Neural coding of attention and attentional set shifting in the rat medial prefrontal cortex
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Chapter 1

Introduction

About the illustration: Multimodal stimulus (MMS) chamber and a model rat with a hyperdrive which is sampling stimuli inside the chamber.
Imagine, you just moved from Amsterdam to Korea for a new job. Your boss invited you and your colleagues for a dinner in a Korean restaurant for your welcoming party. When you arrived in the restaurant, your colleagues took off their shoes and sat on the floor around tables instead of on a chair with shoes. They used a spoon and chopsticks instead of knife and fork. When you tried to cheek-kiss instead of shaking hands for saying goodbye after the dinner, your colleagues were shocked and would run away from you. To ensure your social well-being in the new environment, you would need to adjust your behavior. This is an example of cognitive flexibility, which is the ability to change one’s knowledge and adapt one’s response to varying environmental demands (Spiro et al., 1992), which is a hallmark of executive brain functions.

The term ‘executive function’ is ill-defined because it includes a number of rather heterogeneous phenomena such as control of attention, anticipation, planning, decision making, and inhibitory control over response behavior (Shallice, 1982; Fuster, 1985; Goldman-Rakic, 1995b; Smith and Jonides, 1999; Stuss and Alexander, 2000; Fuster, 2008). Executive functions are thought to be mediated by several cooperating brain areas (Baddeley and Della Sala, 1996; Funahashi, 2001; Miller and Cohen, 2001). Sometimes executive functions are cast in terms of goal-directed behavior (Balleine and Dickinson, 1998; Corbetta and Shulman, 2002; Ridderinkhof et al., 2002; Laurens et al., 2005) or top-down cognitive control (Tomita et al., 1999; Miller and D’Esposito, 2005; Rossi et al., 2007; Buschman and Miller, 2007). In this thesis, executive functioning is predominantly captured by the term ‘top-down cognitive control’, which is defined as the control derived from previous experience, knowledge or internal representations of goals rather than intrinsic properties of stimuli present in the environment (Tomita et al., 1999; Hopfinger et al., 2000; Frith, 2001; Miller and Cohen, 2001; Corbetta and Shulman, 2002; Miller and D’Esposito, 2005; Buschman and Miller, 2007; Johnston et al., 2007).

One of the main brain areas involved in cognitive control is the prefrontal cortex (PFC) and not surprisingly, patients suffering PFC damage or prefrontal dysfunction - such as schizophrenic and Parkinson’s patients - show high distractibility under conditions of attentional conflict (Chao and Knight, 1995; Rogers et al., 1998; Cools et al., 2009) and difficulties in shifting attention when previously irrelevant information
becomes relevant (Goldberg et al., 1987; Downes et al., 1989; Owen et al., 1993; Elliott et al., 1995; Jazbec et al., 2007).

Much previous neurophysiological work on cognitive control focused on its neural correlates in the PFC of primates trained to execute well-controlled behavior with low variability. For instance, neurons in the dorsolateral PFC (dLPFC) showed correlations between their firing rate and working memory - a type of memory that maintains information actively and ‘on-line’ (Fuster, 1973; Goldman-Rakic, 1995a) - and representation of task relevant information (Rainer et al., 1998), goal-directed behavior in time (Fuster et al., 2000), abstract rule learning (White and Wise, 1999; Wallis et al., 2001), representation of action sequences (Averbeck et al., 2006) and top-down control of attention (Bushman and Miller, 2007). Activity in another region of the prefrontal cortex, viz. the orbitofrontal cortex (OFC; Fig.1-1), correlates, amongst others, with reward expectancy (Watanabe, 1996; Tremblay and Schultz, 1999; Hikosaka and Watanabe, 2004), reward value coding (Padoa-Schioppa and Assad, 2006) and emotional (Rolls, 2004) and behavioral inhibition (Szatkowska et al., 2007). The dorsomedially located anterior cingulate cortex (ACC) exhibits response activity correlating with action selection in complicated response conditions (Isomura et al., 2003) and error detection (Ito et al., 2003). In these studies, each cognitive factor was mainly expressed by an enhancement of firing activity of a neuronal subpopulation in the brain area that was recorded from.

Most rodent work on cognitive control was based on brain lesion studies and pharmacological manipulations, applied to several behavioral paradigms that have been developed over the past two decades. Rats with mPFC lesions showed impairments in vigilance, attention and contextually guided behavioral selection (Miner et al., 1997; Broersen, 2000; Birrell and Brown, 2000). In particular, selective and partial inactivation of the prelimbic cortex produced a loss of cognitive control under conditions of response conflict (Marquis et al., 2007). Manipulations of neuromodulatory systems in rat mPFC, particularly of noradrenalin (NA), serotonin (5-HT), acetylcholine (Ach) and dopamine (DA)-releasing fibers have been reported to affect cognitive control (reviews: Dalley et al., 2004; Sarter et al., 2005; Robbins and Arnsten, 2009). Recently, neurophysiological studies on the PFC of the rat were dramatically expanded, owing to the multi-electrode recording technique. These studies focused on varying cognitive capacities, such as working memory (Chang et al., 2002; Baeg et al., 2003; Euston and McNaughton, 2006), operant conditioning (Mulder et al., 2000), place-reward association (Pratt and Mizumori, 2001), egocentric versus allocentric navigation strategies (Rich and Shapiro, 2009) and rule learning (Peyrache et al., 2009). The results showed, amongst others, that the PFC represents relevant task components, mainly by an enhancement of neural activity in mPFC, similar to the results of primate studies. Indeed, although rat mPFC and dLPFC in primates are anatomically distinct (Preuss, 1995), functional similarities between these areas have been indicated (Ongur and Price,
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2000; Brown and Bowman, 2002; Uylings et al., 2003). In rat in vivo electrophysiology, like in primate studies, behavioral variability is required to be controlled as much as possible, in order to dissociate neural correlates of motor behavior from those mediating cognitive control and attentional functions (Euston and McNaughton, 2006; Cowen and McNaughton, 2007).

Altogether, many studies have been conducted to understand the neural basis of cognitive control in humans, non-human primates and rodents. However, dynamic firing patterns of neural ensembles in the brain have been insufficiently characterized in relation to attention and attentional set shifting. Attentional set can be defined as an internal representation of a disposition to respond to particular elements of a task or cognitive problem; attentional set shifting refers to the control processes involved in the flexible shift of attentional set from one to another (Buchwald et al., 1975; Dias et al., 1996b). Moreover, very little is known about the neurophysiological correlates of cognitive control in the rat, particularly under conditions of high behavioral regularity, offering ways to exclude sensorimotor confounds.

Hence, the general aim of this Ph.D. project was to improve our understanding of the neural basis of some cardinal prefrontal functions: directing attention to relevant information while ignoring irrelevant inputs, and shifting of attentional set when a task rule is changed. Before moving into the details of the project, this chapter will first provide some background knowledge, starting with theories of attention and cognitive control.

Theory of attention and cognitive control

Attention is considered a core cognitive function that operates at a systems level, involving many cooperating brain areas (Posner and Petersen, 1990; Cohen et al., 2004). The key characteristic of attention is selection and it can be defined, at a neural level, as enhanced activity for processing a relevant stimulus and suppressed activity for irrelevant stimuli during information processing (Pashler, 1998; Posner, 2004). According to Mackintosh’s theory of selective attention in discrimination learning (Mackintosh, 1975), it is represented as a “chaining” model, in which the subject first attends to a set of stimuli on each trial (sensory aspects of attention) and then produces responses determined by the stimuli attended (executive attention). Which stimuli are relevant or irrelevant is defined by reinforcing feedback. Furthermore, the theory posits an inverse relationship between the probabilities of attending to different stimuli; if the probability for one stimulus goes up, those for others must go down due to any organism’s limited capacity to process information. As an extension of Mackintosh’s theory, Fuster has pointed out that the prefrontal cortex (PFC) is located at the high end
of a hierarchy of the brain’s sensorimotor processing system and bridges temporal gaps between percepts and actions adaptively suited to the current environment (Fuster, 1990). Therefore, the PFC is essential not only for the sensory aspects of attention but also for the motor (executive) and, moreover, temporal aspects of attention. Miller and Cohen proposed that the PFC functions as an integrator of behaviorally relevant information coded in other brain areas and guides behavior by internal representation of a goal (Miller and Cohen, 2001). Moreover, based on previous results (Dias et al., 1996b; Miller et al., 1996; Rainer et al., 1998), they specified that active maintenance and updating of internal representations are requirements for proper attentional control by the PFC as well as for flexibility of cognitive control. Some types of PFC dysfunction can be explained as a consequence of inadequate updating processes, especially when these dysfunctions involve perseveration or increased distractibility (Goldberg et al., 1987; Chao and Knight, 1995; Miller and Cohen, 2001; Cohen et al., 2004). Cohen, Aston-Jones and co-workers have proposed that DA and NE may play a crucial role in these functions (Cohen et al., 1996; Aston-Jones et al., 1999). We will discuss the roles of the neuromodulators in attentional set shifting processes more intensively in chapter 6. Especially, in addition to selective or ‘focussed’ attention, Aston-Jones and colleagues described a “screening” type of attention for updating internal representation under varying environmental circumstances which may be regulated by NA (Aston-Jones et al., 1999). So far, we have indicated four main aspects of attention, i.e. sensory, executive, temporal and screening aspects. Working memory, attentional set shifting and response inhibition are all processes under the control of executive attention and the PFC is believed to play a crucial role in this control process (Fuster, 1990; Miller and Cohen, 2001; Corbetta and Shulman, 2002).

Based on the theories indicated above, we hypothesize that the process of attentional set shifting involves the formation of an altered representation of a current goal, a process to which neural assemblies in mPFC contribute. This formation of a novel goal representation will concur with an inhibition of the ‘old’ set, representing a previous goal, and with the formation of a new stimulus-response association (Mackintosh, 1975; Fuster, 1990; Miller and Cohen, 2001). Moreover, in a hierarchical manner, a specific internal representation would be selectively attracted first by reinforcement feedback and then, the subject would identify which exemplar of this internal representation or dimension is rewarded or punished prior to response selection (Sutherland and Mackintosh, 1971; Robbins, 2007). Regardless of the precise hierarchy of attentional allocation, one may hypothesize that when previously irrelevant dimension becomes relevant, attention needs to be redirected to a specific dimension as well as to a specific stimulus, prior to response selection. In the current thesis we were interested in investigating whether the activity patterns of a set of recorded mPFC neurons are rearranged during this reallocation process.
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The Wisconsin Card Sorting Test and multimodal attentional set shifting tasks as tools to dissociate components of cognitive control

Although related theoretical frameworks of executive function were developed more than three decades ago, only over the past 10 years the research on the neural basis of cognitive controls in human, non-human primate and rodents has increased remarkably. This growth owed a great deal to the design of well-defined cognitive tasks which can dissect different types of cognitive control, such as the Wisconsin card sorting task (Anderson et al., 1991; Heaton et al., 1993; Mansouri et al., 2006), the Stroop task (MacLeod, 1991; MacDonald et al., 2000), task set switching (Rubinstein et al., 2001; Braver et al., 2003; Ragozzino et al., 2003) and tasks for analyzing specific aspects of attentional set shifting (Owen et al., 1991; Dias et al., 1996a; Birrell and Brown, 2000). As this Ph.D. project was mainly concerned with the attentional set shifting paradigm, the Wisconsin Card Sorting Test and attentional set shifting tasks will be dealt with in a more detailed way.

The Wisconsin Card Sorting Test (WCST), developed by Grant and Berg (Grant and Berg, 1948; Heaton et al., 1993), has been widely used to examine cognitive flexibility in human subjects. It requires subjects to find a valid rule for sorting cards displaying items with different properties by trial and error. This sorting rule is often based on three perceptual dimensions (properties) such as the shape, color or number of objects depicted on the cards. An examiner gives feedback to the subject as to whether her way of sorting the cards was correct or not. Once the subject has chosen the correct rule, it must apply it for ten consecutive correct matches. After these trials, the rule changes without any warning. Therefore, the subject is demanded to flexibly shift to another task rule. Although the WCST has often been used to examine PFC function, this test fails to distinguish between frontal and non-frontal lesions (Anderson et al., 1991; van den Broek et al., 1993; Axelrod et al., 1996), amongst others because task performance requires many different executive functions such as monitoring of current external and internal states, integration of feedback, rule learning or formation of an attentional set, and suppression of previous sorting rules (Ridderinkhof et al., 2002; Kopp et al., 2006; Nyhus and Barcelo, 2009). Among the many cognitive components involved in the WCST task, set shifting seems to be specific to prefrontal function (Barcelo, 2001; Rubinstein et al., 2001; Shallice et al., 2008).

To understand different components of cognitive control, well-controlled experiments are required to dissociate a specific component from other cognitive or sensorimotor aspects. Recently, an attentional set shifting task was developed in analogy to the WCST and successfully tested in human, non-human primate and rodents (Owen et al., 1991; Dias et al., 1996; Birrell and Brown, 2000). This task included both intradimensional (IDS) and extradimensional set shifting (EDS).
IDS new stimuli are applied in previously used dimensions, but the relevance or irrelevance of each dimension is maintained. The EDS phase of the task holds that new stimuli are applied in both dimensions and the relevant dimension becomes irrelevant whereas the previously irrelevant dimension becomes relevant (for a more complete overview, see chapter 4). The complete paradigm also examines reversal learning (‘affective shifting’, a previously unrewarded exemplar becomes rewarded, whereas a previously rewarded exemplar becomes unrewarded, while the relevant, reward-predicting dimension remains the same), attentional set formation, and the ability to inhibit and shift attention between stimulus dimensions within a single session with different behavioral phases (from simple discrimination to extradimensional set shifting). In human and non-human primates, the subject is required to discriminate stimuli that are considered ‘simple’ because their differences are set in only one perceptual dimension (e.g., line stimuli; simple discrimination (SD) phase). Next, the subject moves on to compound discrimination (CD), in which another perceptual dimension (e.g. surfaces with specific shapes, in addition to line stimuli) is co-presented with the original dimension, which remains relevant in the sense that stimuli in this dimension remain predictive of trial outcome. Thus, during CD the subject keeps his attention focussed on the same dimension as was relevant in the foregoing SD phase, based on reward feedback. Subsequently, new exemplars are presented to the subject, however the discrimination rule applicable to the SD and CD phases is maintained (intradimensional set shifting, IDS). In extradimensional set shifting (EDS), the subject is required to shift his attention from a previously relevant dimension to a previously irrelevant one (e.g. from line patterns to shapes). Finally, in a reversal learning phase, the subject needs to reverse the previous stimulus-reward associations.

Robbins and co-workers showed that different PFC areas mediate different aspects of set-shifting in schizophrenic and Parkinsonian patients (Downes et al., 1989; Owen et al., 1993) and marmosets (Roberts et al., 1988; Dias et al., 1996b), i.e. the lateral PFC in shifting between abstract perceptual dimensions whereas the OFC appears necessary for shifting of behavioral responses to different stimuli with a specific correlation to reinforcement (‘affective shifting’ as in reversal learning; Dias et al., 1996b). Schizophrenic patients showed abnormally strong perseveration, which is the inability to release attention from a previously relevant perceptual dimension when a current task rule is changed to render a previously irrelevant dimension relevant. In contrast, Parkinson’s disease showed more loss of irrelevant learning, i.e., it revealed an inability to direct attention to a previously irrelevant, but now relevant dimension (Mackintosh, 1975; Owen et al., 1993).

Lesion studies in rats and mice have implicated the medial prefrontal cortex in attentional set shifting, as shown in a paradigm where freely moving animals were allowed to choose between two food bowls filled with a digging medium such as
sawdust and distinguished by dimensions such as odor, digging medium, texture of the bowl’s rim and outer surface. Performance of this task was motivated by a food reward hidden at the bottom of one of the bowls (rat: Birrell and Brown, 2000; Brown and Bowman, 2002; mice: Brigman et al., 2005; Garner et al., 2006). When the mPFC was damaged, the rat showed difficulty in shifting attention when a previously irrelevant dimension became relevant, whereas OFC lesions showed deficits in affective shifting as in non-human primates (Dias et al., 1996b; Brown and Bowman, 2002; McAlonan and Brown, 2003). As suggested by neurophysiological studies on PFC functions in primates and rodents, functions related to attentional set and affective shifting showed area-wise similarities across species - despite the considerable anatomical differences between the PFC in rats and primates (Preuss, 1995; Brown and Bowman, 2002).

Prefrontal cortex as an integrative brain area

As described above, top-down cognitive control needs to operate across various cooperating brain areas. The PFC has anatomical connections with a great variety of brain areas. In this section we will be mainly concerned with the anatomy of rat PFC.

Based on connectivity with the mediodorsal (MD) thalamic nucleus, the rat prefrontal cortex is divided into three topographically different regions, i.e. the medial, orbital and lateral prefrontal cortex (Leonard, 1969; Krettek and Price, 1977; Fig.1-1A). For the interest of this Ph.D. project, medial and orbital PFC will be mainly dealt with here. The medial PFC can be further divided into two distinct areas based on anatomical connections: a dorsal component which consists of the frontal association area (Fr2), the anterior cingulate area (ACg), and the dorsal part of the prelimbic (PLd) area; and a ventral component that includes the ventral prelimbic (PLv), infralimbic (IL) and medial orbital (MO) areas (Heidbreder and Groenewegen, 2003; Steketee, 2003; Fig.1-1B). Although the PL area is well connected with the hippocampal formation and amygdala, the major input to the dorsal mPFC comes from sensorimotor cortical (nonlimbic) and thalamic input (Jay et al., 1989; Gabbott et al., 2005). Inputs to the ventral mPFC originate from limbic cortical and thalamic (MD) structures (Heidbreder and Groenewegen, 2003; Hoover and Vertes, 2007). The medial PFC is densely connected with all cortical sensory systems, motor systems, and many subcortical structures (for general afferent connections, see Conde et al., 1995; Hoover and Vertes, 2007, direct inputs from visual cortex area 2 (V2): Kim et al., 2003, auditory: Van Eden et al., 1992, piriform (olfactory): Datiche and Cattarelli, 1996, insular (taste): Guldin and Markowitsch, 1983, thalamus: Groenewegen, 1988; Wang and Shyu, 2004, amygdala: Cassell and Wright, 1986, hypothalamus: Floyd et al., 2001, and hippocampus: Jay et al., 1989; general efferent connections: Gabbott et al., 2005; Vertes, 2004, striatum: Berendse et al., 1992; Gorelova and Yang,
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A summary is shown in Fig. 1-1B. Moreover, the mPFC receives monoaminergic (Noradrenaline; NA, Dopamine; DA and Serotonin; 5-HT) and cholinergic (Acetylcholine; ACh) innervation from the locus coeruleus (LC), ventral tegmental area (VTA), dorsal raphe, basal forebrain (medial septum), respectively, and projects back to these areas (Steketee, 2003).

Figure 1-1. Diagrams of the rat prefrontal cortex illustrating its structure and connections (adapted from Paxinos and Watson, 1998). (A) Coronal section (3.2 mm anterior to bregma) with a summary of systems functions involving prefrontal subregions (see text for references). From left (red color), middle (green color) to right (yellow color): medial prefrontal cortex,
Selective lesion studies in the rat revealed detailed functional properties of each subdivision of mPFC. In particular, the ACg and dorsal PL appear to be important for the temporal organization of behavioral sequences (Delatour and Gisquet-Verrier, 2001), coordination of movement (Kolb and Whishaw, 1983) and selective attention (Corwin 1986; Passetti et al., 2002). In contrast, the ventral PL is involved in working memory (Ragozzino et al., 1998; Granon et al., 1998), rule learning and habit formation (Killcross and Coutureau, 2003) and attentional set shifting (Ragozzino et al., 1999b; Birrell and Brown, 2000). As a central region in the ventral mPFC, the IL plays a role in autonomic control (Morgan et al., 2003), behavioral inhibition (Chudasama and Robbins, 2003), and control over expression of fear (Morgan and LeDoux, 1995; Quirk et al., 2006, Fig.1-1A).

The orbitofrontal cortex (OFC) consists of three subareas: ventral (VO), ventrolateral (VLO), and lateral orbital (LO) cortex (Krettek and Price, 1977; Reep et al.,
Like the mPFC, the OFC has afferent and efferent connections with all cortical sensory systems, motor systems, and many subcortical structures. The OFC appears to play a unique role in odor-guided learning (Eichenbaum et al., 1980) and social behavior (Kolb et al., 2004). Lesion effects on odor discrimination learning have been linked to deficits in odor detection and in forming odor-reward associations (Slotnick and Schoonover 1992).

Olfactory and visual sensory discrimination learning in the rat

Previously, cognitive functions in the rat have been studied mainly by analyzing processing via the auditory, olfactory and visual modalities; we will focus on the latter two modalities here. The olfactory modality has been studied in investigations of orbitofrontal cortex (OFC) and hippocampal functions (Eichenbaum et al., 1980; Schoenbaum and Eichenbaum, 1995; Feierstein et al., 2006; van Duuren et al., 2007). The OFC is known to receive olfactory input directly from the olfactory bulb (OB; Cinelli et al., 1987). Hodologically, olfactory information is transferred to the mPFC through several pathways (Fig.1-2C). The primary source of olfactory information reaching the mPFC may come from the piriform cortex (PIR), although olfactory input may also be relayed via the OFC and entorhinal cortex (Cinelli et al., 1987; Conde et al., 1995). Other afferent sources may include the mediodorsal nucleus of the thalamus (MD), which receives projections from PIR and entorhinal/perirhinal cortex (Conde et al., 1995; Slotnick, 2001).

Different behavioral tasks have been used to study various types of olfaction-based learning, using automated olfactometers for temporally controlled application of odorized air. Examples of these tasks are Go/NoGo olfactory discrimination to study learning of stimulus-response associations (Eichenbaum et al., 1980), crossmodal associative learning (Lipton et al., 1999), and rule learning in an olfactory matching-to-sample task (Lu et al., 1993). In the current project, a Go/NoGo paradigm was used to train the rats. In this type of task, the rat is required to discriminate two odors and to generate different motor responses based on reinforcement. First, the rat samples an odor stimulus and then moves over to a tray at which reinforcement is delivered: an odor stimulus designated as ‘positive’ (S+) is followed by reward, and a stimulus designated as ‘negative’ (S-) is followed by an aversive outcome (Eichenbaum et al., 1980, Schoenbaum and Eichenbaum, 1995, Kay and Freeman, 1998, Sara et al., 1999, Tronel and Sara, 2002; van Duuren et al., 2007). In general, rats require relatively few sessions for correct odor discrimination performance.
Figure 1-2. **Visual acuity of the hooded rat and a schematic diagram of anatomical pathways relaying olfactory and visual projections to mPFC.**

(A) Comparison of the contrast-sensitivity function for the hooded (Long Evans and Lister hooded) rat, pigeon, cat and human. Adapted from Keller et al., 2000. Pigmented rats such as the Lister-hooded strain have optimal visual acuities between ~0.02 to 1.00 cycles per degree (c/d) based on vertical or oblique gratings (Dean, 1978). (B) Blurring of visual images to illustrate consequences of the rat’s relatively low visual acuity compared to humans. Despite this effect, hooded rats are able to discern larger shapes well enough (lower left). Adapted from Prusky et al., 2002. The original image, taken to illustrate human vision (top-left) has been blurred with a Gaussian filtering function. From top-right to bottom right, acuities of different rat strains with acuities of 1.5 c/d (Fisher-Norway), 1.0 c/d (Lister Hooded) and 0.5 c/d (Wistar) are simulated,
In the rat, the visual modality has been applied less in the investigation of learning and memory as compared to olfaction. When vision is compared across species, the rat has a relatively limited spatial acuity, but can discern larger shapes well enough (Fig.1-2A,B). The transfer of visual information from the retina to the mPFC is rather complicated as compared to olfactory processing (Fig.1-2C). One primary source of visual information may originate from reciprocal connections between mPFC and perirhinal cortex, which receives inputs from the visual cortex. Another source may be the MD nucleus of the thalamus, which receives projections from the superior colliculus (SC; Dean, 1981; Conde et al., 1995).

Lashley showed for the first time that rats can discriminate visual patterns with either a black background or, alternatively, a black and white-striped background pattern (Lashley, 1938). Since then, visual tasks for rats have been advanced with the aid of computer techniques, such as automated touchscreen methods (Bussey et al., 1994), computer-controlled Y-mazes (Simpson and Gaffan, 1999), and ‘visual’ water mazes (Prusky et al., 2000). In contrast to olfactory learning, visual discrimination in rats was reported to require many training sessions (Bussey et al., 1994, Simpson and Gaffan, 1999, Cook et al., 2004, Minini and Jeffery, 2006). In summary, distributed processing of olfactory and/or visual information converges on the mPFC and is characterized by reciprocal interactions between mPFC and modality-specific afferent areas. These circuits are involved in complex sensory and emotional learning. Although rat mPFC and the dIPFC in primates are anatomically distinct (Preuss, 1995), functional similarities between these areas have been indicated (Ongur and Price, 2000; Brown and Bowman, 2002; Uylings et al., 2003).
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Ensemble recordings in freely moving rats performing behavioral tasks

In the current project we hypothesized that attentional set shifting in the rat brain may be regulated by subgroups of distributed neurons and is dynamically controlled by specialized neural networks incorporating the mPFC. Therefore, it was crucial to our experimental approach to record large numbers of individual neurons in the same behavioral session. Multi-electrode extracellular recording techniques have been applied successfully in behaving rats (McNaughton et al., 1983; Wilson and McNaughton, 1993; Gray et al., 1995, in our group: Pennartz et al., 2004; van Duuren et al., 2007; Lansink et al., 2009). The technique we applied is based on so-called tetrodes, which are microbundles consisting of four thin metal electrodes (tetrode diameter: about 25 um, photograph in Fig.1-3B). The design of a multi-tetrode microdrive as used in the project is shown in Fig.1-3A. They are chronically implanted in the brain and yield reliable spike information about a large number of neurons, with single-unit isolation (up to 50 units per session in the work described here). The principle of recording with tetrodes is to use the spatial geometry of the four electrodes making up one tetrode, relying on the fact that the amplitude of a spike recorded on one lead is a function of the distance between the neuron and that lead. Almost all of the units in the vicinity of the tetrode are at a different distance from at least one of the leads of a tetrode. In addition, effective software tools have been developed for spike sorting, which refers to methods for isolating spikes emitted by a single neuron from noise as well as from other, nearby neurons (e.g., Mclust, developed by the group of A.D Redish, and KlustaKwik, developed by K. Harris and colleagues, Harris et al., 2000; Schmitzer-Torbert et al., 2005). An example of sorted spike activity is shown in Fig.1-3D.

General aim of the project and outline of the thesis

To probe the neural basis of attention and attentional set shifting, focusing on the rat mPFC with multi-electrode recording techniques, we first developed a multimodal stimulus (MMS) system which can present an odor and a visual pattern to the rat simultaneously. Based on attentional set shifting tasks previously developed for primates and rats (Owen et al., 1991; Dias et al., 1996b; Birrell and Brown, 2000), we developed a new multimodal set-shifting task especially suited for neurophysiology. The detailed behavioral setup and initial experiments conducted with this apparatus are described in chapter 2. The main challenge and novelty of this task were to allow rats to explore different perceptual dimensions of a stimulus situation while their behavior was required to be regular and relatively stereotyped, to be able to tackle sensorimotor confounds of neurophysiological activity.
Figure 1-3. Overview of “hyperdrive”, tetrodes and an example of sorted spikes. (A) Schematic side view of a ‘hyperdrive’ (multi-tetrode microdrive) with a ‘flat’ tetrode bundle, based on a design described by Gothard et al., 1996 and Lansink et al., 2007. (a) ‘Flat’ bundle of cannulae from which tetrodes are protruding into the brain, (b) one of the drive screws which are used to move each tetrode individually in a dorsoventral direction, and (c) printed circuit board, used for interfacing signals from each electrode wire of all tetrodes with preamplifiers of a headstage. (B) Photograph of 14 tetrodes (d), exiting a bundle of guide cannulae (a). (C) Photograph of a Nissl-stained section showing individual neurons (exemplified by e); microlesion caused by an individual tetrode track is indicated by f. (D) Example of clustered spikes which were recorded from the mPFC with a hyperdrive. Three-dimensional plot of clustered spikes made with a sorting program (MClust) is shown in a two-dimensional projection on the left side. The peak amplitude of spikes is plotted for three leads of a tetrode (each dot represents one spike). Each different color represents a single unit; four units are shown here. Gray clusters represent noise and units lacking sufficient signal relative to noise (axes; in μV, are shown below the gray clusters). The panel at the bottom right shows the averaged spikes waveforms of the unit indicated by the arrow in the left-hand cluster plot (from left to right: lead 1, 2, 3, 4; mean voltage [mV] is shown ± sem values). A histogram of interspike intervals (ISIs) is shown in the upper right panel.
Second, we investigated neural representations of active sensory processing in the mPFC while the rat performed a unimodal to compound transitional task. In the task, rats started with simple odor discrimination in which they were required to distinguish two exemplars in the olfactory dimension, one of which (S+, positive stimulus) was coupled to reward (sucrose solution) and the other (S-, negative stimulus) to an aversive outcome (quinine solution). In the same session, rats then proceeded to compound discrimination (CD) in which irrelevant exemplars (visual patterns) were presented simultaneously with the same exemplars from the first modality. When mPFC neurons engage in processing of stimuli in a certain sensory dimension (e.g. olfaction), the question arises how a crossmodal distractor (e.g. a visual stimulus) alters task-relevant firing patterns in the rat mPFC at the single cell and ensemble level, and how the brain manages to convert relevant sensory information into appropriate behavioral decisions when irrelevant information is presented simultaneously. These questions on handling sensory information and filtering out irrelevant inputs are dealt with in chapter 3.

Third, we asked how firing patterns in the rat mPFC are altered across the successive stages of an attentional set shifting task. These changes are analyzed at the single-unit and ensemble level in chapter 4 and 5, respectively. Finally, this book will be concluded with a general discussion of the results and with future perspectives (chapter 6).