Chapter 8

General Discussion

In a series of behavioural experiments we studied the role of (prefrontal) 5-HT in the ability to express cognitive flexibility. We employed reversal learning paradigms to assess various underlying functions of cognitive flexibility, like stimulus-response learning, inhibition processes, and reward discrimination. As such, reversal learning paradigms provide valuable insights into general cognitive processes. Applying current understanding of subdivisions within the prefrontal cortex (PFC) in relation to cognitive functions, we examined both the medial- and orbital part of the PFC, depending on the specific research question. Following initial experiments specifically aimed at 'simple' reversal learning we studied the role of affect in cognitive flexibility.

To assess the role of 5-HT we transiently manipulated whole-brain availability of the monoamine through dietary depletion of the essential amino acid tryptophan (Chapters 2 and 3), and used local intracerebral infusion of the 5-HT toxin, 5,7-dihydroxytryptamine (Chapter 5), to permanently reduce serotonergic innervation. A specific role of the 5-HT_{2A} receptor in the coding of reward-related information was finally addressed through local application of the 5-HT_{2A} receptor antagonist M100907 (Chapter 7). Below, main aims and findings of these experiments are discussed in relation with current literature.

Prefrontal cortical involvement in cognitive flexibility

The paradigm for cognitive flexibility that we employed throughout this thesis is reversal learning. In a typical operant reversal task a subject is presented with two distinct response options, each associated with a particular outcome (i.e. reward or absence of reward). Following acquisition of the discrimination, indicated by a performance criterion, the response-outcome associations are reversed. Upon the reversal the animal needs to adapt its behaviour by inhibiting the previous response and adopting the previously unrewarded response. In the initial experiments we studied the role of 5-HT in reversal learning of spatial information, i.e. the position of the two levers in an operant box (Chapter 2). Typically reversal learning paradigms employ either such 'spatial' cues or use visual or olfactory stimuli to guide responses.

The prefrontal cortex (PFC) is essential for the successful execution of complex flexible behaviour. As discussed in the introduction, the PFC is considered to organize and direct behaviour based on executive functions like planning, goal selection, attention and performance monitoring (e.g. Kolb 1984; Dalley et al., 2004; Chudasama

and Robbins, 2006). Thus, damage to the prefrontal cortex invariably leads to cognitive deficits and impaired flexibility. Based on a functional-anatomical subdivision within the PFC, both the medial PFC (mPFC) and orbital PFC (oPFC) are of particular relevance to the study of reversal learning.

The orbital area of the PFC (oPFC; Fig. 1., Chapter 1) is considered crucial for the ability to successfully execute a reversal task as lesions of the oPFC have repeatedly been shown to cause serious impairments (e.g. Iversen and Mishkin, 1970; Schoenbaum et al., 2002; McAlonan and Brown, 2003). Functionally, the oPFC is thought to facilitate reversal learning by detecting expected outcome (Schoenbaum and Roesch, 2005; Schoenbaum et al., 2009) and the signaling of this information to the basolateral Amygdala (BLA). The BLA in turn utilizes this information to update response-outcome associations which drive behavioural output. According to this model, lesions of the oPFC disrupt performance on reversal tasks through impaired detection of the expected outcome value (Stalnaker et al., 2009). There are however clear indications that reversal learning is not exclusively mediated by the oPFC, but that oPFC involvement might depend on the pertinent task characteristics like type of stimulus. Typically, oPFC involvement in reversal learning has been shown with paradigms that employ a reversal of olfactory- or visual stimuli, whilst experiments in which reversal of spatial locations were studied point to involvement of the medial PFC (mPFC; Fig. 1, Chapter 1).

An important role for the medial PFC in flexibility in general has long been recognized (e.g. Fuster, 1997) and more recent work has implicated medial PFC areas in functions pertaining to cognitive flexibility that could be involved in reversal learning. Ragozzino and colleagues for instance showed that specific inactivation of the prelimbic area of the mPFC resulted in increases in perseverative errors when rats are required to shift their attention, suggesting an important inhibitory function over previously beneficial behavioural strategies (Ragozzino et al., 2003). The infralimbic area of the mPFC on the other hand has been implicated in habit learning, which is behaviour that is no longer goal-directed and is insensitive to reinforcer devaluation (Coutureau and Killcross, 2003). More specifically, data on reversal learning based on spatial cues, instead of olfactory or visual cues, point to an important role of these mPFC areas. Data by De Bruin et al. (2000) for instance indicates that when spatially separated stimuli are used to guide behaviour in a reversal task a transient inactivation of the medial-, but not the lateral PFC (agranular insular cortex), induced impaired reversal learning, possibly through its interconnection with the hippocampus. These results are in line with both earlier and later work that implicate the mPFC in this type of spatial cognitive flexibility (e.g. Divac, 1971; Kolb et al., 1974; Nonnenman et al., 1974; De Bruin et al., 1994; Bussey et al., 1997; Li and Shao, 1998; Salazar et al., 2004), but not in reversal learning of odour or texture discrimination (Birrell and Brown, 2000).

It should be noted, however, that involvement of the mPFC is not always found. Two recent reports showed that lesions of either the infralimbic or prelimbic area (Boulougouris et al., 2007) and inactivation of the prelimbic area (Floresco et al., 2008), did not interfere with task performance in spatial reversal tasks. Boulougouris et al. furthermore showed that complete orbital cortex lesioning did disrupt reversal learning.

Thus, a strict functional dissociation in medial- versus orbital PFC mediation of reversal learning is not likely, these examples rather point to a functional overlap or interaction between prefrontal areas. Moreover, previous research has implicated sub-cortical areas like the striatum in cognitive functions that can be important to reversal learning. Although a thorough review of cortico-striatal circuitry in relation to reversal learning is beyond the scope of this chapter, it is important to briefly discuss recent findings that implicate the striatum in cognitive flexibility.

Like the PFC, important modulation of flexible behaviour has been shown for several striatal areas (e.g. Divac et al., 1967). In general, the dorsomedial striatum has been shown to be involved in the execution of a chosen response strategy (Ragozzino, 2007) whereas the dorsolateral striatum has been implicated in the formation of habit learning, possibly in conjunction with the infralimbic cortex (Tang et al., 2007). In contrast, the ventral striatum, which is an important projection area of the mPFC, is involved in both the acquisition of operant responding for sucrose, and motivational salience of the reinforcer (Pratt and Kelley, 2004) but has also been implicated in the ability to respond to cues predictive of- or associated with reward (Yun et al., 2004). Encoding of reward-location information has furthermore been shown in ventral striatal neurons (Lansink et al., 2007, see also, Ito et al., 2008) as well as the medial PFC (Hok et al., 2005), both of which areas receive hippocampal input of spatial information. Finally, a recent report on medial-dorsal excitotoxic lesions of the medial striatum in monkeys, showed impairments of reversal learning reminiscent of oPFC lesions (Clarke et al., 2008; see also Castañé et al., 2010).

These few examples point to the importance of cortico-striatal projections in the execution of behavioural flexibility and illustrate the need to interpret involvement of particular areas in a system of functionally interconnected brain areas. The anatomical basis for such a functional interaction of the PFC and striatum is extensively discussed by Groenewegen and Uylings (2010). A recent review by Floresco et al. (2008) moreover provides a functional framework within these circuits in which various functions like associative learning, the control of motor output, and more complex functions like the integration of information and planning of future action takes place, each making distinct contributions to higher cognitive functions (see also, Ragozzino, 2007; Pennartz et al., 2009; Kehagia et al., 2010).

Serotonin involvement in reversal learning

Repeatedly, depletion of 5-HT in rats, both in the PFC in general (Harrison et al., 1999), as well as selectively in the oPFC (e.g. Clarke et al., 2004, 2007) has been shown to impair reversal learning. This impairment is furthermore specific for 5-HT as depletion of oPFC dopamine (DA; e.g. Clarke et al., 2007), and general prefrontal reductions of noradrenaline (NA; Tait et al., 2007) do not affect performance on this task. With regard to the selective involvement of oPFC 5-HT as opposed to DA, these data are in line with tracing studies that show fairly dense innervation of serotonergic fibers (Vertes, 1991) whereas dopaminergic neurons only very sparsely innervate the oPFC (van de Werd and Uylings, 2008).

In human subjects, acute tryptophan depletion (ATD), which presumably reduces 5-HT synthesis and release, can induce reversal learning deficits (e.g. Rogers et al., 1999a) as well as impairments in the processing of emotional content and positive- and negative feedback (e.g. Cools et al., 2008a). Although the effects of ATD are of a general nature, modulation of prefrontal activity through ATD has repeatedly been shown (e.g. Rubia et al., 2005). Genetic studies in both humans and non-human primates have furthermore linked variations in the 5-HT transporter gene to performance on reversal tasks. Izquirda et al. (2007) for instance linked genetic variation in the serotonin transporter, which regulates 5-HT turnover, to impaired reversal learning (see also; Vallender et al., 2009). Involvement of specific 5-HT receptors has not been extensively studied but there are indications that both the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor are important for flexibility (Boulougouris et al., 2008, 2010).

Although monoaminergic modulation of, oPFC mediated, reversal learning appears rather specific to 5-HT, certain aspects of reversal learning outside the PFC have been shown to involve other neurotransmitters. Depletion of DA in the prefrontal cortex has repeatedly been shown to leave reversal learning intact, but a recent study by Izquierdo et al. (2010) related loss of DA transporter function in the dorsal striatum, an area connected to both medial- and orbital PFC areas, to impaired reversal learning, possibly through altered oPFC function (but see Daberkow et al., 2008). Dopamine release has furthermore been shown to increase during initial reversal learning in the medial PFC (van der Meulen et al., 2007). Daw et al. (2002) furthermore have described 5-HT and DA to serve opposing roles in the processing of reward (DA) and punishment (5-HT) (see also Dayan and Huys, 2009). These results suggest that involvement of, for instance DA, is possibly mediated through interaction with prefrontal 5-HT. The interaction of the serotonergic system with DA can be direct through projections of serotonergic neurons from the raphe nucleus to both the substantia nigra (SN) and the ventral tegmental area (VTA) where it can inhibit dopaminergic neurons. Both VTA and SN in turn, project back to the raphe nucleus (see for instance, Kapur and Remington, 1996; Di Matteo et al., 2008) as well as to the striatum where DA modulates both

dorsomedial- and ventral striatal function (e.g. Yun et al., 2004; Haluk and Floresco, 2009). In addition, serotonergic projections have been shown to modulate DA release in the striatum (Jones and Kauer, 1999). Such interactions could facilitate functional differentiation between 5-HT and DA in cognitive functions.

An important aim of the experiments described in this thesis was to further elucidate the role of prefrontal 5-HT in cognitive flexibility. The starting point of our experiments pertained to the question to what extent reversal learning of spatial information (de Bruin et al, 2000), as a measure of flexibility, would rely on 5-HT in general, or more specifically on the serotonergic innervation of the medial PFC, like it has been shown for the oPFC when olfactory or visual stimuli are used.

For the assessment of the role of 5-HT we first used a method of 5-HT depletion that is generally applicable in human and animal studies but has become particularly popular in human research, acute tryptophan depletion (ATD). Through a dietary depletion of the essential amino acid tryptophan, synthesis of 5-HT is reduced (Gessa et al., 1974; Nishizawa et al., 1997) which leads in turn to a lowering of central 5-HT levels (Ashley and Curzon, 1981; Fadda et al., 2000a; Lieben et al., 2004b). When applied acutely, ATD provides a time-window of several hours in which the performance of test subjects can be assessed.

Contrary to our expectations the data presented in chapter 2 showed that despite an ATD induced increase in general locomotor activity, ATD did not affect task acquisition, reversal or extinction learning, suggesting that 5-HT is not strongly involved in the execution of a spatial reversal task. However, as discussed below, the lack of effect of ATD might not be due to a lack of 5-HT involvement.

Evidence for ATD induced reduction of 5-HT release and transmission and implications for the interpretation of ATD-based studies.

Our experimental data failed to implicate 5-HT in reversal learning of spatial information and thus suggests that the involvement of 5-HT in such tasks is stimulus modality dependent. However, the results of a follow-up study on the relation of ATD and changes in 5-HT release cast doubt over the validity of ATD as a method to reduce the release of central 5-HT. That, in turn, implies that an insufficient reduction of central 5-HT release induced by ATD might be responsible for our negative finding.

ATD is generally used as the preferred method in human research to study the role of 5-HT and experiments conducted with this method have implicated 5-HT in a range of cognitive functions, mood and the processing of affective information. Direct evidence for ATD induced reductions of 5-HT release is, however, lacking or indirect at best. To assess the extent to which ATD can reduce the release of 5-HT we combined ATD with measurements of extracellular 5-HT through microdialysis in the

PFC and compared the measured reduction in plasma tryptophan with other studies. As it is conceivable that ATD can affect the availability and release of DA in the brain, through increased central tyrosine availability or direct 5-HT – DA interactions, we also included measurements of DA-release in our experiment. Moreover, knowledge regarding the effects of ATD on central DA release would provide valuable insight into the way ATD affects cognitive and affective functioning.

Surprisingly, our data opposed the general assumption that ATD reduces central 5-HT release. In our experiment we observed a rapid decrease in plasma tryptophan levels, similar to those observed in other studies, but we did not observe a reduction of 5-HT release in the medial PFC. These results bear several implications. With regard to the results of our reversal study and our conclusion that 5-HT is not critically important for spatial reversal learning, these findings suggested that the ATD manipulation did not reduce 5-HT release sufficiently well to induce impairments in cognitive flexibility. This would imply that our finding that 5-HT is not involved in spatial reversal learning might not be entirely correct. A second implication that potentially has a far greater impact is that the relation between ATD and its behavioural effects is more complex than what is often assumed. ATD might not exert its effects through a synthesis-induced reduction of central 5-HT release and transmission. In an attempt to reconcile this finding with current literature we reviewed both behavioural and cognitive effects of ATD described in the literature as well as the neuropharmacological evidence for the effect of ATD on 5-HT release.

Through the use of ATD, 5-HT has repeatedly been implicated in cognitive functions like decision-making, memory function (e.g. Rogers et al., 1999a; Finger et al., 2007; Park et al., 1994, Lieben et al., 2004a), but also affective states like depression and aggression (Fadda, 2000; Riedel et al., 2002). Functional magnetic resonance imaging has furthermore shown functional changes in prefrontal activity following ATD (Rubia et al., 2005; Allen et al., 2006; Evers et al., 2005). There is thus ample evidence of ATD mediated effects on both brain activity as well as behavioural output. However, effects on cognitive functions are not always reproducible and the effects on mood have primarily been shown in human subpopulations that show vulnerability for 5-HT dysfunction, like remitted depressed patients (Bremner et al., 1997; Young and Leyton, 2002; Neumeister et al., 2003) and people who show variations in the 5-HT transporter gene (Roiser et al., 2006; Finger et al., 2007). The latter suggests that the effectiveness of ATD to manipulate 5-HT release depends on a subject's sensitivity to 5-HT depletion, which is a possible reason for the moderate effects of ATD on central 5-HT when groups are compared.

Neurochemically there is limited support for the effectiveness of ATD to reduce 5-HT release; while reductions have been reported after chronic trypthophan depletion or co-administration of selective serotonin reuptake inhibitors (SSRI's), our data is,

to the best of our knowledge, the first to measure prefrontal 5-HT release in a setting similar to that employed in human research. Our data show that reductions in plasma concentrations of tryptophan, similar in extent to levels observed in human studies, do not necessarily reflect central reductions of 5-HT efflux (van der Plasse et al., 2007a). In terms of the consequences of these findings for the interpretation of ATD-induced effects on cognition and behaviour, and the validity of the assumption that ATD-induced effects are (central) serotonergic in nature, caution is warranted. Despite the fact that it is now widely reported that ATD is mainly effective in inducing behavioural effects in vulnerable groups and thus might not functionally affect 5-HT function in all test subjects, effects of ATD are still more often than not interpreted as reflecting central serotonergic function in all subjects. Although such an interpretation might (partially) hold for vulnerable groups, it is unclear to what extent 5-HT release has been reduced following ATD, if at all. Despite these concerns ATD-based studies still form the basis of novel theories of the role of 5-HT in behaviour and cognition (e.g. Cools et al., 2008b; Robinson and Sahakian, 2009; Robinson et al., 2010; Ruhé et al., 2007; Crockett et al., 2009).

The question that remains is, if not through 5-HT depletion, how ATD exerts it effect on cognition and affective processing. One possible explanation involves (central) interactions of 5-HT and other neurotransmitters (e.g. Fusar-Poli et al., 2006; Praschak-Rieder et al., 2004). Evidence for this, however, is sparse and rodent studies aimed at measuring direct effects of ATD on other central and peripheral monoamines show that ATD neither affects central concentration of NA and DA (Ardis et al., 2009; Lieben et al., 2004b) nor the release (Fadda et al., 2000a; van der Plasse et al., 2007a). The levels of tyrosine, the precursor to the catecholamines DA en NA, were not consistently altered either (Lieben et al., 2004b; Jans et al., 2007a). Similar findings were reported in human CSF measurements, suggesting that the effects of ATD can not be explained by changes in NA or DA. Alternatively, experiments by Kilkens et al. (2004) for instance point out that 5-HT is a key transmitter in the bidirectional communication between 'cognitive and emotional centers in the brain, the neuroendocrine centres, the enteric nervous system and the immune system'. This suggests that ATD can influence central processes through peripheral action and as such can link somatic and psychiatric processes (Russo et al., 2003). The notion that ATD affects other processes than central 5-HT and can thus act through peripheral systems is not unlikely as serotonin has, for instance, been implicated as an important modulator of cardiovascular and gastrointestinal activity.

Further experiments in which the effects of ATD are assessed in conjunction with candidate (peripheral) mediators can clarify the mechanism through which ATD affects cognition and affect. Alternatively, further neurochemical measurements following ATD, e.g. microdialysis studies in other brain areas, can shed light on the neurochemical effects of ATD.

In terms of our hypothesis that 5-HT mediates reversal learning of spatial information, the current ATD results did not provide conclusive evidence that 5-HT is not involved in spatial reversal learning. We therefore abandoned ATD as a method to study 5-HT function and switched to lesioning of serotonergic terminals in the mPFC by means of intracerebral microinfusions of the toxin 5,7-dihydroxytryptamine (5,7-DHT).

Depletion of medial prefrontal 5-HT; effects on cognitive flexibility

Following our ATD studies we retested our hypothesis that 5-HT in the medial PFC is important for reversal learning of spatial information. In addition we tested our hypothesis that serotonin is especially important when affect guides decision-making (i.e. the ability to evaluate and integrate emotional content, see for instance, Hariri et al., 2006) by manipulating the reward value. This hypothesis stems in part from clinical studies that show that modulation of serotonergic neurotransmission can effectively treat affective disorders like major depression and obsessive compulsive disorder. Also ATD studies in humans have linked 5-HT depletion to impaired reward discrimination (Rubinsztein et al., 2001; Rogers et al., 2003; see also Roberts and Wallis 2000) and recently to specific impairments in the prediction of aversive signals (Cools et al., 2008a; Crockett et al., 2009, but see, Rogers et al., 1999a for contrasting results). Such changes in affective processing (see also Remijnse et al., 2005) have, however, not yet been reported in studies involving selective prefrontal 5-HT lesions in either primates or in rats. Moreover, given the negative results of our ATD studies it is of great importance to find evidence for a role of 5-HT in affective processing in non-human animals. An important note is that we consider affect as different from mood, in that affect involves transient 'emotion-like' states brought on by particular stimuli (like preferred and non-preferred rewards) whereas mood can be considered as a more stable, 'background' state, like the altered mood that is present in for instance chronic depression (Robinson and Sahakian, 2009).

Microinfusion of the 5-HT toxin 5,7-dihydroxytryptamine successfully reduced 5-HT concentrations in the medial PFC without affecting neighbouring areas or inducing depletion of dopaminergic or noradrenergic innervation. Following lesioning we subjected the animals to two reversal learning paradigms, based on spatial- and olfactory stimuli. Reversal learning of visual information in primates and of olfactory- and tactile information in rodents has been shown to depend on the serotonergic innervation of the orbital PFC. However, as discussed previously ("Prefrontal cortical involvement in cognitive flexibility") the necessity of 5-HT transmission might not be limited to reversal learning of visual and olfactory stimuli, but also when stimuli need to be reversed based on their location.

In addition to the results from our ATD reversal learning experiment, the data from the lesion experiment provided evidence against our hypothesis by showing that reversal learning per se is not mediated by medial prefrontal 5-HT. The data showed that lesioned animals are fully capable of adapting their behaviour in both a spatial- and olfactory reversal task despite mPFC 5-HT depletion. Moreover, 5-HT depletion did not impair response inhibition, in either the no-go trials or when rewards are no longer given (extinction learning). However, our idea that 5-HT in this area of the PFC is important for reversal learning when affect guides decision-making was supported by our data. In both the spatial- as well as the olfactory task we observed that depletion of medial PFC 5-HT prevented the impairment of reversal learning when the reward identity was changed. This was shown in two different experiments. Firstly sham control animals suffered impaired performance in the spatial task when the reward was changed from a non-preferred to a preferred pellet, due to an increase in erroneous responding. Secondly we observed reduced motivation during the subsequent olfactory task in which sham animals showed an increase in omissions after repeated testing. In both cases, the lesioned animals performed better than controls. Although these data suggest that mPFC 5-HT depletion is beneficial for the animal as it prevents an impairment of flexible behaviour, what it actually illustrates is the importance of 5-HT in the medial PFC for adapting behaviour when the reward value changes. We thus interpret this finding as an inability of lesioned animals to accurately use reward value cues to guide decision-making.

These data seem to corroborate findings in humans that 5-HT plays an important role in the processing of reward and affective information (e.g. Rogers et al., 2003; Remijnse et al., 2005; Hariri et al., 2006). Of special interest in this context are the results of two recent studies on mood and affect. The first is a meta-analysis of human monoamine depletion studies (Ruhé et al., 2007) that indicates that mood is not affected by ATD treatment in healthy male volunteers (but is in women). The second is a study by Robinson and Sahakian (2009) that shows a dissociation between affect and mood in human subjects after ATD. These authors report that 5-HT is important for the processing of affective information, and support previous findings that ATD does not exert its effect on affect through modulation of mood state. Importantly it should be noted that this does not mean that mood state can not affect ATD-induced cognitive effects (Robinson et al., 2010). A matter that remains to be adequately addressed in these and other ATD studies is, that ATD might not functionally reduce 5-HT in all subjects and thus, that conclusions about the serotonergic nature of ATD induced (cognitive) effects remains speculative.

The current data supplements these findings in human subjects by implicating specifically mPFC 5-HT in the processing of affective information. Although lesions and measurements of this prefrontal area have previously been shown to impair the

processing of reward-related information (e.g. Cardinal et al., 2001; Hok et al., 2005), to the best of our knowledge this is the first study to directly show medial PFC 5-HT mediated involvement in affective processing. Despite the fact that the human medial PFC and rodent mPFC show a partial functional and anatomical overlap (Uylings et al., 2003), it is important to note these areas are not considered as fully homologous. The data do, however, fit with the notion that the human PFC acts as a control center that regulates the 'active maintenance of patterns of activity that represent goals and the means to achieve them' (Miller, 2000). More precisely, it is hypothesized that the human mPFC uses reward information to signal lateral areas of the PFC to adapt behavioural output (Ridderinkhof et al., 2004). With respect to such a monitoring and signaling role for medial PFC, our data, although not assessed directly, does show that depletion of mPFC 5-HT can affect behaviour of a 'typical' oPFC reversal task, namely olfactory reversal learning. Additional (disconnection) studies could further be employed to examine this interaction in greater detail.

Interestingly, it is the orbital PFC that has been shown to be a specialized region for the processing of reward and punishment information (e.g. Kringelbach, 2005; Feierstein et al., 2006). As such, neurons in the oPFC have been shown to respond to the motivational salience of reinforcers and integrate this with current goals and actions. In addition to the oPFC, the current findings suggest that the medial PFC for the rat, and specifically mPFC 5-HT, is important for the processing of reward-related information too, as lesions of serotonergic terminals in this area impair the ability to respond to changes in reward value. In contrast, 5-HT depletion did not affect the ability to extinguish responding in the absence of reward. These data indicate that the mPFC and oPFC mediate complementary functions, although direct mPFC – oPFC projections are sparse, conversion of projections in striatal areas, as discussed previously, possibly underlies behavioural integration (Ongür and Price, 2000; Uylings et al., 2003; Floresco et al., Groenewegen and Uylings, 2010).

Neural correlates of reversal learning; role of the 5-HT$_{2A}$ receptor in the encoding of reward-related information in the orbital PFC.

Following the experiments aimed at spatial reversal learning and the role of 5-HT therein, and in light of our finding that medial PFC 5-HT depletion does not impair reversal learning per se, we shifted our focus on the orbital PFC and the coding of reward-related information. The choice for the orbital PFC in these experiments is based on involvement of the orbital PFC in the processing of reward-related, affective information (Tremblay and Schultz, 1999) and reversal learning, as discussed in detail previously. Neurons of the oPFC have been shown to code specific reward-size, relative value, and reward identity. Moreover work by Rolls et al. (1996) has shown

that individual neurons in the oPFC can reverse their cue-selective firing in response to a task reversal (see also Thorpe et al., 1983; Schoenbaum et al., 1999; Stalnaker 2006). The exact role of these neurons is however unclear as a negative correlation has been observed between the number of neurons that show adaptive coding and task performance (Schoenbaum et al., 2009; see also, Stalnaker et al., 2009). In addition, neurons in the oPFC are found to be capable of coding the expected outcome which could be utilized as a trigger to change behaviour when predicted- and actual outcome do not match (e.g. Rosenkilde et al., 1981). The orbital PFC can thus function as an area that enables the 'updating' for current stimulus-outcome associations, code the affective value of a certain outcome, and use this information to guide behaviour.

As mentioned in previous sections, oPFC 5-HT is important for reversal learning and related functions like perseverative responding (Beninger and Phillips, 1979; Morgan et al., 2003) and impaired impulse inhibition (Winstanley et al., 2004a,b, 2005). However, relatively little is known about the way in which 5-HT is involved in reversal learning on the level of individual neurons and thus, how 5-HT acts on a neuronal level. In light of our aim to clarify the involvement of 5-HT in cognitive flexibility and the coding of affective information, we studied how 5-HT interacts with oPFC neurons in encoding reward-related information.

Although in vitro measurements of neural responses to 5-HT can provide important information, these studies provide no insight into the role of 5-HT on a cellular level, in a behaving animal. For this reason we developed a measuring device, i.e. the *combidrive*, which allows the simultaneous recording of multiple neurons during the local administration of selective drugs, in freely moving animals (van Duuren et al., 2007b).

Our validation experiments of the combidrive, described in chapter 6, illustrate that the drive developed for this task is capable of measuring the effect of drug perfusion on neuronal activity in freely moving animals. The validation experiments made a comparison of three commonly used inhibitors of neuronal activity, tetrodotoxin (TTX), lidocaine and muscimol. The results showed the differential inhibitory effect on firing frequency and the subsequent recovery of activity and led to insight in the, in vivo, duration and potency of effects of each of these inhibitors on neuronal activity. These experiments have shown that the combidrive offers a new method to study neuronal function with the advantage over 'in vitro' experiments that it is possible to study drug-cell interaction in the intact brain, and the possibility to do so in relation to behaviour.

Following the validation experiments we used the combidrive in an experiment that aimed to measure the effect of the selective 5-HT$_{2A}$ antagonist M100907 (Knauer et al., 2008) on the encoding of (affective) reward-related information during an olfactory discrimination and reversal learning task. As discussed in detail before, the importance

of the orbital PFC for reversal learning of olfactory information and coding of reward information is well established. However, if 5-HT modulates reversal learning on a single cell level through alterations in the encoding of reward-related information is not known.

As set out in the introduction of this thesis, at least 14 receptor subtypes for 5-HT are identified (Barnes and Sharp, 1999) of which several are known to be of importance for cognitive functions. Although the specific contribution of each subtype in affect and cognition is poorly understood and reports are to some extent contradictory, there are indications that 5-HT_{2A} receptor activation affects both affect and cognition. 5-HT_{2A} receptor antagonism as augmentation strategy in depressed patients has been shown to decrease depression scores (e.g. Marek et al., 2003; Sato et al., 2009), and specific cognitive deficits associated with depression, like impaired memory function and attentional deficits (through increased impulsivity) improve following 5-HT_{2A} receptor antagonism (Berg et al., 2008). If 5-HT_{2A} antagonism improves the, often poor, performance on reversal learning in depressed patients, is unclear, although a recent experiment by Boulougouris et al. (2008) in rats suggests that this might not be the case. In their study the effect of 5-HT receptor contribution to spatial reversal learning was examined. They compared the behavioural effects of 5-HT_{2A} and 5-HT_{2C} receptor antagonism on cognitive flexibility and reported impaired reversal learning following antagonism of the 5-HT_{2A} receptor, while antagonism of the 5-HT_{2C} receptor increased performance on the same measure.

In our reversal experiment we tested rats daily on an olfactory reversal task. Each day rats were trained in an acquisition session to discriminate new odour-outcome associations and reverse these in a subsequent reversal session. Throughout the day individual neurons were recorded during both sessions and their firing activity was correlated to events in the behavioural task. To assess the role of 5-HT_{2A} receptor in the oPFC in the coding of reward-related information we compared the effect of the locally applied selective antagonist M100907 (Knauer et al., 2008) with a control of artificial cerebrospinal fluid (aCSF), through reverse microdialysis.

Our data is the first to show that antagonism of the 5-HT_{2A} receptor in the oPFC directly affects the encoding of trial outcome. During the acquisition of the odour discrimination (morning session) control animals showed a greater number of correlates for the rewarded trials than unrewarded trials. In contrast, following perfusion of the 5-HT_{2A} antagonist during task acquisition this differential firing was not found, causing an equal number of neurons to respond to rewarded and unrewarded trials. No effect of drug perfusion was found for either the total number of cells that showed task-related activity or the relative number of correlates these cells showed. These results suggest that drug perfusion specifically interfered with the initial encoding of the stimulus – reward association. Behaviourally, the unilateral drug perfusion did not affect task

performance as both groups showed equal task performance during acquisition and reversal sessions. Differences were found only on a neuronal level, neither changes in number of cells per session nor firing rate were observed; only the distribution of event-related firing over rewarded and unrewarded trials differed. During both control and drug sessions all types of responses were found.

In line with the study described in Chapter 5 we included two rewards in these experiments that were differentially preferred. In this case the purpose was to study how 5-HT$_{2A}$ receptor blockage affects the encoding of reward-related and affective information.

Despite the fact that rats clearly showed differential preference for the two rewards, this preference was not reflected in the neuronal activity. Whereas Watanabe (1996) reported that a sub-set of (medial) prefrontal neurons increased their firing activity with increased preference (see also Tremblay et al., 1999, oPFC), a unidirectional response to the differentially preferred rewards in the current experiment was not observed. We did observe that individual neurons discriminated between preferred- and non-preferred rewards in terms of firing frequency, but these neurons responded with either an in- or decrease in firing activity following reward-presentation. No differences in terms of total number of neurons that responded to reward-type were observed either. These data are consistent with earlier data (van Duuren et al., 2007a) suggesting that coding of reward preference in the rat oPFC is qualitatively different from coding in the primate cortex. Drug perfusion did not affect this response pattern. Of interest is the contrast with data presented in chapter 5 that showed mPFC 5-HT involvement in the processing of reward-value (preferred vs. non-preferred) rather than outcome (positive-negative).

These data thus suggest a possible dissociation in serotonergic function between mPFC mediated processing of affective value and oPFC involvement in the encoding of stimulus – outcome information. In contrast to our expectation, receptor blockade did not affect the encoding of the affective value of the reward, but rather the positive- or negative outcome. Based on the possible involvement of 5-HT in the processing of affective information (e.g. Rogers et al., 1999a) and the role of the oPFC in the encoding of reward preference (Tremblay and Schultz, 1999) we expected that 5-HT$_{2A}$ receptor blockage would affect the former rather than the latter. Watanabe (1996) did, however, report that the encoding of relative reward preference is also observed in the medial PFC.

The current data are reminiscent of tryptophan depletion studies in humans that reported altered feedback processing following 5-HT depletion (e.g. Cools et al., 2008b; Roiser et al., 2006; Rogers et al., 2003; see also Bari et al., 2010). Based on these studies 5-HT is thought to modulate sensitivity to positive- and negative feedback, a reduction

serotonergic availability is suggested to increase sensitivity to negative feedback. The current data provide a possible mechanism through which 5-HT depletion can induce such a bias.

In light of our aim to clarify the role of oPFC 5-HT in reversal learning, we aimed to study the effect of 5-HT$_{2A}$ receptor blockade on adaptive coding in cue-sensitive neurons. Previously Rolls (1996) described neurons in the cortex that reverse their stimulus – outcome association following a task reversal (e.g. Stalnaker et al., 2006). Although the importance of such neurons for successful reversal learning is unclear (Schoenbaum et al., 2009) 5-HT might modulate task performance through these neurons. However, the absence of a significant number of neurons that reversed their response following task reversal, in both control- and drug sessions, prevented such a comparison. Based on the lack of effect of local blockade of 5-HT$_{2A}$ receptors in the oPFC (Boulougouris et al., 2010) and the apparent inverse relation that reversal-neurons have on task performance (e.g. Stalnaker et al., 2006) it was, however, expected that no differences would be found.

A clear effect of the antagonist on reversal learning that could explain the observed effects of oPFC 5-HT depletion on reversal learning (e.g. Clarke et al., 2004, 2007) was not found. The data showed that perfusion of the oPFC with the antagonist during reversal learning did not induce any observable difference in event-related firing compared with control sessions. These results suggest that although the 5-HT$_{2A}$ receptor is important for the initial encoding of stimulus – outcome associations it has no direct role in the mediation of 5-HT mediated reversal learning. Such a conclusion is supported by recent findings of Boulougouris and colleagues (Boulougouris and Robbins, 2010). These authors previously reported impaired reversal learning following systemic administration of the 5-HT$_{2A}$ antagonist M100907 (Boulougouris et al., 2008) but have recently shown that local infusions of the same antagonist in the mPFC, oPFC and nucleus accumbens does not interfere with behavioural performance on a spatial reversal task (Boulougouris and Robbins, 2010).

However, based on these data, a role for the 5-HT$_{2A}$ receptor in reversal learning can not be excluded. An 'over expression' of cues associated with unrewarded trials like observed in the current experiment might not interfere with behavioural performance during task acquisition as it conceivably strengthens the association between stimulus and outcome without affecting the speed or accuracy of task acquisition. However, such a strengthened association could in turn, impair performance during the reversal phase of the task when the stimulus – reward association needs to be reversed. Whereas involvement of 5-HT in the acquisition of stimulus – reward associations has been shown before (e.g. Ward et al., 1999; Harrison et al., 1999), the effect of 5-HT$_{2A}$ antagonism on performance on task acquisition has not been addressed. Moreover, it is

known that performance during task acquisition can affect reversal-learning (Robinson and Storm, 1978), possibly through altered stimulus – reward association. Exactly what the relation is between stimulus – reward coding in the oPFC and success of reversal learning on a behavioural level remains to be examined.

Taken together, these findings indicate that 5-HT, through 5-HT$_{2A}$ receptor mediated action, can modulate the encoding of stimulus - outcome information in the oPFC. As such, these data are particularly relevant for the understanding of 5-HT mediated effects on cognitive flexibility and the processing of reward-related information.