Chapter 1

General Introduction

Cognitive flexibility is the ability to adapt behaviour in response to changes in environmental demands. In other words, when desired goals are no longer obtained through previously successful strategies, cognitive flexibility enables us to abandon those strategies and find alternative approaches. As such, cognitive flexibility is of vital importance for the proper execution of goal-directed behaviour and impairments in its function will invariably lead to maladaptive behaviour.

Cognitive flexibility is not a unitary function, but is the result of the integration of a multitude of functions that monitor the current goal and behavioural performance, direct attention, and initiate responses while inhibiting others. A disturbance, or failure in any of these processes can impair flexibility and in turn, optimal decision making. The study of cognitive flexibility therefore relies on the investigation of these individual processes, and consequently their neural substrate. A typical behavioural approach that is often used in studying cognitive flexibility is reversal learning. In this paradigm subjects are trained to choose one of two possible response options, each of which is linked to a positive or negative outcome. After learning the relation between the action and outcome, the contingencies are reversed and the subject is required to make a behavioural adaptation to obtain the reinforcement and prevent negative outcome. Behavioural flexibility can be assessed with tasks like these which are often combined with neurophysiological measurements to gain insight in its neural substrate.

The prefrontal cortex (PFC) has long been known to be of critical importance for these functions. Through connections with thalamic and striatal areas as well as cortico-cortical projections the PFC can serve as a relay station in which information converges to produce goal-directed behaviour (e.g. Floresco et al., 2009). A functional differentiation within the PFC is furthermore apparent from studies across species. Despite the clear anatomical differences, various aspects of cognitive flexibility have been shown to be mediated by similar PFC areas across (e.g. Fuster, 1997).

The PFC and thalamo-cortico-striatal system are under modulatory control of monoaminergic input, and as such, serotonin (5-HT), but also dopamine (DA), are recognized to be important for certain, separable, aspects of cognitive flexibility, like the ability to shift attentional focus (Crofts et al., 2001) and reversal learning (Clarke et al., 2005). In addition, 5-HT has been associated with emotional and motivational processes, a function that is perhaps most apparent in relation to affective disorders like anxiety and depression. Drugs that act specifically on the serotonergic system are

therapeutically beneficial in these disorders, and 5-HT also bears a close relation to the severe cognitive inflexibility in depression, schizophrenia and obsessive-compulsive disorder. Although it is not surprising that affective processes are important for decision-making, only relatively recently the relation between affect and cognitive flexibility has become a subject of study. The experiments described in this thesis were performed with the aim to deepen our understanding of the role of 5-HT in the interaction between affective processes and cognition. By means of a behavioural paradigm typically employed in studies of cognitive flexibility, namely reversal learning, we assessed the effect of 5-HT manipulations on behavioral performance and the coding of reward-related information in the PFC. In addition we studied the impact of 5-HT depletion on performance on this task following a shift in affective value of the reward.

The following sections will give an overview of the functional differentiation within the PFC in relation to cognitive flexibility and the putative role of 5-HT.

Prefrontal cortex and behaviour

In studies of cognitive flexibility the prefrontal cortex (PFC) is of particular importance as it is considered to be the primary location of 'executive'- or 'higher' cognitive functions (e.g. Kolb 1984; Dalley et al., 2004; Chudasama and Robbins, 2006). These functions include processes like the selection of, and attention to relevant environmental stimuli, goal selection and performance monitoring, planning, cognitive control (Miller, 2000; Fuster, 2001) and response choice (Chudasama and Robbins, 2006). As such, damage to the PFC has been associated with loss of specific cognitive functions, while the cognitive deficits that are observed in psychiatric diseases like schizophrenia, depression and OCD have often been associated with prefrontal (functional and/or structural) abnormalities.

Historically the prefrontal cortex was considered to be exclusively present in primates until Rose and Woolsey (1948) defined the prefrontal cortex in terms of projections from the medial dorsal nucleus of the thalamus. With respect to the anatomical delineation of the PFC, the presence of a PFC in other species, however, is now generally accepted.

In an attempt to describe the rat PFC, Uylings et al. (2003) incorporated both an anatomical and functional approach to defining PFC boundaries in rat brain (Fig. 1). In their paper they conclude that the rat does possess a PFC which, moreover, has functional characteristics of the primate medial- and orbital PFC as well as the dorsolateral PFC. Notwithstanding the anatomical and functional differences between the rat and (human) primate brain, we argue that the functional and anatomical overlap between these species is such that conclusions can be drawn from non-primate experiments that are important for our understanding of (primate) prefrontal function.

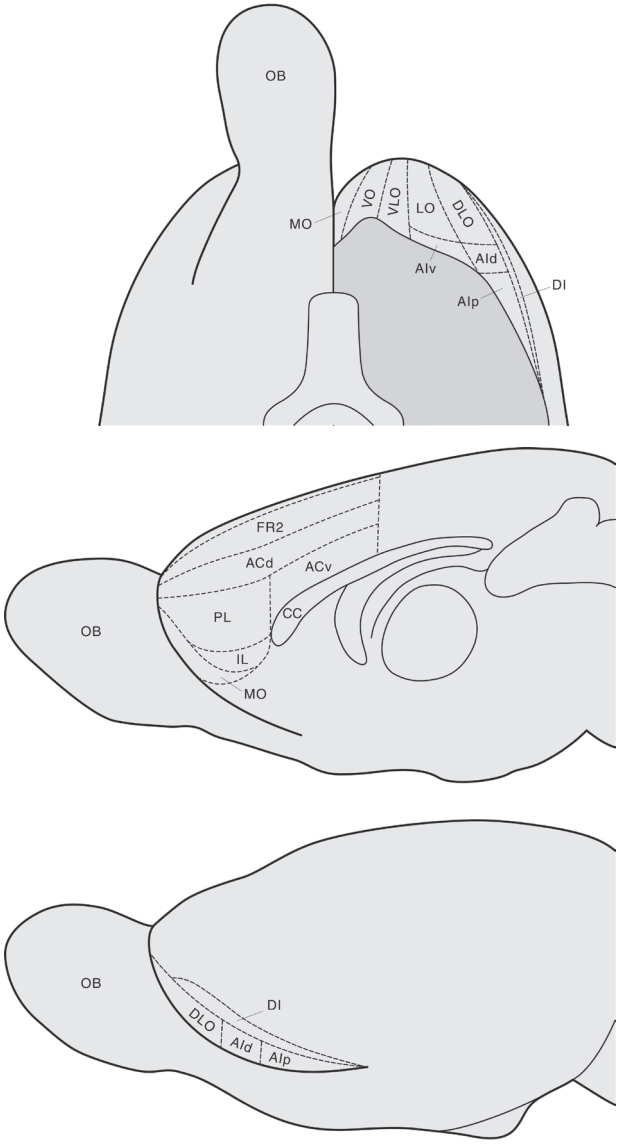

Fig. 1. Schematic view of the medial (top), lateral (middle) and ventral (bottom) areas in the frontal lobe of the rat brain. The ventral view (lowest panel) shows both hemispheres; the olfactory bulb (OB) and olfactory tract have been removed from beneath the left hemisphere to display the ventral orbital and insular cortical areas. Abbreviations: *Medial prefrontal cortex*: IL Infralimbic area, PL Prelimbic area, ACd Dorsal anterior cingular area, ACv Ventral anterior cingluar area, Fr2 Frontal area 2. *Orbital prefrontal cortex*: DLO Dorso-lateral orbital area, LO Lateral orbital area, VLO Ventrolateral orbital area, VO Ventral orbital area, MO Medial orbital area. *Lateral prefrontal cortex*: AId Dorsal agranular insular area, AIv Ventral agranular insular area, AIp Posterior agranular insular area. *Other*: CC Corpus callosum, DI Dysgranular insular area, OB Olfactory bulb. (Adapted from; van de Werd and Uylings, 2008).

As mentioned in the previous section, the PFC not only receives (sensory) input from other (sub)cortical areas, but can be seen as an integrative brain-area of partly separate circuits. Alexander et al. (1986) proposed a parallel organization of segregated circuits that connect subareas of the PFC to subcortical areas that form functional 'loops' that hold partially separate functions. It is considered that these projections to subcortical areas and back to the PFC, along with modulatory input from monoaminergic projections like dopamine (DA) and serotonin (5-HT), provide the basis on which the PFC can organize and drive behavioural output and support 'higher' or executive, cognitive functions.

A functional differentiation within the a PFC has been recognized early on in the observation of patients with PFC damage who displayed wide variety of behavioural abnormalities while remaining fully functioning in other domains (e.g. Fuster, 1997). Similar cases are observed still today, and with advanced brain imaging techniques these patients have provided important insight into PFC functioning. Although non-invasive imaging techniques have become available to study cognitive functions in human (and rodents), these methods can not address many of the questions related to, for instance, the role of certain neurotransmitters in cognitive tasks. Experimental measurements and manipulations of the living brain in humans remains, for the obvious ethical reasons, limited. To resolve this issue animal research continues to provide essential information on PFC function.

Through the use of complex behavioural tests for cognitive flexibility in combination with measurements and manipulations of brain function, important functional dissociations within the PFC have been made. The role of the PFC in cognitive flexibility has been studied by examination of both complex functions like reversal learning and attentional set-shifting and 'singular' functions that make up these complex behaviours, like response discrimination and extinction. Whereas reversal learning measures the animal's ability to respond to a reversal of cue-associated outcomes, attentional set-shifting assesses the ability to shift attention from one stimulus domain to another.

Current literature points to a key role for the orbital part of the PFC (see Fig. 1) in the ability to solve reversal learning problems (e.g. Iversen and Mishkin, 1970; Rolls et al., 1994; Schoenbaum et al., 2002; McAlonan and Brown, 2003) by enabling the inhibition of a previously rewarded action in favour of a response that was previously unrewarding. In contrast, medial prefrontal areas (see Fig. 1) have been found to facilitate attentional shifting (e.g. Birrell and Brown, 2000; see also Dalley et al., 2004; Rich and Shapiro, 2009). In these, a subject shifts its focus of attention from one stimulus property or stimulus dimension to another so that previously insignificant properties or dimensions acquire a functional relevance. Although these examples illustrate a possible functional differentiation within the PFC, there are indications that PFC functions overlap and that contributions of PFC subareas depend on task specific

parameters. De Bruin et al. (2000), for instance, report medial PFC mediated reversal learning of spatial information and this is supported by others, as well (e.g. Kolb et al., 1974; Li and Shao, 1998; Salazar et al., 2004). In a similar vein, the amygdala (e.g. Schoenbaum et al., 1998), hippocampus (Watson and Stanton, 2009; Shohamy et al., 2009), and striatum (e.g. Ferry et al., 2000; Clarke et al., 2008; Cools et al., 2009) have been shown to be involved in the execution of various aspects of reversal tasks. These examples illustrate that the understanding of the underlying functions of complex behaviour, like reversal learning and attentional set-shifting, is imperative. Chapters 2 and 5 deal specifically with these issues and aim to study both reversal learning and functions that are important for performance on these tests, like acquisition of response-outcome associations, response-inhibition, and extinction-learning.

Serotonin

Clinically, prefrontal serotonergic dysfunction has been suggested to be closely related to affective disorders like anxiety and depression, but also schizophrenia and obsessive-compulsive disorder. Interestingly, cognitive flexibility is impaired in the latter three, showing dysfunctions like perseverative responding and an inability to adapt behaviour despite explicit knowledge of the inappropriateness of the behavioural choices. As such, understanding of the role of 5-HT in relation to prefrontal function is of great interest.

Midbrain serotonergic neurons project throughout the brain and provide a fairly homogenous innervation of the PFC, through input from the dorsal raphe nucleus. This broad innervation, in combination with a total of at least 14 different 5-HT receptor subtypes (Barnes and Sharp, 1999), supports the possibility of a great variety of functions. Furthermore, the PFC is the only cortical region that modulates its own monoaminergic innervation, and that of other cortical areas, through descending projections to the monoaminergic nuclei. In a similar fashion 5-HT modulates, and is modulated by, both dopaminergic and noradrenergic innervation (e.g. Esposito et al., 2008, Di Giovanni et al., 2008). However, despite this apparent interdependence of monoaminergic neurotransmission, functional dissociations have been made with regard to the role of 5-HT in cognitive functions.

A popular approach in 5-HT functional research in both primate and rodent studies is acute tryptophan depletion (ATD). The hydroxylation of the essential amino-acid tryptophan by tryptophan-hydroxylase is the rate-limiting step in the synthesis of 5-HT and dietary depletion of tryptophan has been shown to lower central 5-HT levels (Fadda et al., 2000a; Lieben et al., 2004a). Although this effect is relatively modest, this approach is particularly popular in human studies as ATD can be easily applied to lower whole-brain 5-HT in a reversible way. In both patient- and healthy volunteer studies ATD has been shown to affect a number of 'typical' prefrontal tasks like reversal learning (Rogers et al., 1999a; Finger et al., 2007) and set-shifting paradigms (Park et al., 1994).

In addition, ATD has effects on memory consolidation and affective processes like the ability to evaluate and integrate emotional content (Fadda, 2000; Riedel et al., 2002) and processing of positive and negative signals (e.g. Rubinsztein et al., 2001; Rogers et al., 2003; Cools et al., 2008a). Functional MRI studies moreover report changes in prefrontal activity following ATD that are reminiscent of PFC dysfunction observed in depressed patients and people suffering from OCD (Rubia et al., 2005; Allen et al., 2006; Evers et al., 2006).

Although ATD has been employed in rodent studies (see also chapter 2) and has clear advantages over lesioning such as reversibility of the depletion and the comparibility with depletion studies in humans, methodological issues regarding the extent to which ATD induced effects reflect changes in central 5-HT release, in addition to peripheral 5-HT changes, warrant the use of more selective measures to study the role of 5-HT in confined areas (see chapters 3 and 4 for a more detailed discussion).

A method that allows for selective reduction of 5-HT innervation in a specific target area is the local infusion of the toxin 5,7-dihydroxytryptamine (5,7-DHT). This toxin is taken up into the cell body after infusion through the serotonin transporter and induces the breakdown of nerve terminals (Baumgarten and Björklund, 1976). Neurochemical measurements after treatment are then taken to confirm loss of serotonergic innervation. This method of 5-HT manipulation has been used in both primate and rodent experiments and confirms, and extends, earlier reports of the role of 5-HT in cognition.

Clarke et al. (2004, 2005), for instance, used this method to deplete prefrontal 5-HT in the marmoset and observed impaired reversal learning without impairments in attentional set-shifting. The reversal impairment was attributed to an inability to refrain from responding to a previously rewarded stimulus. Further studies confirmed that this effect was due to serotonergic, but not dopaminergic transmission, and located this deficit to a specific sub-area of the PFC, the orbital PFC (Clarke et al., 2007). Central 5-HT depletion in rats, furthermore, indicate that 5-HT depletion leads to impaired flexibility (Harrison et al., 1999), increased perseverative responding (Beninger and Phillips, 1979; Morgan et al., 1993) and impaired impulse inhibition (Harrison et al., 1997; Winstanley et al., 2003).

Together, these, and other (rodent) studies have shown that certain aspects of cognitive flexibility critically depend on central 5-HT. Furthermore, these impairments appear to be fairly specific to the mentioned brain area, but also the serotonergic innervation. This is particularly surprising as the PFC is innervated by a multitude of neuromodulators that have been shown to interact on synaptic and circuit levels (e.g. Di Pietro and Seamans, 2007).

Thus, although clinical observations indicate that 5-HT is an important modulator of cognitive functions, its role in affective processing (see also Remijnse et al., 2005) has

yet to be clarified. Therefore, the elucidation of the neural substrates underlying this special role of 5-HT in affective processing is one of the aims of the current study (see also chapters 5 and 7).

General overview

The overall aim of this thesis is to deepen our understanding of the role of prefrontal serotonin (5-HT) in cognitive flexibility and affect. To this end we have performed a series of behavioural experiments that assess reversal learning, as well as functions that are thought to be essential for cognitive flexibility, like response selection and inhibition, extinction learning and reward encoding. We studied the relation of these behavioural functions with 5-HT by means of manipulations of (central) 5-HT function, e.g. acute tryptophan depletion (ATD) and lesioning, in combination with behavioural measurements, microdialysis, and in vivo electrophysiology. The specific aims that were investigated are described per chapter below.

Chapter 2 describes a set of experiments in which we investigated the role of 5-HT in serial reversal learning of a spatial discrimination. Previous research suggested that the medial prefrontal cortex (PFC) is involved in reversal learning of spatial information, especially when the task is new. As direct evidence for the involvement of 5-HT in reversal learning of a spatial discrimination is lacking we aimed to investigate the role of 5-HT in serial reversal learning early in training, when the task is new, and late in training, when the task is well learned. In line with previous research by others, we employed the method of acute tryptophan depletion

Chapter 3 deals with the effectiveness of ATD to reduce 5-HT release in the prefrontal cortex. Despite an abundance of literature that describes reductions of plasma tryptophan following ATD as well as a wide range of treatment effects on mood and cognition, there is only indirect evidence for ATD induced reductions in central 5-HT release.

In order to address this issue and provide evidence for ATD-induced reductions of 5-HT, and DA release, we performed microdialysis measurements for these monoamines in the PFC following ATD treatment.

In Chapter 4 we review the literature on ATD and the evidence for ATD-induced reductions in 5-HT release. Despite the fact that ATD continues to be a popular method to study the role of 5-HT in mood and cognition, evidence for actual decreases in central 5-HT release is lacking. Our data presented in chapter 3 stress the importance of this question by showing that ATD-induced reductions in plasma tryptophan levels, comparable to those reported in human and rodent literature, do not necessarily indicate reductions in (prefrontal) 5-HT release. In this chapter we reviewed the ATD literature and provided alternative explanations to the interpretation of ATD-induced behavioural effects.

Chapter 5 describes an experiment in which we assessed the role of medial PFC serotonin in reversal learning of two stimulus modalities and the impact of affective value of the reinforcer. Previous research has shown that prefrontal 5-HT is crucial for behavioural adaptation when stimulus-reward contingencies are reversed and behaviour needs to be adapted accordingly. These observations however, pertain to certain stimulus modalities (i.e. visual and olfactory) and have not been shown for spatial information. One part of this study examines the impact of a selective lesion of 5-HT terminals in the medial PFC on reversal learning of spatial- and odour information. As previous research has implicated 5-HT in affective processing and reward discrimination, a second aspect of this chapter deals with the possible role of prefrontal 5-HT in the affective aspects of cognitive flexibility.

To this end we made selective lesions of serotonergic terminals in the medial PFC and tested the impact of the lesion on reversal learning performance. In addition we assessed the effect of changed reward value during reversal learning, to clarify the impact of 5-HT depletion on affective processing.

In chapter 6 we introduce a new method for studying the relation between neurotransmission and neuronal activation. Current understanding of the relation of 5-HT in cognitive flexibility is to a great extent based on lesion- and microdialysis studies in combination with operant behaviour. In addition, electrophysiological measurements of prefrontal neurons in behaving animals have provided important information about the coding of reward-related information in reversal learning. Both lines of evidence point to the importance of the PFC in cognitive flexibility, but convergence of these lines of research is lacking.

To study a direct relation between 5-HT receptor activation and neural (encoding) activity in behaving animals we developed a measuring device that allows for the recording of neural activity during operant behaviour, whilst applying specific drugs that act on the neurons that are recorded. This experiment describes this device and the validation experiments.

Finally, chapter 7 describes an experiment in which we aim to investigate the importance of 5-HT_{2A} receptor activation for the encoding of reward-related information.

As described previously, both 5-HT and the PFC are of crucial importance for cognitive flexibility in situations where previously relevant stimulus-reward contingencies change and need to be updated. Although the function of different 5-HT receptors in cognitive flexibility is not well understood, 5-HT_{2A} receptors are heavily expressed in the PFC and recent reports have implicated the 5-HT_{2A} receptor in reversal learning (Boulougouris et al., 2008). In these experiments we aimed to directly measure neural activity during a reversal learning experiment and measure the effect of specific 5-HT_{2A} receptor blockade on the encoding of reward-related information. To examine

the role of the 5-HT_{2A} receptor on the processing of affective information, we offered animals two types of rewards, a preferred- and non-preferred reward. The approach we chose for this study was the measuring device described in chapter 6.

These chapters are followed by a general discussion in which the results of each of the chapters is reviewed and an attempt is made to answer the posed questions and come to general conclusions.