during task execution. Altered DA signaling is a potential contributor to changes in corticostriatal activity during cognitive processing in OCD patients.

In Chapter 3, we described how DA signaling in the ventromedial striatum (VMS), a striatal subregion that receives projections from limbic brain regions, changes when animals adapt their behavior in a reversal learning task. Successful adaptation of behavior following reversal requires the ability to implement a change in behavioral strategy based on reinforcing feedback. We hypothesized that DA would be involved in the rapid updating of response-reward information essential for successful reversal learning. We showed that during the modification of an established response preference, DA signaling in the VMS rapidly adapts to reversed response-reward contingencies. Phasic DA selectively reflected the receipt of positive, but not negative feedback.

In addition, we showed that individual differences in the performance of task execution could be related to differences in striatal DA signaling: Animals that reversed their responding faster showed an effect of positive feedback on cue-evoked DA release, whereas this was absent in animals that did not learn to reverse their behavior. This DA signal following positive feedback may support the stabilization of adaptive behavior. Overall, these findings suggest that DA is a neural correlate that facilitates the adaptation of established behavior.

In Chapter 4 we extended these findings by reporting DA changes in reversal in a different striatal region: The dorsolateral striatum, a striatal subregion that receives projections from sensorimotor brain regions. Together with our previous results obtained in the VMS (Chapter 3), these findings reveal distinct phasic DA release patterns during adaptation of established behavior in these striatal subregions. Whereas the VMS DA signal rapidly adapted to a reversal of response-reward contingencies, DLS DA release patterns did not, but instead remained stable. The receipt of positive feedback was reflected in the VMS DA signal, whereas trial outcome was not reflected in the DLS DA signal. These findings suggest that during behavioral adaptation in reversal learning, VMS DA may facilitate behavioral adaptation, whereas DLS DA reflects a action signal independent of outcome. This DLS signal, may enable initiation and execution of previously learned operant responses or the motivation to perform them.
Effects of deep brain stimulation on dopamine release and cognition

Chapter 5 reports for the first time the feasibility of combining deep brain stimulation (DBS) with measurements of phasic DA release in awake, freely moving rodents. We described the effect of DBS of in the medial forebrain bundle/ventral tegmental area, a target area for DBS in depression, on DA release in the VMS. Onset of deep brain stimulation in this area induced an increase in DA release in the striatum that was sustained for at least 40 seconds. Next to this immediate effect of DBS onset, we looked at the effect of continued stimulation on phasic DA release dynamics. The amplitude and frequency of spontaneous fluctuations in striatal DA concentration (transients) were not influenced by ongoing stimulation. MFB DBS also did not influence reward-evoked DA release. Together, these findings suggest that onset of DBS in the MFB/VTA region with clinically relevant stimulation parameters induces DA release, but that continued stimulation in this region does not modify spontaneous or reward-induced phasic DA dynamics in the VMS of normal rats.

In Chapter 6, we investigated the cognitive effects of DBS in the orbitofrontal cortex (OFC). The orbitofrontal cortex could be hypothesized as a target for DBS, provided stimulation in this area does not induce adverse side effects. Here, we report that DBS of the lateral OFC impairs cognitive flexibility in a spatial reversal learning task. After reversal, stimulated rats showed (transient) perseverative responding to the previously rewarded lever, suggesting that behavioral inflexibility increases after OFC stimulation. These findings showed that DBS in the OFC may induce unwanted side effects in disorders with behavioral inflexibility as a core symptom.
GENERAL DISCUSSION

PART I – Dopaminergic control of cognitive flexibility

Cognitive flexibility is the ability to adapt goal-directed behavior to changes in the environment. This ability is an important component of everyday life and allows us to adapt our behavior when circumstances demand this. This type of behavior requires integrity of corticostriatal circuits that consist of reciprocal connections between cortex, striatum and thalamus. Several neurological and psychiatric disorders are characterized by dysfunctional activity in corticostriatal circuits, resulting in behavioral inflexibility or rigidity (Remijnse et al., 2013; Millan et al., 2012; Cools et al., 2001; Chamberlain et al., 2008). Thus, studying the underlying neurobiological mechanisms of cognitive flexibility will not only increase our understanding of our everyday functioning, but may also provide an indication of the neurobiological changes underlying psychiatric symptoms.

Dopaminergic control of cognitive flexibility

Imaging studies from human subjects and lesioning studies in experimental animals showed the importance of connections between prefrontal regions and the striatum in the regulation of adaptive behavior: Connections between orbitofrontal cortex (OFC) and ventral or medial parts of the striatum are implicated in reversal learning (Ghahremani et al., 2010; Bellebaum et al., 2008; Castane et al., 2010; Clarke et al., 2008; Dias et al., 1996a; McAlonan and Brown, 2003; Divac, 1971; Rogers et al., 2000), whereas the connections between dorsolateral prefrontal regions (or the medial PFC in rodents which is in this task functionally equivalent) and more dorsal striatal areas are important for set- and task switching performance (Dias et al., 1996a; Dias et al., 1996b; Ragozzino, 2007; Graham et al., 2009; Owen et al., 1991; Manes et al., 2002; Sohn et al., 2000; Birrell and Brown, 2000). Because these circuits show consistent similarities between primates and rodents (Mailly et al., 2013), translational research can provide more information about the neurobiological substrates of cognitive flexibility.

Dopamine (DA) is an important neuromodulator that regulates activity in corticostriatal circuits. In Chapter 2 we reviewed the available literature that has investigated the contribution of DA in regulating cognitive flexibility. The combined evidence from human and animal studies suggest that DA is indeed actively involved in the performance of tasks requiring cognitive flexibility: task performance is associated with increased DA release and is impaired by DA depletion. In addition, pharmacological interference with DA transmission can influence task performance and activity in corticostriatal circuits. Similarly, human studies suggest the influence of genes affecting DA function on activity in ventral striatal regions and connectivity between PFC and ventral striatum during reversal learning. Thus, findings from both human and animal studies suggest that DA in ventral regions of the striatum contributes to reversal learning, whereas DA in more dorsal striatal regions may be more important for cognitive flexibility tasks with a higher order complexity, such as attentional set shifting and task switching.

Both DA receptor types (D1 and D2 receptors) contribute and appear to be cooperatively involved in discrimination learning and the flexible adaptation of behavior. Inactivation of D2 receptors may allow switching of behavioral patterns whereas disturbed signaling through D1 receptors may impair cognitive flexibility by reducing the ability to use negative feedback (Frank et al., 2007). Thus, the ability to use negative feedback may support successful adap-
Summary and general discussion

Facilitation of behavioral adaptation by deactivation of striatal D2 receptors and facilitation of the acquisition of the 'new' behavioral response by striatal D1 activation suggest the importance of biphasic fluctuations in striatal DA levels when adapting response patterns.

Although these findings from human and animal studies suggest that DA in striatal regions indeed contributes to the regulation of cognitive flexibility, there are some points requiring further attention:

1. Characterization of phasic DA release patterns during reversal learning

Various manipulations show the involvement of DA in reversal learning, but most use manipulations that affect the DA system are long-term (i.e., will affect fast and slow changes in DA concentration). There have been surprisingly few studies that investigated the release during task execution and it is still an outstanding question how phasic DA changes during behavioral adaptation, e.g. reversal learning. Thus, we used a detection technique with sufficient time resolution to directly monitor fast changes in DA that occur in response to specific events when adapting an established behavioral response. Moreover, this allows direct testing of the hypothesis that biphasic fluctuations in DA might be important during behavioral adaptation (i.e. whether the receipt of positive and negative feedback are indeed reflected in a biphasic DA signal).

2. Differences between striatal regions

It is not well known how DA release in different subregions of the striatum alters during adaptive behavior. Both ventral and dorsal striatal regions have been linked to cognitive flexibility. However, ventromedial and dorsolateral striatum receive input from separate cortical regions (Webster, 1961; McGeorge and Faull, 1989) and these parallel corticostriatal circuits are differently involved in the control of behavior (Voorn et al., 2004; Yin and Knowlton, 2006). Moreover, DA release patterns are not uniformly broadcast throughout the entire striatum, but show regional differences in response to natural rewards and reward-related stimuli (Brown et al., 2011; Cacciapaglia et al., 2012; Shnitko and Robinson, 2015; Willuhn et al., 2012). This may be related to differences in origin, synaptic input and intrinsic properties of the DA neurons (see below). Comparing DA release patterns in the ventromedial and dorsolateral striatum during reversal learning can indicate how DA release in different striatal subregions contributes to adaptive behavior.

3. Relate performance to DA release patterns

Human studies have particularly shown the importance of individual differences in the DA system. Individual differences in DA synthesis capacity influence both task performance and effects of manipulations to the DA system in different types of flexibility. This type of approach can also be used in animal research, for example by investigating if there is a relation between
the amount of DA release or the pattern of DA release and performance of reversal learning. By linking behavioral performance to DA release patterns, we can use variation within a normal population to study neurobiological correlates that contribute to reversal learning.

**Studying the neurobiology underlying cognitive flexibility**

In the first part of this thesis, we initiated two experiments to further unravel the neurobiology underlying cognitive flexibility. In these experiments, we used a spatial reversal learning task designed to measure cognitive flexibility (De Bruin et al., 2000). In this task, rats learned that a lever press on one side was always rewarded, whereas a lever press on the other side was never rewarded. The rewarded side was not cued to the animals (cue lights were only presented to indicate trial onset), thus the rats had to use feedback to learn which side was rewarded. After learning this discrimination, a reversal was presented - the previously rewarded side was now unrewarded whereas the previously unrewarded side was rewarded. This reversal occurred within a session, and was not cued. Rats thus had to use the change in reinforcing feedback to adapt their behavior: Use negative feedback to inhibit a previously rewarded response and to use positive feedback to adapt behavior to the newly rewarded side.

As mentioned previously, performance of reversal learning and execution of other motivated behavior relies on integrity of corticostriatal circuits. These circuits consist of converging input from different cortical areas and midbrain DA neurons to projection neurons in the striatum. Although this converging input is a universal organizational principle throughout the striatum, there are some distinctions between striatal subregions. Thus, ventromedial striatum (VMS) and dorsolateral striatum (DLS) receive input from separate cortical regions and distinct DAergic nuclei. Whereas the VMS receives DAergic input from the ventral tegmental area (VTA), projections to the DLS originate in the substantia nigra (SN) (Bjorklund and Dunnett, 2007; Beckstead et al., 1979). These DA cell groups are differently innervated by afferent input from other regions (Watabe-Uchida et al., 2012) and show different intrinsic properties (Lammel et al., 2008), which results in heterogeneous responses to rewards and reward-predicting stimuli (Bromberg-Martin et al., 2010; Lerner et al., 2015). In addition, the projections from cortical regions to the striatum are organized topographically: limbic regions innervate the VMS, whereas sensory and motor regions innervate the DLS (Webster, 1961; McGeorge and Faull, 1989). These different innervation patterns, as well as other local differences (see Chapter 4) can induce different DA release patterns between striatal regions during the execution of goal-directed behavior (Brown et al., 2011; Cacciapaglia et al., 2012; Shnitko and Robinson, 2015; Willuhn et al., 2012). Thus, to compare DA release patterns in different striatal subregions, we performed DA measurements in both VMS and DLS.

**Ventromedial striatum**

Chapter 3 describes phasic DA release patterns in the VMS during reversal learning. The VMS thought to be involved in motivation and reinforcement for natural rewards (Kelley, 2004). Receiving input from various brain areas implied in cognition, memory, emotional behavior and learning (i.e. prefrontal cortex, hippocampus, amygdala and mediodorsal thalamus) (Kelley, 2004), this area seems well positioned to function as a relay station where input from different modalities (cognitive, affective and sensory) is put into behavioral actions (Mogenson et al., 1980).
Phasic DA patterns in the VMS rapidly reflected a reversal of response-reward contingencies. During successful responding in the discrimination phase (before reversal), cue presentation (indicating trial onset) induced phasic DA release, whereas reward delivery did not. Thus, VMS DA did not reflect the receipt of positive or negative feedback (i.e. presence or absence of a sucrose pellet). Moreover, the receipt of positive or negative feedback did not influence cue-evoked DA signaling on the trial that followed, suggesting that when performing a well learned discrimination (i.e. an established behavioral response pattern), the cue-evoked DA signal might induce incentive motivation and promote behavioral actions irrespective of trial outcome (Flagel et al., 2011; Wise, 2004; Berridge et al., 2009).

During adaptation of choice behavior following reversal of response-reward contingencies, DA release patterns changed: reward delivery now evoked DA release, whereas cue-induced DA was temporarily lower. During reversal learning, we saw a selective effect of positive, but not negative feedback on DA release. The selective effect of positive feedback on DA release was twofold: 1) direct receipt of positive feedback induced DA release when reward was unexpected, and 2) receipt of positive feedback was immediately reflected in the cue-evoked signal on subsequent trials. In contrast, absence of reward in the initial incorrect trials after reversal (negative feedback) did not induce a decrease in DA signaling and did not affect cue-evoked DA on subsequent trials. Such a decrease was only detected across the entire reversal session, suggesting that this effect develops slowly or that the decrease in DA after reward omission is relatively small, requiring a larger number of trials to be detected. Although pauses in DA neuronal firing rate following omission of expected rewards have been repeatedly reported in rats and primates (Pan et al., 2005; Schultz et al., 1997), reports of corresponding decreases in extracellular DA concentrations have been inconsistent (Owesson-White et al., 2008; Stuber et al., 2005; Sunsay and Rebec, 2014; Hart et al., 2014). Although studies using aversive stimuli suggest that FSCV can report decreases in DA concentration (Roitman et al., 2008; Oleson et al., 2012), this technique may not sensitive enough the record a small decrease in extracellular DA that results from a pause in DA neuron firing on a single trial basis, whereas such a decrease may be sufficient to change receptor binding and have downstream effects. It is also possible that more experience with a non-rewarded outcome is necessary to counteract an already established learned association. A final possibility is that an independent, non DAergic system codes for negative reward prediction errors (Bayer and Glimcher, 2005; Daw et al., 2002).

An interesting finding was that variation in the cue-evoked DA response exclusively following positive feedback predicted individual differences in performance of reversal learning: Animals that were faster to reverse their responding showed an effect of positive feedback on subsequent cue-evoked DA signal, whereas this was absent in animals that were slower to reverse their behavior. Together, this suggests that the modification of established behavior is facilitated by updating cue-evoked DA release as a consequence of positive feedback. Reward-induced DA release following the first couple of responses on the newly rewarded side could drive learning about the newly reinforced response, whereas the subsequent feedback-induced increase in cue-evoked DA may help to sustain motivation to regularly sample and consolidate responding to the newly rewarded side. It is still an open question whether these phasic increases following positive feedback indeed effect behavioral output exclusively through D1 receptor mediated signaling, as proposed by modeling studies (Hong and Hikosaka, 2011; Frank and Claus, 2006); see also Chapter 2).
Dorsolateral striatum

The dorsolateral striatum (DLS) receives input from sensorimotor cortex and has long been associated with stimulus-response learning. The DLS is not just involved in the acquisition and performance of operant actions and habit formation (Faure et al., 2005; Amalric and Koob, 1987; Beninger and Ranaldi, 1993; Robbins et al., 1990; Robinson et al., 2007), but also in cognitive flexibility (Chamberlain et al., 2008; Sawamoto et al., 2008; Clarke et al., 2011; Cools et al., 2001).

In the DLS, DA release was not associated with task-related events, such as a presentation of a cue signaling reward availability. Cue-evoked DA release was small, whereas pronounced DA release was seen around the time of lever press, both on rewarded and non-rewarded trials. This was not an increase associated with motor responses in general, but occurred selectively during a learned operant response initiated to obtain a reward. DA release patterns did not change following a reversal of response-reward contingencies: DA to cue presentation remained small, whereas DA release increased around the time of lever press. Increased release around the time of lever press may enable initiation and execution of an operant response that was previously learned, or provide the motivation to initiate such responses (Howe et al., 2013).

Together with the results from the VMS, these findings suggest that DA may be a neural correlate that facilitates the modification of established behavior. The VMS DA signal, which rapidly adapts to reversal response-reward contingencies and is sensitive to positive feedback, may provide a teaching signal and represent motivational properties of the stimulus to promote reward seeking actions (Flagel et al., 2011; Montague et al., 1996; Schultz et al., 1997; Steinberg et al., 2013; Waelti et al., 2001; Berridge et al., 2009). In contrast, the stability of DLS DA patterns during adaptation of behavior suggests that DLS DA is not consistent with a teaching signal, but instead is associated with the execution of operant responses or the motivation to perform them (Howe et al., 2013), and may encode the value of an operant response (Yin et al., 2008). The observed differences between DLS and VMS DA dynamics during the performance of behavior are consistent with the suggested dissociation between the involvement of sensorimotor circuits (associated with the DLS) and limbic circuits (associated with VMS or medial striatum) in the control of behavior (Voorn et al., 2004; Yin and Knowlton, 2006). The DLS and its associated circuitry are essential to initiate an action that is driven by a reward-related stimulus, whereas limbic circuitry is associated with learning about the relation between expected and received rewards following a certain response and the stimuli predicting reward (Corbit and Janak, 2007; Yin et al., 2008). However, these circuits do not act in complete isolation. Instead, it is thought that limbic circuitry can influence the sensorimotor system through a series of connections between striatal projection neurons and the DA system (Haber et al., 2000; Nauta et al., 1978). This serial connectivity enables the interaction between striatal subregions, and may be the neural substrate that enables the integration of motivation and motor outcomes (Haber et al., 2000).

Future studies

One way to extend the type of experiments described in this thesis would be the use of chronically implanted recording electrodes (Clark et al., 2010). Chronically implanted recording electrodes allow monitoring of the same animal over an extended period of training allowing long term characterization of DA release patterns. This can be used to investigate whether individual differences in DA release during early learning stages (e.g. during lever press training) can be related to performance (and DA release) during later stages (e.g. during multiple reversals). For example, although we did not observe any differences in DA release
between learners and non-learners during performance of a well learned discrimination, we cannot exclude that they may have shown different DA release patterns during initial lever press training. Moreover, long term measurements would allow measurements in multiple striatal subregions in the same animal, to investigate if non-learners also show different release patterns in the DLS.

Another question apparent from our results is – can we improve reversal learning in animals that perform poorly? For example, would an additive positive feedback signal help the non-learners to adapt behavior faster, and similarly, would inhibition of the positive feedback signal impair performance in animals that have learned the reversal? The DA response to positive feedback appeared to be intact in non-learners: reward-induced DA release on the first couple of trial after reversal was similar between learners and non-learners. However, cue-evoked DA signaling on the trial that followed positive feedback was not updated in these animals. Brief electrical stimulation of the VTA induces transient VMS DA elevations and can trigger lever press responding (Phillips et al., 2003), and phasic optogenetic activation of DA neurons can increase approach behavior (Ilango et al., 2014). Thus, perhaps an artificial boost of VMS DA activity during cue presentation may help the non-learners to reverse faster.

Optogenetic studies showed that selective activation of DA neurons (mimicking a positive feedback signal) is sufficient to sustain operant responding (Kim et al., 2012; Witten et al., 2011) and mediates reversal of reward-seeking behavior (Adamantidis et al., 2011). However, in this group, 25% of animals did not reverse responding, even though they appeared to have sampled the newly reinforced side receiving direct activation of DA neurons (Adamantidis et al., 2011). Thus, sufficient experience with the newly rewarded response may be needed to consolidate responding. Moreover, it is possible that although phasic DA release following reward delivery (or induced DA release) is similar between learners and non-learners, the downstream effects of this phasic DA increase to reward differs. A phasic increase of DA in striatal regions is thought to selectively strengthen input of particular cortical or limbic afferents onto striatal medium spiny neurons, supporting the association between specific sensory events and behavioral responses (Kelley et al., 2003; Schultz, 2002) allowing reorganization of response patterns during adaptation of behavior (Goto and Grace, 2005; Kelley et al., 2003; Hong and Hikosaka, 2011). It is therefore possible that even though learners and non-learners show similar DA release to (unexpected) reward delivery in the initial correct trials after reversal presentation, they may different activity in the cortical or limbic afferents to the striatum (see also section PFC-DA interactions below), resulting in different response patterns.

A last line of further research would be the use of cognitive flexibility tasks that reflects a higher order level of processing, such as attentional set shifting or task switching (Monsell, 2003; Sohn et al., 2000; Rogers et al., 2000; Birrell and Brown, 2000). As described in Chapter 2, these paradigms rely on connections between more dorsal striatal regions and dorsolateral prefrontal cortex DA in striatal subregions may be differently involved in the regulation of these types of flexibility. Moreover, OCD patients show impaired performance during task execution in these tasks in combination with altered functional activity in more dorsal striatal regions and associated cortical regions (Remijnse et al., 2013; Gu et al., 2008; Page et al., 2009); see also Chapter 2), thus, studying DA release VMS and DLS in these types of paradigms may provide more information on the neurobiological basis of these impairments. Attentional set-shifting and task switching can be successfully implemented in rodent research (Haluk andFloresco, 2009; Leenaars et al., 2012; Birrell and Brown, 2000). Thus, combining these behavioral paradigms with DA measurements in VMS and DLS, as described for reversal
learning, may enhance understanding of the potential differences in DA regulation in reversal learning versus higher order flexibility tasks.

**PFC-DA interactions and relevance for psychiatric disorders**

Both MDD and OCD are characterized by disturbances in prefrontal cortical areas and connected striatal regions (Mayberg, 1997; Menzies et al., 2008; Harrison et al., 2009). In addition, both disorders may be associated with disturbances in the DA system (Kapur and Mann, 1992; Russo and Nestler, 2013; Denys et al., 2004b), albeit in opposite fashion. Whereas MDD (or anhedonia in MDD) is associated with reduced DA function, OCD has been linked to DA hyperactivity. In addition, both MDD and OCD patients show blunted activity to rewards and during reward anticipation (Greenberg et al., 2015; Figeé et al., 2011; Jung et al., 2011; Marsh et al., 2015; Pizzagalli et al., 2009), suggesting that they also show impairments in phasic DA reward processing. An open question is whether this dysregulation in DAergic function is a primary dysfunction or whether it is secondary to the hyperactivity in cortical regions. Moreover, it is incompletely understood how disturbances in prefrontal activity can influence DA signaling, for example in relation to reward-related learning.

A recent study described that increased excitability of the medial prefrontal cortex (mPFC) reduces striatal BOLD responses to stimulation of DA neurons (Ferenczi et al., 2016). Moreover, this manipulation of mPFC activity (modeling hyperactivity observed in MDD) induced anhedonic behavior and attenuated conditioned place preference that was induced by stimulation of DA neurons. These findings suggest that the mPFC may interact with DA input to the striatum, affecting reward-related behavior (Ferenczi et al., 2016). Moreover, Jo & Mizumori (2015) recently showed that inactivation of mPFC activity not only reduces spontaneous activity of VTA DA neurons, but also enhances phasic DA activity to reward-related cues. Extending these types of studies that combine manipulations of mPFC activity with measurements of either DA neuronal activity or phasic DA release patterns, may provide more information about the relation between mPFC hyperactivity and reward-related DA function relevant for MDD.

In relation to OCD, it would be interesting to further investigate the link between OFC and DA. OCD patients show hyperactivity in the OFC during rest and symptom provocation (Nakao et al., 2005; Saxena et al., 1998). The OFC is thought to code the value of expected outcomes (Schoenbaum et al., 1998; Wallis and Miller, 2003; Tremblay and Schultz, 1999; Takahashi et al., 2009). Thus, disturbed activity in this region may influence the capability to learn from differences between actual and expected outcomes (Takahashi et al., 2009), resulting in reward dysfunction (Figeé et al., 2011) and altered adaptive behavior in OCD patients (Remijnse et al., 2009). Because the OFC innervates both the striatum (Schilman et al., 2008; Haber et al., 1995) and the VTA (Watabe-Uchida et al., 2012), it has the potential to interact with the DA system both on the level of the cell bodies, and locally in the projection regions. Thus, OFC projections to the striatum may provide a top-down influence on response selection processes (Frank and Claus, 2006), whereas projections from the OFC to VTA DA neurons may influence phasic DA responses related to reward (Schoenbaum et al., 2009). In fact, OFC might be one of the afferents that provide VTA DA neurons with information necessary to calculate differences between expected and received rewards resulting in the phasic DA teaching signals necessary to guide behavior (Schoenbaum et al., 2009). Indeed, lesions and temporal activation of the OFC not only influence spontaneous activity of VTA DA neurons, but also attenuates phasic DA responses to unexpected rewards and reward-related cues (Takahashi et al., 2011; Jo and Mizumori, 2015).
Together, these findings suggest that interactions between prefrontal regions and the DA system may be important for the regulation of reward-related behavior. Increased understanding about the interaction between different regions of the corticostriatal circuits during reward-related learning and adaptation of response behavior is relevant, not only to provide insight into the neural mechanisms underlying maladaptive behavior in psychiatric disorders, but also to understand our everyday behavior.

PART II – Deep Brain Stimulation in corticostriatal circuits – effects on dopamine and cognitive flexibility

Corticostriatal circuits consist of reciprocal connections between cortex, striatum and thalamus and regulate motivation and goal-directed behavior. Patients with psychiatric disorders, such as major depressive disorder (MDD) and obsessive-compulsive disorder (OCD), show structural and functional changes in these circuits, which may underlie the core symptoms of these disorders (Mayberg, 1997; Harrison et al., 2009; Menzies et al., 2008). First-line treatment of these disorders is the use of behavioral or pharmacological therapies (or a combination of both) and successful symptom reduction using these conventional treatments results in normalization of dysfunctional activity in corticostriatal circuits (Saxena et al., 1998; Mayberg et al., 2000; Nakao et al., 2005). Approximately 30% of MDD (Rush et al., 2006) and 10% of OCD patients (Denys, 2006) do not respond to the standard treatment options available. In this group of treatment-resistant patients, DBS appears to be a promising approach. Clinical studies investigating DBS in psychiatry report response rates of 50% (or higher) in MDD and OCD patients that were otherwise not responsive to treatment. Successful symptom reduction can be achieved with stimulation in different target regions within corticostriatal circuits. Such stimulation does not only affect neural activity in the target region immediately surrounding the electrode, but also affects activity in distal regions (Mayberg et al., 2005; Figee et al., 2013). Thus, it appears that DBS can modulate activity throughout a neural circuit and may exert its treatment effect by normalizing the aberrant network activity underlying MDD and OCD symptoms (Anderson et al., 2012; Lujan et al., 2008; Deniau et al., 2010; McIntyre and Hahn, 2010). However, it is not yet understood which specific physiological effects of DBS are responsible for the changes in network activity and result in successful treatment. Preclinical animal studies have been initiated to increase our understanding of the physiological effects of DBS. The use of animals permits direct measurement of neural activity and neurotransmitter release following DBS in homologous regions to those used in patient groups. Studies reporting the effects of DBS on neurotransmitter release in clinical conditions are rare. Yet, some of those studies suggest that DA release may indeed be altered by DBS (Figee et al., 2014; Nimura et al., 2005; Kuhn et al., 2012). One of the main advantages of animal DBS research is the possibility to perform invasive measurements in both normal and pathological conditions (Feenstra and Denys, 2012). Moreover, using these measurements in freely moving animals allows studying effects of DBS in different cognitive and behavioral paradigms.

Acute effects of DBS on neurotransmitter release

Stimulation of the superior branch of the medial forebrain bundle (MFB) was recently introduced as a novel target for DBS in MDD (Schlaepfer et al., 2013; Schlaepfer et al., 2014). This target appears promising, as the antidepressant effect occurred rapidly after onset of
stimulation and applying a low current was sufficient to reach treatment effect. With connections to all previously described target sites, the MFB is well positioned to influence activity throughout corticostriatal networks (Dobrossy et al., 2015; Coenen et al., 2011). The authors of this clinical study specifically hypothesized that dopamine (DA) release in the cortex and striatum might contribute to the treatment effect observed. In chapter 5 we directly investigated this hypothesis by measuring DA release in the striatum during MFB DBS. Previous preclinical studies had already shown that DBS in the MFB/VTA reduces depressive symptoms in animal models of depression (Bregman et al., 2015; Furlanetti et al., 2015a; Edemann-Callesen et al., 2015; Friedman et al., 2009) and that continuous DBS in this region does not have adverse effects (Furlanetti et al., 2015b). Onset of MFB DBS induced DA release in the striatum that lasted for at least 40 seconds. However, our recording equipment did not enable us to measure whether DA elevation is sustained beyond these 40 seconds, throughout the stimulation period, or gradually diminishes over time. Alternatively, the use of other detection techniques, such as microdialysis and fast-scan adsorption voltammetry (Atcherley et al., 2015) not to be mistaken for the here used fast-scan cyclic voltammetry) allows monitoring of longer lasting changes in DA concentration. However, as the effects of DBS onset might be small, and the duration of the increase is unknown, the time resolution of these measurements should be sufficiently short (i.e. 1-5 min samples) to be able to detect whether the increase is sustained over a longer time period or fluctuates. Because of its better chemical selectivity, the use of microdialysis would also allow the monitoring of changes in other neurotransmitters, such as serotonin or noradrenaline as well as measuring release in other brain regions, such as the prefrontal cortex. Future studies with these techniques will shed light on the more long term effects of DBS on DA release.

Another outstanding question is how DBS causes DA release to be elevated. Electrical stimulation can directly influence activity of DA neurons, resulting in DA release in projection regions (Garris et al., 1997; Hernandez et al., 2006; Nakahara et al., 1989; Young and Michael, 1993). However, direct activation of DA axons with the stimulus parameters used in DBS (high frequency, low pulse width) may not be the reason for the DBS-induced elevation of DA release (Yeomans et al., 1988; Yeomans, 1989). Axons of DA neurons are unmyelinated and may require longer pulse widths (or higher currents) for direct activation (Yeomans et al., 1988; Yeomans, 1989). Because DA neurons encompass only a small fraction of all fibers present in the MFB, it is possible that MFB DBS directly activates other fibers which may in turn result in indirect activation of the DA system. Schlaepfer et al (2013; 2014) hypothesized that indirect activation of the DA system may result from recruitment of glutamatergic fibers that originate in the PFC and project to DA neurons in the VTA. DA neurons receive direct input from PFC regions (Sesack and Pickel, 1992; Frankle et al., 2006; Watabe-Uchida et al., 2012; Beier et al., 2015) and manipulating mPFC activity affects both spontaneous and burst firing of VTA DA neurons (Lodge, 2011; Jo et al., 2013; Jo and Mizumori, 2015). The idea that modulation of PFC activity can induce DA release is particularly attractive as normalization of PFC activity is associated with successful treatment effects for MDD (Mayberg et al., 2000). Moreover, a recent study reported that increased excitability of the mPFC (modeling hyperactivity observed in MDD) interacts with DA input at the level of the striatum (Ferenczi et al., 2016). Thus, a future study may investigate whether modulation of PFC activity during MFB DBS is responsible for the increase in DA release to DBS onset. Indeed, pharmacological manipulation of PFC activity can influence striatal DA through the VTA (Karreman and Moghaddam, 1996) and it was recently shown that selective activation of prefrontal terminals in the VTA...
Manipulating activity in the PFC long term (by lesion or pharmacological inhibition) may provide a general indication of whether the PFC is involved in the induction of DA release following DBS onset. However, these manipulations affect all PFC efferents, making it difficult to determine the specific projections responsible for any possible effect. Optogenetics or pharmacogenetics may be used to specifically silence excitatory input from the prefrontal cortex to DA neurons in the ventral tegmental area during the onset of MFB DBS. The benefit of this approach would be the superior selectivity of manipulation of the projection of interest.

**Does elevated DA contribute to treatment effects of DBS?**
As mentioned above, the way in which DBS exerts its treatment effects are incompletely understood. Although we showed that MFB DBS acutely elevates DA in the striatum, does this also imply that DA contributes to the treatment effect of DBS in MDD (and OCD)? In this section, we explore some options to further investigate the contribution of DA to the treatment effect of DBS in psychiatry.

**Chapter 5** described the acute effect of DBS on DA release for a short period after onset of stimulation. As already mentioned above, the use of other detection techniques, such as microdialysis or fast-scan adsorption voltammetry (Atcherley et al., 2015) can be implemented to investigate if the increase can sustain over time. Previous studies also showed acute effects of DBS in different target regions on neurotransmitter release (Hamani et al., 2010; van Dijk et al., 2012; Paek et al., 2013). Interestingly, clinical studies report acute effects of DBS onset on mood and anxiety (Denys et al., 2010; Greenberg et al., 2010; Malone, Jr. et al., 2009; Mayberg et al., 2005; Schlaepfer et al., 2014) that occur within seconds to minutes after onset of stimulation (Denys et al., 2010; Greenberg et al., 2010). These acute effects are observed in both responders and non-responders, suggesting that acute effects do not necessarily predict chronic treatment effects (Denys et al., 2010; Malone, Jr. et al., 2009). Thus, one possibility is that acute elevation of neurotransmitters following DBS onset (van Dijk et al., 2012; Hamani et al., 2010; Paek et al., 2013; **Chapter 5**) contributes to these acute effects of DBS onset on mood and anxiety reported in the clinic. Whether changes in neurotransmitter levels can contribute to longer term treatment effects is currently unknown, as preclinical studies investigating the effect of chronic stimulation on neurotransmitter release levels are lacking. In fact, this is a more general limitation in animal studies used to investigate the effects of DBS. Most of these studies only look at acute effects of stimulation, whereas chronic stimulation (e.g. over days) is rarely studied. Use of wireless DBS devices can be used to apply DBS in freely moving rodents over longer time periods (Paralikar et al., 2015). Two recent studies report time-dependent changes in the effect of DBS, some of which develop over days or weeks, illustrating the importance of initiating studies with chronic stimulation to more carefully separate acute and chronic effects of DBS (Ewing and Grace, 2013; Chassain et al., 2016).

The contribution of DA to DBS treatment effects can be further explored in animal models of depression. By using an animal model, we could study if DBS with stimulation parameters than can reduce depressive symptoms is also associated with DA release, and whether such release is required for antidepressant efficacy. For example, Hamani et al (2010) showed that integrity of the serotonergic system is required for the anti-depressant like effects of DBS in the ventromedial PFC, whereas integrity of the noradrenergic system is not. There are indications that activity of VTA DA neurons contributes to depressive symptoms in animal
models. For example, rats of the Flinders sensitive line, a rodent depression model, show altered burst firing which can be restored by direct VTA stimulation, albeit with very specific stimulation parameters that diverge from commonly used DBS parameters (Friedman et al., 2009; Friedman et al., 2012). Long lasting exposure to aversive and stressful stimuli, for example during social defeat stress or chronic mild stress, induces depressive symptoms in rodents (Willner, 2005; Krishnan et al., 2007). The development of symptoms is accompanied by altered VTA burst firing activity and an increase in spontaneous DA transients in the nucleus accumbens (Anstrom et al., 2009), and these changes can be long-lasting (Razzoli et al., 2011). These studies suggest that phasic DA release patterns in the ventromedial striatum can be altered during depressive states in rodents. In Chapter 5, we did not find an effect of MFB DBS on the occurrence of spontaneous transients in healthy animals; however, it is possible that DBS can modify these characteristics in an altered system, which can be studied with these animal models.

Chronic mild stress and social defeat stress are particularly interesting animal models to use in DBS research related to depression, for two reasons. First, not all animals are susceptible to repeated social defeat stress, and resilient animals show different neurobiological characteristics than animals that are susceptible (Krishnan et al., 2007). Thus, studying neurobiological characteristics of these animals before exposing them to a depression-inducing paradigm may provide information about possible biomarkers indicative of a pathological state. Second, the group of susceptible animals, that develop depressive symptoms, can be separated in responders and non-responders to chronic SSRI treatment (Jayatissa et al., 2006; Christensen et al., 2011). Thus, there is a group of treatment-resistant animals which may more accurately model the patient population relevant for DBS studies. For example, Dournes et al (2013) used the chronic mild stress model and showed that DBS in the anterior cingulate cortex reduced depressive symptoms in mice that did not respond to chronic fluoxetine treatment. Extending these types of studies by comparing neurobiological characteristics of animals that are sensitive to chronic pharmacological treatment and animals that do not respond to these treatments may provide clues to the specific neurobiological changes related to treatment resistance and the specific working mechanisms of DBS in treatment resistant patients.

The ability to study the effects of DBS on DA release is not just relevant for the mechanisms of action of DBS in depression. OCD is also associated with dysfunctions of the DA system. In Chapter 2 we reviewed some of the alterations in the DA system described for OCD patients. For example, a hyperactive DA system was proposed following the finding that OCD patients show reduced binding to D2/3 receptors, in particular in the ventral striatum (Denys et al., 2004a; Perani et al., 2008; Schneier et al., 2008; Denys et al., 2013), and because of the efficacy of DA antagonist as additive treatment to SSRI’s (Dougherty et al., 2004; McDougle et al., 2000; Denys et al., 2004b). Moreover, OCD patients also show altered activity in the ventral striatum during reward-related learning (Figee et al., 2011; Jung et al., 2011; Marsh et al., 2015), which may be an indirect indication of altered DA reward-processing.

A recent study reported changes in D2/3 receptor binding potential following DBS in the nucleus accumbens/anterior limb of the internal capsule, suggesting that DBS may increase striatal DA (Figee et al., 2014). This may seem counterintuitive in light of the proposed hyperactivity of the DA system described above. However, these authors suggest that their sample of treatment refractory OCD patients (which did not respond to DA antagonist treatment)
may have shown an underlying DA deficit, rather than DA hyperactivity (Figee et al., 2014). Indeed, the neurobiological correlates separating treatment resistant patients from patients that do respond to treatment are incompletely understood and need further investigation. Although changes in binding potential to D_{2,3} receptors may indicate increased DA release, they can also result from reduced binding affinity or reduced availability due to receptor downregulation. Directly measuring DA release in animals may provide us with more information. The combination of techniques described in Chapter 5 can be used to directly study the effect of stimulation in target regions relevant for treatment of OCD on DA release. Studying the effects of DBS in different target regions on DA release may provide insight into potential different physiological effects of stimulation between regions.

The use of an animal model for OCD is somewhat complicated because one of the core symptoms of the disorder, the occurrence of unwanted and intrusive thoughts (obsessions), is a symptom that is inherently human and impossible to model in animals. Therefore, existing animal models of OCD predominantly reflect the compulsive acts (repetitive, excessive and inappropriate behaviors) of OCD patients (Wang et al., 2009; Korff and Harvey, 2006; Fineberg et al., 2011; Albelda and Joel, 2012b). Manipulations of the DA system have been associated with compulsive or repetitive behavior in animals (as reviewed in Chapter 2). Different types of compulsive behavior (e.g. compulsive checking and grooming) can be induced by direct manipulations that result in DA hyperactivation (Berridge et al., 2005; Campbell et al., 1999; Szechtman et al., 1998), and involvement of DA mechanisms has also been shown for other validated OCD models (Joel and Doljansky, 2003; Presti et al., 2003; Albelda and Joel, 2012a; Moreno and Flores, 2012). Two genetic mouse models, the SAPAP3 and Slitrk knockout mouse lines, show specific deficits in corticostriatal circuits inducing compulsive grooming behavior and anxiety (Welch et al., 2007; Shmelkov et al., 2010), but DA release in these animal models has not been studied. Sesia et al (2013) recently reported altered VTA burst firing activity after induction of compulsive behavior with quinpirole. However, for other OCD animal models, VTA firing activity and phasic DA release patterns have not been characterized, and require further investigation. Future studies can use FSCV to characterize phasic DA release patterns in animal models of OCD, such as the SAPAP3 knockout mouse. Moreover, although several studies reported a reduction of compulsive behavior following DBS in different target regions (Klavir et al., 2009; Winter et al., 2008; Mundt et al., 2009; Klavir et al., 2011), it has not yet been investigated whether DA is necessary for such effects.

Another aspect that needs further attention in animal models for OCD is treatment resistance. To date, there are no studies that report a separation in treatment-responsive and treatment-resistant animals, for example following chronic SSRI treatment. This line of research is necessary in order to gain more understanding of the potential underlying neurobiological changes related to treatment resistance, which is particularly relevant for studies investigating DBS effects, which is only used for treatment-resistant patients.

To summarize, there is still too little evidence to conclude that effects on DA contribute to chronic treatment effects of DBS in psychiatry. This section provided some ideas to further investigate this relation. Further characterization of DA signaling in animal models for OCD is necessary to clarify the role of DA in this disorder. Moreover, the combination of FSCV and DBS in freely moving animals (as first described in Chapter 5) can be used to further characterize the effects of DBS in different targets and in different behavioral situations on DA.
release and might ultimately be used to investigate the effects of DBS on DA release in MDD and OCD animal models.

**Exploring new target regions for DBS – effects of OFC stimulation**

Preclinical animal research does not just provide a useful tool to enhance our understanding of the working mechanism of DBS by directly studying effects of stimulation on neural activity or neurotransmitter release, these types of studies can also delineate specific effects of DBS on core symptoms of psychiatric disorders (e.g. mood, anxiety, compulsivity) as well possible effects on cognition (Hamani and Temel, 2012). For example, van Dijk et al (2013) showed that conditioned and unconditioned anxiety can be differentially modulated by stimulation in different target regions, and that conditioned anxiety is exclusively reduced by stimulation of the internal capsule. Effects of DBS on cognition are important to study; not only because DBS in psychiatry may restore normal cognitive functioning when that is disturbed, but also to investigate possible cognitive side effects of stimulation in new target regions.

In Chapter 6, we investigated the cognitive effects of deep brain stimulation in the OFC. OCD patients show hyperactivity of the OFC during rest and symptom provocation, and this hyperactivity can be reduced by following successful pharmacological and behavioral therapy (Nakao et al., 2005; Saxena et al., 1999). OFC hyperactivity is also normalized following DBS in the ventral striatum and subthalamic nucleus (Rauch et al., 2006; Le Jeune et al., 2010), suggesting that modulation of OFC activity is important for symptom reduction in OCD. Therefore, we hypothesized that the OFC could be a potential novel target region for DBS. As several studies suggest OFC control of DA activity (Lodge, 2011; Jo and Mizumori, 2015; Takahashi et al., 2009; Takahashi et al., 2011), the OFC may also be a putative DBS target to restore abnormalities in DA function.

Preliminary results suggested that DBS in the OFC can rescue compulsive behavior in a mouse homolog of obsessive compulsive disorder (de Haas, 2012). We used a cognitive task that depends on integrity of the OFC to test the cognitive consequences of stimulation in this region. Direct stimulation of the OFC impaired acquisition of a spatial reversal learning task. Initially, stimulated animals showed perseverative behavior: they kept lever pressing on the side that was originally rewarded and took longer to switch to the other side, suggesting an inability to use negative feedback to switch responding. A recent study shows that OFC inactivation may affect learning from both positive and negative feedback during discrimination and reversal learning (Dalton et al., 2016). Moreover, manipulating OFC activity can influence both spontaneous activity of VTA DA neurons and their response to rewards and reward-predicting stimuli (Jo and Mizumori, 2015; Takahashi et al., 2011; Lodge, 2011). As we reported in Chapter 3, phasic DA dynamics in the striatum are associated with positive, but not negative feedback during reversal learning. Further studies may investigate whether alterations in phasic DA during reward-related learning and feedback processing may contribute to impaired cognitive flexibility following dysregulation of OFC activity. Moreover, the combination of DBS and FSCV during execution of behavioral paradigms, such as reversal learning, may be used to investigate whether DBS in different target regions can restore reward processing deficits associated with psychiatric disorders (Figeé et al., 2011; Pizzagalli et al., 2009; Greenberg et al., 2015).

The observed impairments following DBS in the OFC resemble deficits in reversal learning observed following OFC lesions (Boulougouris et al., 2007; Dias et al., 1997; Schoenbaum...
et al., 2002). This suggests that DBS may inhibit activity in the OFC, or disrupts OFC output to other cortical or subcortical areas, such as the striatum and basolateral amygdala (Stalnaker et al., 2007; Schoenbaum et al., 2009), resulting in behavioral inflexibility. By using DBS to manipulate OFC activity during a behavioral paradigm that depends on OFC integrity, we showed that OFC DBS induces unwanted cognitive side effects. Considering that behavioral inflexibility is a core symptom of compulsive disorders, the OFC does not appear to be a first choice target for DBS in these disorders. However, it is not yet known if the DBS-induced inflexibility sustains upon continuous stimulation. Moreover, the effects of OFC DBS in a model of OFC hyperactivity, where OFC inhibition may be beneficial, may provide more information on the OFC as a potential target region for DBS in compulsive disorders.

Together, the studies described in this section showed some examples of how preclinical studies can be used to enhance our understanding of the working mechanism of DBS for psychiatric disorders and to characterize novel stimulation targets. These types of experiments may be used to help increase our understanding of some of the outstanding questions in the clinical field.
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Appendix

Nederlandse samenvatting
PhD portfolio
About the author
Dankwoord
Neuromodulatie in cortico-striatale circuits – effecten van diepe hersenstimulatie en dopamine

Deel I – De rol van dopamine in cognitive flexibiliteit

Cognitieve flexibiliteit is het vermogen gedrag aan te passen aan een veranderende omgeving. In het dagelijks leven voeren we continu handelingen uit met een bepaald doel voor ogen, maar we zijn ook in staat ons gedrag aan te passen als blijkt dat het doel dat we voor ogen hadden niet meer bereikt wordt. Het vermogen om flexibel om te gaan met dit soort veranderingen is verstoord bij patiënten met psychiatrische aandoeningen, zoals depressiviteit en obsessief-compulsieve stoornis. De prefrontale cortex (PFC) is een belangrijk hersengebied dat betrokken is bij de regulering van doelgericht gedrag en cognitieve flexibiliteit. Verstoorde activiteit of beschadiging van de PFC kan leiden tot verminderde cognitieve flexibiliteit. De PFC is echter niet als enig hersengebied betrokken bij de regulering van doelgericht gedrag. Verbindingen vanuit de PFC naar andere hersengebieden zoals de thalamus en het striatum (en vice versa) spelen daarbij ook een belangrijke rol. Dit netwerk wordt ook wel het cortico-striatale circuit genoemd. Deze cortico-striatale circuits zijn betrokken bij bijvoorbeeld beloningsgericht gedrag en zijn daarbij sterk afhankelijk van dopamine regulatie. Bij verschillende psychiatrische aandoeningen zijn verstoringen in zowel structuur als functie van cortico-striatale circuits gevonden. Door meer te weten te komen over de neurobiologische grondslag van cognitieve flexibiliteit kunnen we dus niet alleen meer te weten komen over ons alledaags gedrag, maar ook over de neurobiologische veranderingen die een rol kunnen spelen bij psychiatrische aandoeningen.

Dopamine wordt veel in verband gebracht met motivationeel of beloningsgericht gedrag. De celkernen van dopamine neuronen bevinden zich in de middenhersenen. Vanuit de middenhersenen onstaan wijdvertakte projecties naar zowel corticale als subcorticale gebieden. Dopamine neuronen zijn continu actief en geven continu dopamine af in de projectiegebieden. Deze constante afgifte van dopamine is noodzakelijk voor motorische handelingen, maar is ook belangrijk voor cognitieve processen, zoals het werkgeheugen, het nemen van beslissingen of motivatie. Naast deze basale activiteit, kunnen dopamine neuronen ook kortdurende (milliseconden) verhogingen in activiteit laten zien. Deze kortdurende verhogingen kunnen spontaan voorkomen, maar worden ook vaak gezien als er een belangrijke gebeurtenis plaatsvindt, zoals het ontvangen van een onverwachte beloning. De kortdurende verhogingen in activiteit van dopamine neuronen worden ook wel ‘fasische’ dopamine genoemd, en gaan gepaard met een verhoogde afgifte in de projectiegebieden die enkele seconden aanhoudt. Er wordt gedacht dat de fasische dopamine activiteit belangrijk is voor het aanleren van beloningsgericht gedrag. Het aanbieden van een beloning aan een dier zorgt voor een kortdurende verhoging in de afgifte van dopamine (een dopamine ‘transient’) in het striatum. Indien een neutrale stimulus (bijvoorbeeld een lampje) meermals gevolgd wordt door een beloning zal er ook bij presentatie van deze stimulus (cue stimulus) een dopamine transient te zien zijn. Dit kortdurende dopamine signaal codeert als het ware de verwachting van een te ontvangen beloning. De fasische dopamine activiteit is dus belangrijk voor het opmerken van beloningen en om gebeurtenissen te onthouden die als voorspellers voor beloningen optreden. Er zijn aanwijzingen dat dopamine niet alleen belangrijk is voor het aanleren van beloningsgericht gedrag, maar dat het ook betrokken kan zijn bij het aanpassen van gedrag.
In het eerste deel van dit proefschrift onderzochten we in welke mate dopamine betrokken is bij de regulatie van cognitive flexibiliteit.

**Hoofdstuk 2** bevat een literatuuroverzicht over de rol van dopamine in cognitive flexibiliteit. Zowel farmacologische als genetische studies in mensen en proefdieren tonen aan dat dopamine inderdaad betrokken is bij verschillende aspecten van cognitive flexibiliteit. Deze studies laten zien dat het aanpassen van gedrag gefaciliteerd wordt door een verminderde activiteit van dopamine op D2-type receptoren, terwijl het aanleren van een nieuwe response tijdens het aanpassen van gedrag gefaciliteerd wordt door verhoogde activiteit van dopamine via D1-type receptoren. Dit wijst erop dat kortdurende veranderingen in de concentratie belangrijk kunnen zijn voor het aanpassen van gedrag: een verlaging in dopamine zou ervoor kunnen zorgen dat de activiteit via D2 receptoren vermindert, terwijl een verhoging in dopamine ervoor kan zorgen dat de activiteit via D1 receptoren toeneemt. Uit deze studies blijkt echter ook dat hoewel dopamine het aanpassen van gedrag lijkt te faciliteren, het niet per se noodzakelijk is. Dopamine heeft dus een modulerend effect – het heeft misschien geen direct effect maar zorgt ervoor dat andere effecten sneller plaatsvinden. Het vermogen om gedrag aan te passen vermindert, maar is niet geheel afwezig na manipulaties in het dopamine systeem.

In dit hoofdstuk bekeken we ook of verminderde cognitieve flexibiliteit bij patiënten met obsessief compulsieve stoornis verklaard zou kunnen worden door een verstoring van het dopamine systeem. Patiënten met obsessief compulsieve stoornis laten niet altijd een verminderde prestatie laten zien op gedragstaken die cognitieve flexibiliteit meten. Er zijn echter wel veranderingen in activiteit te zien in corticostriatale hersencircuits als zij deze taken uitvoeren. Een verstoring in het dopamine systeem zou kunnen bijdragen aan deze functionele veranderingen tijdens het uitvoeren van cognitieve taken bij patiënten met obsessief-compulsieve stoornis.

De studies beschreven in **hoofdstuk 2** hebben laten zien dat dopamine inderdaad betrokken is bij het reguleren van cognitieve flexibiliteit. Veel van de studies die hierin beschreven zijn maken echter gebruik van farmacologische of genetische manipulaties die het dopamine systeem langdurig beïnvloeden. Er zijn nooit metingen van korte veranderingen in dopamine concentratie gedaan in het striatum tijdens het uitvoeren van een taak die cognitieve flexibiliteit meet. Tijdens het aanpassen van beloningsgericht gedrag is het noodzakelijk om snel informatie te verwerken over ontvangen beloningen en gebeurtenissen (stimuli) die deze beloningen kunnen voorspellen. Het is daarom interessant om te bekijken hoe fasische afgifte van dopamine verandert tijdens het aanpassen van beloningsgericht gedrag. We kunnen dan bovendien onderzoeken of het patroon van fasische afgifte, of de hoeveelheid dopamine die vrijkomt, de snelheid waarmee gedrag wordt aangepast kan beïnvloeden.

In de twee hoofdstukken die volgen beschrijven we twee experimenten die zijn opgezet om de rol van fasische dopamine in cognitieve flexibiliteit te onderzoeken. We gebruikten daarvoor een elektrochimische meetmethode (fast-scan cyclic voltammetry; FSCV) met een zeer hoge tijdssresolutie (10Hz, elke 100 msec een meetpunt). Het gebruik van deze techniek stelt ons in staat om korte veranderingen (seconden) in de extracellulaire concentratie van dopamine te detecteren. Vanwege de hoge tijdssresolutie is het mogelijk om te zien hoe het aanbieden van stimuli (zoals een lampje of een beloning) en het uitvoeren van
handelingen (zoals het indrukken van een pedaal) het dopamine niveau in de hersenen beïnvloedt. Om cognitieve flexibiliteit te meten gebruikten we een ‘reversal-taak’. Deze taak is erop gebaseerd dat ratten aanleren dat één van twee mogelijke handelingen een beloning oplevert en dat ze bij de omkering van de respons-beloningsrelatie (reversal) hun gedrag moeten aanpassen door de andere, eerder niet-beloonde handeling te kiezen. De twee mogelijke handelingen zijn het drukken op de linker- of rechterpedaal in een operante box, de keuze berust dus op het aanleren van een spatiale discriminatie (onderscheid maken op basis van de plaats van iets). De mogelijkheid om een beloning te kunnen krijgen na pedaalgedruk wordt aangegeven door een lampje (cue). Door het uitvoeren van dopamine metingen tijdens deze taak konden we onderzoeken hoe dopamine betrokken is bij de verschillende fases van een beloningsgerichte respons: detectie van de cue, voorbereiding voor de handeling, de handeling (pedaalgedruk), nose-poke om de beloning te pakken, eten van de beloning.

Hoofdstuk 3 beschrijft hoe dopamine in het ventromediale striatum fluctueert tijdens het uitvoeren van reversal leren. Voor het met succes uitvoeren van een reversal leren taak is het nodig om veranderingen in feedback op te merken en het gedrag daarop aan te passen: het ontblijven van een beloning na omkering van de respons-beloningsrelatie is een vorm van negatieve feedback, terwijl het krijgen van een beloning na het uitvoeren van de voorheen niet-beloonde handeling gezien kan worden als positieve feedback. We verwachtten dat dopamine betrokken zou zijn bij het leren van feedback en bij het coderen van informatie over de relatie tussen een bepaalde handeling en een beloning die volgt op deze handeling tijdens reversal leren. De metingen in het ventromediale striatum lieten zien dat het patroon van dopamine afgifte in dit gebied zich snel aanpast als dieren hun gedrag aanpassen. Voor de reversal zagen we een kortdurende dopamine verhoging tijdens het aanbieden van een cue stimulus en geen dopamine verhoging na het aanbieden van een beloning. Na de reversal was dit patroon omgekeerd: het signaal op de cue stimulus verlaagde, terwijl er een duidelijke verhoging te zien was na het ontvangen van een beloning. Na de reversal zorgde het ontvangen van positieve feedback (het krijgen van een beloning) dus voor een verhoging van dopamine. Bovendien beïnvloedde het ontvangen van positieve feedback het cue-signal in de daaropvolgende trial. Het ontvangen van negatieve feedback (het ontblijven van een verwachte beloning) was niet direct zichtbaar in het dopamine signaal.

Als laatste werd gevonden dat individuele verschillen in het patroon van dopamine afgifte op positieve feedback gerelateerd kunnen worden aan de prestatie op de reversal taak: bij dieren die hun gedrag sneller aanpast en na de reversal zorgde het ontvangen van positieve feedback voor een verhoging in dopamine afgifte tijdens het aanbieden van een cue stimulus, dit werd niet gezien bij dieren die hadden hun gedrag aan te passen. Deze resultaten tonen aan dat dopamine tijdens een reversal-taak betrokken is bij het leren van positieve feedback en dat dopamine in het ventromediale striatum het aanpassen van gedrag faciliteert.

Veel onderzoek naar de rol van dopamine in motivationeel gedrag is gericht op dopamine metingen in het ventromediale striatum. Er zijn echter aanwijzingen dat ook het dorsolateraal striatum belangrijk is voor het aanleren/onderhouden van motivationeel gedrag en cognitieve functies. Zowel het ventraal als dorsaal striatum ontvangt dopaminerge input, maar de precieze dopaminerge populaties die naar deze striatale gebieden projecteren verschillen. Hetzelfde geldt voor projecties vanuit de PFC naar het striatum: beide striatale gebieden
ontvangen prefrontale input, maar niet uit dezelfde corticale gebieden. Het ventromediale striatum ontvangt vooral projecties van limbische gebieden die betrokken zijn bij emotie, motivatie en geheugen, en is belangrijk voor het aanleren van beloningsgericht gedrag. Het dorsolateraal striatum ontvangt juist projecties van gebieden die betrokken zijn bij het verwerken van sensorische en motorische informatie en wordt daarom meer in verband gebracht met beweging. Het lijkt er dus op dat er verschillende, parallele cortico-striatale circuits zijn die elk belangrijk kunnen zijn voor verschillende aspecten van motivationeel gedrag.

In Hoofdstuk 4 werden de resultaten uit hoofdstuk 3 uitgebreid met metingen in een tweede gebied in het striatum: het dorsolaterale striatum. Door gebruik te maken van dezelfde gedragstaak (reversal-taak), konden we een directe vergelijking maken tussen het dopamine signaal in het ventromediale striatum en het dopamine signaal in het dorsolaterale striatum. In het dorsolaterale striatum zagen we een ander dopamine afgiftepatroon dan in het ventromediale striatum: het aanbieden van een cue stimulus zorgde niet voor een verhoging in dopamine, terwijl er een duidelijke dopamine verhoging te zien was tijdens het uitvoeren van een beloningsgerichte handeling (pedaaldruk). Dit afgiftepatroon werd niet beïnvloed door het aanbieden van een reversal. Bovendien werd er in zowel beloende als onbeloende trials een verhoging van dopamine gevonden tijdens het uitvoeren van de pedaaldruk, wat erop wijst dat dopamine in het dorsolaterale striatum niet gevoelig is voor de uitkomst van een bepaalde handeling. Dopamine afgifte in het dorsolaterale striatum zou dus betrokken kunnen zijn bij de motivatie om een handeling uit te voeren, of belangrijk kunnen zijn voor het initiëren van een handeling die bedoeld is om een beloning te krijgen. Samen met de resultaten uit hoofdstuk 3 laat dit zien dat dopamine afgifte tijdens het aanpassen van ongeleerd gedrag sterk verschilt tussen deze striatale gebieden. Het dopamine signaal in het ventromediale striatum volgde een reversal van handeling en beloning, terwijl het dopamine signaal in het dorsolaterale striatum niet beïnvloed werd door het wisselen van de beloonde kant. Bovendien was het dopamine signaal in het ventromediale striatum gevoelig voor het ontvangen van positieve feedback, terwijl de uitkomst van de trial niet zichtbaar was in het dopamine signaal in het dorsolaterale striatum. Dit suggereert dat dopamine in het ventromediale striatum het aanpassen van gedrag faciliteert en dat het dopamine signaal in het dorsolaterale striatum de initiatie en uitvoering van beloningsgerichte handelingen mogelijk maakt.

Deel II – Effecten van diepe hersenstimulatie op dopamine afgifte en cognitie

In het eerste deel van dit proefschrift onderzochten we de neurobiologische grondslag van cognitieve flexibiliteit. Dit leert ons niet alleen meer over ons alledaags functioneren, maar kan ook aanwijzingen geven over verstoringen die zorgen voor cognitieve problemen in psychiatrische ziektebeelden. In het tweede deel van dit proefschrift onderzochten we de neurobiologische en cognitieve effecten van een relatief nieuwe behandeling binnen de psychiatrie: diepe hersenstimulatie (DHS). DHS laat veelbelovende resultaten zien bij patiënten die niet reageren op enige andere vorm van therapie. Er zijn echter nog veel vragen over de manier waarop DHS zorgt voor vermindering van symptomen. Bovendien lijken verschillende doelgebieden voor plaatsing van de DHS elektrodes mogelijk om tot een effectieve behandeling te komen. Al deze doelgebieden maken deel uit van of hebben connecties met het cortico-striatale circuit. Er wordt daarom gedacht dat DHS verstoorde activiteit binnen cortico-striatale circuits kan beïnvloeden. DHS zou er voor kunnen zorgen
dat de afwijkingen in de corticostriatale circuits genormaliseerd worden met normalisatie van gedrag als gevolg.

In een recente studie is bij een kleine groep patiënten met depressie DHS in de medial forebrain bundle uitgevoerd. De medial forebrain bundle is een vezelbundel met projecties vanuit de middenhersen naar het limbisch systeem en bevat onder andere de projectievezels van dopamine neuronen. De medial forebrain bundle zorgt daarmee voor verbindingen tussen verschillende hersengebieden die betrokken zijn bij motivationeel gedrag en stemming. In een kleine groep patiënten werd een zeer snel effect van DHS in de medial forebrain bundle gezien (>50% reductie depressieve symptomen na een week stimulatie) en kon er met een lagere intensiteit gestimuleerd worden. Gedacht wordt dat DHS in de medial forebrain bundle kan leiden tot verhoogde dopamine activiteit in striatale en prefrontale gebieden. In hoofdstuk 5 hebben we een studie opgezet om te onderzoeken of DHS in de medial forebrain bundle inderdaad de afgifte van dopamine beïnvloedt. We onderzochten het acute effect van het aanzetten van de stimulatie op dopamine afgifte in het striatum en bekeken ook of langer duurande stimulatie spontane (niet-taakgerelateerd) dopamine afgifte beïnvloedt. De dopamine concentratie in het ventromediale striatum neemt meteen toe na het aanzetten van DHS, en deze verhoging duurt tenminste 40 seconden. Verder onderzoek is nodig om te zien of de verhoging langdurig aanhoudt. De stimulatie had geen invloed op de spontaan verkomende fluctuaties in het niveau van dopamine in het striatum. Ook dopamine afgifte tijdens beloningsgericht gedrag werd niet beïnvloed door DHS in de medial forebrain bundle. De bevindingen in dit hoofdstuk tonen aan dat het aanzetten van DHS in de medial forebrain bundle met klinisch relevante parameters onmiddellijk zorgt voor een verhoging van dopamine in het striatum. Langer duurande stimulatie heeft geen invloed op parameters van fasische dopamine afgifte of dopamine afgifte die volgt op een beloning. De combinatie van technieken (FSCV en DHS) zoals beschreven in dit hoofdstuk kan in vervolgonderzoek gebruikt worden om te onderzoeken hoe DHS in verschillende hersengebieden dopamine afgifte kan beïnvloeden. Het uiteindelijke doel is om in diemodellen voor psychiatrische aandoeningen te onderzoeken hoe DHS neuronale activiteit en dopamine afgifte binnen cortico-striatale circuits beïnvloedt en of DHS ervoor kan zorgen dat verstoringen in beloningsgericht gedrag weer normaliseren.

Hoofdstuk 6 beschrijft het effect van DHS in een nieuw doelgebied op cognitie. Patiënten met obsessief-compulsieve stoornis laten hyperactiviteit zien in de orbitofrontale cortex en deze hyperactiviteit vermindert na succesvolle behandeling. De orbitofrontale cortex zou daarom een mogelijk nieuw doelgebied voor DHS kunnen zijn, mits er bij stimulatie in dit gebied geen bijwerkingen optreden. DHS in de orbitofrontale cortex zorgde voor verminderde cognitieve flexibiliteit in een reversal taak. Na de reversal hadden gestimuleerde ratten meer moeite om hun gedrag aan te passen, ze bleven de pedaal indrukken die niet meer beloond werd. Stimulatie in de OFC lijkt dus voor te zorgen dat er (tijdelijk) cognitieve inflexibiliteit optreedt. Dit is een onwenselijke bijwerking voor aandoeningen die al gekenmerkt worden door cognitieve inflexibiliteit, zoals obsessief compulsieve stoornis. Er is meer onderzoek nodig naar de langdurige effecten van DHS in dit gebied voordat dit hersengebied overwogen kan worden als nieuw doelgebied voor DHS bij patiënten met obsessief-compulsieve stoornis.
PHD PORTFOLIO

Marianne Klanker  
June 2009 – July 2015  
Supervisors: prof. dr. Damiaan Denys, dr. Matthijs Feenstra, dr. Ingo Willuhn

PUBLICATIONS

PUBLICATIONS IN THESIS

Published


Klanker M, Feenstra MG and Denys D (2013). Dopaminergic control of cognitive flexibility in humans and animals. Frontiers in Decision Neuroscience, vol 7: 201


In preparation/submitted


OTHER PUBLICATIONS


### PHD TRAINING

#### COURSES/WORKSHOPS/SUMMERSCHOOLS

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<td>Introductory course ONWAR graduate school</td>
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#### Workshops/Summerschools

| Attendance annual meetings ONWAR graduate school                       | 2009-2012 | 100          |
| ECNP Workshop on Neuropsychopharmacology for young scientists in Europe | 2012     | 50           |
| FENS-IBRO Summerschool “Cognition and action: Systems neuroscience approaches to understanding complex behaviour” | 2010     | 65           |

### PRESENTATIONS

#### Oral presentations

| Changes in phasic dopamine release during spatial reversal learning  | 2014 | 14 |
| Endo-Neuro-Psycho meeting, Lunteren, the Netherlands                |      |    |
| Phasic dopamine release in the ventral striatum during spatial discrimination and reversal learning Monitoring Molecules in Neuroscience: 14th international conference, London, UK | 2012 | 14 |

#### Poster presentations

| Dynamic changes in dopamine release during spatial reversal learning | 2013 | 14 |
| Society for Neuroscience, Annual Meeting, San Diego, CA, USA       |      |    |
| Dynamic changes in dopamine release during spatial reversal learning | 2013 | 14 |
| EBBS Meeting, Munich, Germany                                        |      |    |
### PRESENTATIONS

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<td>High frequency stimulation in the rat orbital prefrontal cortex impairs spatial reversal learning</td>
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<td>Distinctive effects of medial and orbital prefrontal cortex deep brain stimulation on reversal learning in rats</td>
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<td><em>Society for Neuroscience, Annual meeting, San Diego, CA, USA</em></td>
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<td>Dopamine transients during classical conditioning</td>
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<td><em>Endo-Neuro-Psycho meeting, Lunteren, the Netherlands</em></td>
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### (Inter)national Conferences

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<td>Monitoring Molecules in Neuroscience, London, UK</td>
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<td>Dutch Endo-Neuro-Psychomeeting, Lunteren, the Netherlands</td>
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<td>Animal models in psychiatry, University of Amsterdam</td>
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<td>Supervising</td>
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<td>Eva Gadet – Fast scan cyclic voltammetry measurements in the striatum during and in the absence of deep brain stimulation in the lateral habenula and internal capsule.</td>
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<td>Debra Schrader – The effect of deep brain stimulation on cognitive flexibility in a spatial reversal learning paradigm in rats.</td>
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<td>Ger Post – Flexibility and the prefrontal cortex. The effects of sleep deprivation and deep brain stimulation in the ventromedial and orbital prefrontal cortex on behavioural flexibility.</td>
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<td>Iris Lange – Influence of deep brain stimulation of the lateral habenula on negative reward-related responses.</td>
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### PARAMETERS OF ESTEEM

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