Neural representation of reward information: coding by single cells and populations in rat orbitofrontal cortex
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Chapter I

General Introduction
“Het is nu zes uur ‘s avonds, het werk zit erop. Ik kan nu een wandeling gaan maken, of ik kan naar de club gaan; ik kan de toren beklimmen om de zon onder te zien gaan, maar ik kan ook naar het theater gaan; ik kan bij deze vriend op bezoek gaan, maar ook bij een andere; ik kan zelfs de stadspoort uitlopen, de wijde wereld in en nooit meer terugkomen. Dit hangt allemaal alleen van mij af; ik heb daartoe de volledige vrijheid. Toch doe ik dat allemaal niet: ik ga even vrijwillig naar huis, naar mijn vrouw.

Dat is precies hetzelfde als dat het water zou zeggen: ik kan hoge golven maken (jazeker, in zee tijdens een storm), ik kan wild omlaag stromen (jazeker, in de bedding van een rivier), ik kan schuimend en bruisend naar beneden storten (jazeker, in een waterval), ik kan in een straal recht de lucht inspuiten (jazeker, als fontein), ik kan ten slotte zelfs verkoken en verdwijnen (jazeker, bij 80° Réamur). Toch doe ik dat nu allemaal niet: ik blijf gewoon vrijwillig als stil en helder water hier in deze spiegelende vijver.

Net zoals het water dit allemaal alleen kan doen door tussenkomst van de oorzaken die het op een of andere manier bewerken, kan die man wat hij meent te kunnen alleen onder diezelfde voorwaarde. Zonder de oorzaken is het hem onmogelijk; zijn de oorzaken echter aanwezig, dan moet hij wel – net zoals het water, zodra het in de juiste omstandigheden wordt gebracht. (...) Dit alles is volmaakt verenigbaar met het ‘Ik kan doen wat ik wil’ van ons zelfbewustzijn, waarin tot op de dag van vandaag sommige hersenloze filosofasters de vrijheid van de wil menen te bespuren en deze dienovereenkomstig als een vaststaand feit van het bewustzijn doen gelden.”

A. Schopenhauer, 1839

At this time, it is still a matter of debate in both philosophy and science whether man truly possesses ‘free will’, or whether his actions are determined. To be able to resolve this controversy, one needs to get insight in the process of how we exercise ‘free will’, that is, how we make our choices. To this end, we need to explore the neural mechanisms underlying the process of decision-making.

One area in the brain considered to be involved in decision-making is the prefrontal cortex, which is located in the rostral pole of the frontal lobes. Initially, insight in the
functional properties of the prefrontal cortex was provided by deficits observed in human patients with prefrontal damage. The most famous patient who sustained frontal damage was Phineas Gage, a railroad foreman whose prefrontal cortex was damaged by an iron bar penetrating through his head during a work-related accident in 1848. Although Gage had no impairments in retrograde and anterograde long-term memory, the alterations in his social and cognitive abilities were so severe that people described him as ‘no longer Gage’. Over the years following this accident, Gage, as noted by his physician, turned into a ‘fitful, irreverent person, indulging at times in the grossest profanity, but little deference for his fellows, impatient of restraint or advice when it conflicts with his desires’ (O’Driscoll and Leach, 1998). Additional research with prefrontal patients revealed that they have normal IQ scores, but suffer from a variety of social and emotional disturbances, have personality changes and display impulsiveness and alterations in goal-directed behavior and decision-making (Bechara 1994, 2004; Brazzelli et al., 1994; Rolls et al., 1994; Verin et al., 1993). These impairments are indicative of the importance of the prefrontal cortex in human functioning, especially in the optimization of complex behavior.

Dysfunction of the prefrontal cortex is also implicated in a number of psychiatric disorders, including schizophrenia and obsessive-compulsive disorder. One of the most consistent abnormalities observed in schizophrenic patients is a decreased volume of the prefrontal cortex (Antonova et al., 2004). In addition, schizophrenia is associated with hypofrontality, meaning that these patients fail to activate the prefrontal cortex during executive functioning (Glahn et al., 2005; Ragland et al., 2007). In contrast, hyperactivity of the orbitofrontal cortex, located in the ventral part of the prefrontal cortex, is demonstrated in patients with obsessive-compulsive disorder (Breiter et al., 1996; Rauch et al., 1994). Patients with obsessive-compulsive disorder have impaired executive functioning and cognitive flexibility, which is consistent with the idea of the orbitofrontal cortex being necessary for the guidance of goal-directed behavior (Cavedini et al., 2006).

This thesis focuses on this particular part of the prefrontal cortex, the orbitofrontal cortex. The orbitofrontal cortex is specifically implicated in the guidance of goal-directed behavior, and hence decision-making, for which it uses predictive information about future outcomes. The following overview will provide, besides general information regarding connectivity and functionality of the prefrontal cortex, in more detail the behavioral and neurophysiological evidence for involvement of the orbitofrontal cortex in the coding of expectations for future rewards and its involvement in the guidance of behavior. Finally, the aims and outline of this thesis will be presented.
The prefrontal cortex: anatomy and connectivity

*Demarcation, subdivision and comparative anatomy*

The prefrontal cortex is located in the most rostral area of the frontal lobes and is part of the frontal cortex. Historically, the frontal cortex of primates has been divided into two regions: the electrically excitable primary motor and premotor area, which produces motor activity on stimulation, and the prefrontal cortex (Kolb, 1984, 1990). This division further relates to the presence of a granular layer IV, which is absent in the primary motor and premotor cortical areas. However, based on the observation that this granular layer is absent in rodents, other criteria were needed to be able to delineate the prefrontal cortex across different species. Another anatomical characteristic used to define the prefrontal cortex has been originally proposed by Rose and Woolsey (1948), and considers the prefrontal cortex as that part of the frontal cortex receiving projections from the mediodorsal thalamic nucleus. According to this definition, almost all mammalian species probably do have a prefrontal cortex, which in primates and rodents includes the entire cortex of the dorsolateral, orbital and medial frontal lobe rostral to the precentral motor cortex (Öngür and Price, 2000).

The prefrontal cortex in primates can be roughly subdivided into a medial, orbital (or orbitomedial) and dorsolateral region (Fuster, 1997). Whereas the dorsolateral area is implicated in higher cognitive processes, the medial and orbital parts are thought to be more involved in the conduct of emotional behavior. Within these regions, a large variety of cytoarchitectonically distinct subareas can be distinguished, which may contribute differentially to the cognitive and emotional processes mediated by this area (Barbas, 1996; Carmichael and Price, 1994; Fuster, 1997). The rat prefrontal cortex can be subdivided into a number of areas as well, including (i) a medial part, consisting of the medial agranular cortex (area Fr2), the dorsal and ventral anterior cingulate and the prelimbic, infralimbic and medial orbital cortices, (ii) a lateral part that comprises the dorsal and ventral agranular insular cortices and the lateral orbital area, and (iii) a ventral part that encompasses the ventral orbital and ventral lateral orbital cortices (Kolb, 1984; Uylings and Van Eden, 1990) (Fig. 1).

Differences in prefrontal cytoarchitecture and connectional characteristics between primates and rodents make it difficult to establish homologies between the different prefrontal cortical areas in these species. However, based on an overall similarity in anatomy and function, the prelimbic, infralimbic, and orbital areas and the lateral prefrontal cortex in the rat are considered homologous to the primate prefrontal cortex (Brown and Bowman, 2002; Kolb, 1984; Öngür and Price, 2000; Uylings et al., 2003). Whether the prefrontal cortex in the rat contains an equivalent of the primate dorsolateral prefrontal cortex is still a matter of debate. According to
Preuss (1995) there is no such equivalent in the rat, but Uylings et al. (2003) argued that, based on anatomical and functional data, regions of the prefrontal cortex in rats (i.e. Fr2 and the prelimbic region) do display features that resemble characteristics of the primate dorsolateral prefrontal cortex, although it should be kept in mind that in the rat the prefrontal cortex is not as differentiated as in primates.

![Diagram of prefrontal cortex subdivisions](image)

**Figure 1.** Diagram illustrating the subdivision of the rat prefrontal cortex. (a) Lateral view, (b) coronal section at the location indicated by the arrow in (a). Different shadings represent the major subdivision of the prefrontal cortex in a medial, ventral and lateral part. Abbreviations: ACg, anterior cingulate cortex; AID, dorsal agranular insular cortex; AIV, ventral agranular insular cortex; AOM, medial anterior olfactory nucleus; AOV, ventral anterior olfactory nucleus; cc, corpus callosum; Cg2, cingulate cortex area 2; gcc, genu of corpus callosum; IL, infralimbic cortex; LO, lateral orbital cortex; M1, primary motor area; MO, medial orbital cortex; OB, olfactory bulb; PrL, prelimbic cortex; PrC, precentral cortex; VLO, ventrolateral orbital cortex; VO, ventral orbital cortex. Adapted from Dalley et al., Neurosci Biobehav Rev 28: 771-784, 2004.

**Connectivity**

The main excitatory neurotransmitter in the prefrontal cortex is the amino acid glutamate, which is provided to the prefrontal cortex amongst others by means of its widespread cortico-cortical connections. Both in rats and primates the various subareas of the prefrontal cortex are highly interconnected (Carmichael and Price,
1996; Öngür and Price, 2000). In primates, the distinction between a dorsolateral and an orbitomedial part is also reflected in the pattern of cortico-cortical connections (Fig. 2). The dorsolateral prefrontal cortex receives, besides input from (pre-)motor and orbitomedial areas, input from most sensory modalities, as well as from the parietal, temporal and cingulate cortices. There is a functional distinction between a dorsal and ventral information stream towards the dorsolateral prefrontal cortex, directed to respectively areas located in and around the principal sulcus, and to the inferior convexity in ventrolateral prefrontal cortex (Ungerleider, 1998). The dorsal stream contains fibers from primarily the dorsal parietal lobe, conveying information related to spatial orientation, whereas the ventral streams contain more fibers from the temporal lobe, transmitting information related to the identification and recognition of objects. Hence, the representation of these aspects of the external world in the prefrontal cortex is considered important for the function this area fulfills in cognitive functions such as working memory and the guidance of complex behaviors (Ungerleider, 1998). The cortico-cortical connections of the primate medial and orbital areas characterize the caudal orbital area as visceral- and olfactory-related, and more rostrolateral orbital areas as related more strongly to exteroceptive senses (i.e. vision, audition) (Price et al., 1996).

In rats, the pattern of cortico-cortical connections of the prefrontal cortex is less differentiated as compared to those in primate prefrontal cortex. Area Fr2 and the dorsal anterior cingulate cortex have reciprocal connections with the somatosensory, motor and visual cortices and with temporal association cortices, such as the perirhinal cortex. The prelimbic, infralimbic, and ventral anterior cingulate cortex are reciprocally connected with the perirhinal and entorhinal cortices, as well as with the lateral part of the prefrontal cortex. Furthermore, predominantly the lateral and ventral parts of the prefrontal cortex receive olfactory and gustatory inputs (Carmichael and Price, 1996; Groenewegen and Uylings, 2000; Uylings et al., 2003). In this respect, Fuster (1997, 2004) views the interaction between prefrontal cortex and other cortices as a 'perception-action cycle', in which the cycle represents the circular flow of information by which an organism relates to its environment. In this hierarchical concept, the prefrontal cortex is connected with other cortices as long as the behavior contains novelty, uncertainty or ambiguity and has to bridge time intervals using short-term memory. As soon as these constraints disappear and behavior becomes automatic, these functional interactions disappear or weaken: the action is then executed via lower brain structures (Fuster, 2004).
Figure 2. Diagram illustrating the organization of prefrontal affenter and efferent projections in primates. A distinction is made between the dorsolateral prefrontal cortex and the orbitomedial part of the prefrontal cortex. Note that connections of the prefrontal cortex in rats are less differentiated as compared to those in primate prefrontal cortex (see text). Adapted from Groenewegen and Uylings, PBR vol. 126: 3-28, 2000.

A large number of other brain areas is reciprocally connected with the prefrontal cortex. As already noted, both in rats and primates a topographically organized reciprocal connection exists between the prefrontal cortex and the mediodorsal nucleus of the thalamus, as well as with the intralaminar and midline nuclei, the rostral part of the ventral thalamic complex and the anterior medial thalamic nucleus (Barbas et al., 1991; Berendse and Groenewegen, 1991; Ray and Price, 1992; Reep et al., 1996). In both species, the dorsomedial prefrontal cortex
receives via the mediodorsal thalamic nucleus more exteroceptive sensory input, whereas the ventromedial, orbital and agranular insular areas receive mostly limbic, visceral and olfactory inputs (Groenewegen and Uylings, 2000). In primates, reciprocal connections with the amygdala are organized with a medial to lateral topography across the main amygdaloid nuclei (the basal, accessory basal and lateral amygdala) and prefrontal areas, with medial areas in both structures projecting to each other and lateral areas projecting to lateral areas (Carmichael and Price, 1995). In rats, projections to the PFC primarily originate from the basolateral amygdala, terminating in the medial and lateral subdivisions of the prefrontal cortex (McDonald, 1991). Hippocampal input originating from area CA1 and the subiculum is also topographically organized and projects in both rats and primates to the medial prefrontal cortex, especially the prelimbic, infralimbic and medial orbital areas, and the lateral prefrontal cortex (Jay and Witter, 1991; Jay et al, 2002). The prefrontal cortex projects to the dorsal and ventral striatum, receiving indirect input from the striatum via the pallidum and thalamus (Groenewegen et al., 1999). There is also a connection with the hypothalamus that is reciprocated, a projection that both in rats and primates primarily applies to the ventral and medial prefrontal cortex (Rempel-Clower and Barbas, 1998; Saper, 1985; Sesack et al., 1989). This connection of specific parts of the prefrontal cortex with the hypothalamus implies the involvement of the prefrontal cortex in autonomic and endocrine functions (Robbins, 2000). In addition, both in rats and primates, the prefrontal areas projecting to the hypothalamus also project directly to various areas in the brainstem that are targeted by the hypothalamus as well, including the periaqueductal grey, the peribrachial nuclei, the nucleus of the solitary tract and parts of the reticular formation (Sesack et al., 1989; Uylings and Van Eden, 1990).

The prefrontal cortex is reciprocally connected with the main sources of the forebrain monoaminergic and cholinergic neurotransmitter systems, including dopamine containing cells in the ventral tegmental area, the noradrenergic cells of the locus coeruleus, serotonergic neurons of the raphe nucleus and the cholinergic magnocellular basal nucleus (Robbins, 2000). These projections enable the prefrontal cortex, by means of its excitatory glutamatergic projection neurons, to exert control over processes within all of these structures, and to regulate input coming from these areas (Robbins, 2000). For example, stimulation of the prefrontal cortex would be expected to excite dopaminergic neurons in the ventral tegmental area, producing an increase in prefrontal dopamine. This was suggested by Jedema and Moghddam (1996), who showed that stimulation of the medial prefrontal cortex with AMPA (which mimics the effect of glutamate), causes an elevation of dopamine within this area. Furthermore, Sara and Herve-Minvielle (1995) demonstrated a strong inhibitory control of prefrontal neurons on the activity of neurons within the locus coeruleus.
Prefrontal cortical functions

Executive functions and working memory

The prefrontal cortex is generally considered to mediate executive functions, which are cognitive control processes that are needed to optimize intended, complex behaviors. These control processes include attentional selection and resistance to interference, monitoring, behavioral inhibition, task switching, planning and decision-making (Dalley et al., 2004). The mediation of cognitive control requires all kinds of information, and the prefrontal cortex, because of its widespread connectivity, is a suitable area to integrate all information needed for the complex sequencing of behavior. This idea is supported by deficits that occur when the prefrontal cortex is damaged: impairments are most apparent in situations in which cognitive control is needed. Prefrontal patients show altered social and goal-directed behavior, impulsiveness or disinhibited behavior and misinterpretation of other peoples moods, and they suffer from personality changes (Bechara, 1994, 2005; Brazzelli et al., 1994; Rolls et al., 1994; Verin et al., 1993). These impairments are indicative of the involvement of the prefrontal cortex in impulsive control, behavioral inhibition and decision-making, which are considered executive functions necessary to optimize behavior.

In addition, the prefrontal cortex is thought to play an important role in working memory, which is a temporary memory system used for the active maintenance and elaboration of memory representations for future use, and intimately related to executive functioning. Studies in rodents and primates examining the involvement of the prefrontal cortex in working memory make use of paradigms with delayed response contingencies, including spatial delayed alternation tasks in rats or delayed non-matching to sample tasks in rats and primates. Rats with lesions of prelimbic and infralimbic areas show delay-dependent disruption of task performance (Delatour et al., 1999; Robbins et al., 2000). Furthermore, electrophysiological studies in primates demonstrate the activation of neurons in the dorsolateral prefrontal cortex specifically during the delay (Fuster, 1973; Kojima and Goldman-Rakic, 1982). This activity persists from the time a cue is presented until the response is performed, and is also found to be specific for locations within the visual field (Goldman-Rakic, 1996). These activations are thought to reflect information that is held ‘on-line’, so that it is directly available to be used in decision-making processes (Funahashi, 2006; Goldman-Rakic, 1995). Brain imaging studies in humans have consistently demonstrated prefrontal activations during the performance of variants of the delayed matching-to-sample task as well (see for reviews: D’Esposito et al., 2000; Fletcher and Henson, 2001; Passingham and Sakai, 2004). Furthermore, patients with frontal lobe lesions show impairments in a variety of working memory tasks as well (Müller and Knight, 2006).
It is known that working memory processes are modulated by the dopaminergic system (Dalley et al., 2004; Robbins, 2000). Dopaminergic manipulations in rats and primates that either decrease or increase dopamine levels in the prefrontal cortex produce deficits in working memory tasks, which implies that working memory in the prefrontal cortex may depend on an optimal level of dopamine, presumably according to an inverted ‘U-shaped’ function (Robbins, 2002; Williams and Goldman-Rakic, 1995).

**Attentional processes and attentional set shifting**

The prefrontal cortex has a prominent role in attentional functions (Dalley et al., 2004). A behavioral paradigm that is widely used to assess attentional processes and executive functions in rats is the 5-choice serial reaction time task. In this paradigm, a brief visual stimulus that signals reward can be presented in a one of five spatial apertures. Upon a response of the animal in this aperture within a certain period after illumination, a reward is presented. This task captures different aspects of attention and inhibition, including attentional capacity, as indexed by the accuracy of responding, and inhibitory response control, such as compulsive (perseveration) and impulsive (premature) responding (Chudasama et al., 2003b). Lesions of different subareas of the prefrontal cortex produce differential effects on performance in this task: lesions encompassing the cingulate and prelimbic cortices produce deficits in attentional selectivity and a slower latency to respond correctly. Lesions comprising the infralimbic area cause deficits in response inhibition (impulsive premature responding), whereas orbitofrontal lesions increase perseverative responding, but only when the inter-trial interval is long and unpredictable (Chudasama et al., 2003b; Muir et al., 1996).

Other paradigms used to examine attentional processes make use of attentional set shifting. For example, in a primate analogue of the Wisconsin Card Sorting Test, a series of compound visual discriminations is used that requires the monkey either to maintain attention on a certain dimension (e.g. focusing on the color of items presented), and to transfer behavioral control from one pair of exemplars to another within that same relevant perceptual dimension, for example from one pair of blue shapes to another (intra-dimensional shift), or to shift its attentional set from a previously relevant perceptual dimension to another, previously irrelevant dimension, for example from a pair of blue shapes to a pair of white lines (extra-dimensional shift) (Dias et al., 1996). Lesions of the dorsolateral prefrontal cortex, but not the orbitofrontal cortex, impair extra-dimensional attentional set shifting, whereas lesions of the orbitofrontal cortex produce deficits in reversal learning (in which the previously acquired stimulus-reward contingencies are reversed). Similar findings are obtained in an attentional set shifting paradigm in rats: animals with lesions of the medial prefrontal cortex trained to discriminate bowls containing food on the basis
of odor, digging medium or the texture covering the bowls, are impaired in extra-
dimensional set shifting, whereas lesions of the orbitofrontal cortex do not impair
the acquisition of intra- or extra dimensional set shifting, but do cause deficits in
reversal learning (Birrell and Brown, 2000; Brown and Bowman, 2002). These
behavioral findings support the idea that the medial prefrontal cortex is necessary to
preserve attentional selectivity to relevant stimulus features during behavior, whereas
ventral and lateral regions appear to be more critical for inhibitory response control
or behavioral adaptation to previously established, but altering reward contingencies.

The ascending monoaminergic and cholinergic systems are found to
contribute to different aspects of the 5-choice serial reaction time task (Robbins,
2002). The observation that lesions of the cholinergic neurons of the magnocellular
basal nucleus produce deficits in performance of the 5-choice serial reaction time
task is in line with the finding in rats that neuronal activity associated with increased
attentional demands within the medial prefrontal cortex is modulated by cholinergic
afferents (Gill et al., 2000). Depletion of noradrenalin and dopamine from the
medial prefrontal cortex also results in attentional impairments in the 5-choice serial
reaction time task (Robbins, 2002), suggesting the involvement of multiple
neurotransmitter systems during task performance.

**Associative learning: action-outcome associations, habits and extinction learning**

During conditioning, animals learn contingencies between particular stimuli
or actions and outcomes, which allow the prediction of events occurring in the
environment. When animals perform a goal-directed action, which corresponds to
the human-oriented concept of ‘intended’ behavior, they learn the contingency
between performing a certain behavioral act and the outcome (also called action-
outcome learning). When the outcome is as desired, an action-outcome association is
learned. This in contrast to stimulus-response learning, a form of learning that does
not integrate knowledge of outcome in the learned association and which can result
in habit formation by overtraining, in which actions that were originally goal-
directed become automatic and habitual.

The prelimbic cortex is required for the detection of action-outcome
relationships, but seems less important for tasks that can be acquired through habit
formation (Cardinal et al., 2002). As demonstrated by Ostlund and Balleine (2005)
in a devaluation task used to discriminate between stimulus-response and action-
outcome learning, the prelimbic medial prefrontal cortex is specifically involved in
the acquisition of action-outcome representations, but not in the expression of
action-outcome associations during instrumental learning. Furthermore, animals with
lesions of prefrontal medial prefrontal cortex or orbitofrontal lesions within this task
are impaired in adjusting their behavior upon the changed incentive value of the
reinforcer (i.e. they exhibit habit-like task performance) (Balleine and Dickinson,
1998; Corbit and Balleine, 2003; Killcross and Coutureau, 2003; Ostlund and Balleine, 2007). A possible neural correlate of action-outcome learning has been described in the medial prefrontal cortex by Mulder et al. (2003) during a lever-press task in rats. In contrast to the prelimbic cortex, lesions of infralimbic medial prefrontal cortex caused animals to remain goal-directed (Killcross and Coutureau, 2003) while temporary inactivation of the infralimbic medial prefrontal cortex reinstated goal-directed responding (Coutureau and Killcross, 2003).

The involvement of the prefrontal cortex in classical conditioning has been studied less than its involvement in instrumental conditioning (but see Mulder et al., 2003). In fear conditioning, lesions of the prefrontal cortex generally do not affect acquisition (Morgan et al., 1993). Effects consist of an increase in fear responses to the conditioned stimulus or context (Morgan and LeDoux, 1995). Furthermore, lesions of rat orbitofrontal cortex impair the acquisition of Pavlovian autoshaping, whereas lesions of the infralimbic medial prefrontal cortex do not (Bussey et al., 1997; Chudasama et al., 2003a). However, lesions of the infralimbic medial prefrontal cortex were found to enhance recovery and reinstatement of an appetitive Pavlovian response after extinction (Rhodes and Killcross, 2004, 2007). As regards extinction learning, from lesion studies during extinction of conditioned fear it became apparent that the ventral medial prefrontal cortex (i.e. prelimbic and infralimbic parts of the prefrontal cortex) is involved in the extinction of fear conditioning, whereas the dorsal part of the medial prefrontal cortex does not seem to be essential for this type of learning (Morgan and LeDoux, 1995, 1999). Involvement of the infralimbic cortex in extinction of conditioned fear is furthermore supported by the finding that neuronal firing within this area correlates with extinction of responding to an aversively paired cue (Milad and Quirk, 2002).

An interesting point concerns the functional heterogeneity in the prefrontal cortex in relation to the different components of executive functioning. The behavioral studies in rats and primates as described above are consistent with the notion of functionally dissociable regions within the prefrontal cortex (Bussey et al., 1997; Chudasama et al., 2003, 2004; Dias et al., 1996; Passetti et al., 2002), but it remains largely unknown how these regions are organized, for example in a hierarchical manner or as a heterarchical group (Fuster, 1997b). Since the primary focus of this thesis concerns the rat orbitofrontal cortex, which is a part of the prefrontal cortex that appears to be specifically required for adapting already established behavioral responses in reaction to changes in the environment, the connectivity and functionality of this part of the prefrontal cortex are discussed in more detail below.
The orbitofrontal cortex: connectivity and functions

Connectivity

Thalamic afferents to the various subareas of the orbitofrontal cortex are topographically organized, and originate primarily from the submedial and mediodorsal nuclei (Reep et al., 1996). The ventrolateral area of the orbitofrontal cortex receives projections from the entirety of the submedial nucleus, whereas the other orbital areas receive projections from the periphery of this nucleus. In addition, each orbital area is connected with a particular segment of the mediodorsal nucleus, including the dorsocentral and the central and the lateral segments. In the rat, the medial and central regions of the mediodorsal thalamic nucleus receive direct afferents from a number of structures, including the amygdala, which is a complex of nuclei involved in the processing of affective and motivational aspects of learning, and the piriform cortex sending olfactory input (Ray and Price, 1992). Hence, olfactory and associative information is provided to the orbitofrontal cortex through this thalamic input. These types of information also reach the orbitofrontal cortex directly by means of its reciprocal connections with the basolateral nucleus of the amygdala and piriform cortex (Ray and Price, 1992). The direct and indirect pathways from the piriform cortex converge on the same region in the orbitofrontal cortex (lateral and medial orbitofrontal cortex), but originate from neurons that differ in their morphology (Ray and Price, 1992). Neurons from piriform cortex projecting to the orbitofrontal cortex through the mediodorsal nucleus of the thalamus are large multipolar neurons, whereas cells projecting directly to the orbitofrontal cortex are small and superficially located within the piriform cortex (primarily layers II and III). The existence of these two pathways suggests that olfactory information is carried by two different information streams. A similar pattern is demonstrated by the direct and indirect pathway originating from the basolateral amygdala: neurons projecting via the mediodorsal nucleus of the thalamus appear to be larger and better suited to integrate activity over a wide region than those projecting directly to orbitofrontal cortex (Ray and Price, 1992).

Cortico-cortical connections of the orbitofrontal cortex in rats are more extensive for the ventral and ventrolateral areas than for the medial and lateral orbital areas (Reep et al., 1996). The ventral and ventrolateral parts are connected with the cingulate cortex, FR2, and several areas of the visual and somatosensory cortices (Par1, Par2, PPC, Oc2M and Oc2L). The medial orbital area has connections with the cingulate cortex, FR2 and the posterior parietal cortices, whereas the connections of the lateral part are limited to the granular and agranular insular cortices and the somatosensory cortex. In addition, the orbitofrontal cortex is reciprocally connected with other subareas of the prefrontal cortex (Carmichael and Price, 1996; Ongür and Price, 2000), and provides a strong efferent projection to the nucleus accumbens.
(part of the ventral striatum) that overlaps with innervations from limbic structures, including the amygdala (Berendse et al., 1992; Groenewegen et al., 1990; McDonald, 1991). The nucleus accumbens is thought to act as an interface between the limbic and motor system, integrating information from sensory modalities and cortical areas to prepare for and invigorate behavioral actions (Swanson and Morgenson, 1981; Pennartz et al., 1994).

**Orbitofrontal cortical functions: behavior**

Based on its position in the circuitry outlined above, the orbitofrontal cortex is able to integrate sensory, motivational and associative input with information regarding planned actions, and applies this information to the guidance of goal-directed behavior. This hypothesis is consistent with the effects on behavior when the orbitofrontal cortex is damaged. Humans with lesions of the orbitofrontal cortex show alterations in emotional state, a lack of concern for the future and socially inappropriate or disinhibited behavior (Bechara, 1994, 2004; Berlin et al., 2004; Damasio, 1994; Hornak et al., 2003; Rolls et al., 1994). In formal testing situations, patients with orbitofrontal damage are unable to rapidly learn reversals of previously acquired stimulus-reinforcer associations (Fellows and Farah, 2003; Hornak et al., 2004; Rolls et al., 1994). Interestingly, patients can report verbally that task contingencies have changed, but are unable to alter their behavior accordingly (Rolls et al., 1994). This type of dysfunction, the inability to alter established behavior in response to a change in reinforcer associations, may in part underlie the social deficits found in those patients, since social and emotional interaction often requires the inhibition of inappropriate responses. In addition, it is suggested that emotion-based biasing signals that arise from the body are integrated within the orbitofrontal cortex to regulate decision-making in complex and uncertain behavioral situations (Damasio, 1994; Bechara et al., 1996). These presumed ‘somatic marker’ signals (skin conductance responses or SCRs) were found to be absent in patients with orbitofrontal lesions when they anticipated future outcomes, linking the presence of SCRs to successful performance on a decision-making paradigm in healthy subjects. However, questionability of both the behavioral task used and the interpretation of the findings in these experiments, together with a shortage of evidence for causally linking peripheral feedback to the performance in the task used, make the empirical evidence for this hypothesis rather weak (for a critical review of the somatic marker hypothesis, see Dunn et al., 2006). Support for the idea that the orbitofrontal cortex is involved in the mediation of emotions that influence decision-making comes from recent imaging studies that implicate the orbitofrontal cortex in the experience of regret, which is a negative emotion affecting choice behavior (Coricelli et al., 2005). For example, in a gambling task, normal subjects learned from regret caused by their suboptimal choices and tried to minimize future regret, whereas subjects with
orbitofrontal lesions reported to not experience regret and were unable to anticipate the negative outcomes of their future choices (Camille et al., 2004).

Deficits in reversal learning arising upon orbitofrontal damage as displayed by humans occur in other species as well. Both rats and monkeys are able to normally acquire stimulus-reward associations, but are impaired when the contingencies are altered (Bohn et al., 2003; Chudasama and Robbins, 2003a; Dias et al., 1996; Fellows and Farah, 2003; Ferry et al., 2000; Izquierdo et al., 2004; Kim and Ragozinno, 2005; McAlonan and Brown, 2003; Meunier et al., 1997; Schoenbaum et al., 2002, 2003b). Since the initial acquisition of the stimulus-reward associations remains unaffected and responses at this stage can be inhibited when appropriate, this impairment in reversal learning cannot be explained by a simple and general deficit in response inhibition due to orbitofrontal damage. Hence, the orbitofrontal cortex is thought to be involved not only in the representation of information regarding the incentive value of conditioned and unconditioned stimuli (Pears et al., 2003), but also in the process by which conditioned stimuli access a specific representation of the incentive value of the response outcome to guide behavior (Gallagher et al., 1999; Roberts et al., 2006). According to this hypothesis, damage to the orbitofrontal cortex disables the representation of the conditioned stimulus to access the representation of the associated reinforcer, which is needed to use the motivational guidance provided by the response outcome when environmental changes demand an adaptation in behavioral responses.

Besides the deficits reported in reversal learning, more evidence for this idea is provided by reinforcer devaluation paradigms. In these tasks, rats are trained to associate e.g., a light with a reinforcer. After conditioned responses to the light are established, the incentive value of the food is reduced (‘devaluated’) by pairing it with a toxin in the home cage in the absence of the light, to form a conditioned taste aversion. Upon re-exposure to the light, normal animals spontaneously decrease their conditioned response to the light cue, in accordance with the low value of the food reward. Rats with orbitofrontal lesions show normal acquisition of conditioned responding to the light and a normal reduction in food consumption after food-toxin pairing, but fail to show a decrease in conditioned responding to the light after reinforcer devaluation (Gallagher et al., 1999), which is an effect that is also observed when lesions are made later in the task, namely after associative learning (Pickens et al., 2003). This indicates that the orbitofrontal cortex is not only involved in the acquisition of the cue-outcome associations, but also in the control of conditioned responding according to the new representation of the devaluated response. Similar results are obtained in a devaluation task in primates (Izquierdo et al., 2004b), in which monkeys are trained to discriminate different visual objects to obtain different rewards. After devaluation of one of the rewards by food satiation, monkeys are offered a choice between the visual objects associated with the
devaluated versus the non-devaluated reward. Normal monkeys bias their choice towards the objects that are not associated with the devaluated reward, whereas in monkeys with lesions the effect of reward devaluation is attenuated (Izquierdo et al., 2004b).

It has been demonstrated that normal performance in devaluation paradigms requires an interaction between basolateral amygdala and orbitofrontal cortex (Baxter et al., 2000; Izquierdo et al., 2004a). Disconnection lesions of the basolateral amygdala and orbitofrontal cortex (orbitofrontal cortex damaged in one hemisphere and the amygdala in the other) or combined lesions of these two structures cause a failure in reducing conditioned responding after devaluation (Baxter et al., 2000; Izquierdo et al., 2004a). Examination of the specific contribution of these two structures in the devaluation paradigm reveals that both (bilateral) lesions of the orbitofrontal cortex or basolateral amygdala made prior to learning causes impairments in the devaluation paradigm (Hatfield et al., 1996; Gallagher et al., 1999), but when basolateral amygdala lesions are made after the conditioned response to the light is established no effect on behavior is observed, whereas lesions of the orbitofrontal cortex made at this stage continue to cause behavioral effects (Pickens et al., 2003). Lesions of the orbitofrontal cortex continue to have an effect on behavior even when they are made after the stage when the reinforcer is devaluated (Pickens et al., 2005). This suggests that the basolateral amygdala is involved in coding of the original associations, while the orbitofrontal cortex is involved in monitoring and updating the current value of the reinforcer and to use that information to guide behavior. This dissociable role for the amygdala and orbitofrontal cortex is also evident from an odor-guided discrimination and reversal task, in which lesions of either basolateral amygdala or orbitofrontal cortex exerted different effects on performance (Schoenbaum et al., 2003a). Neither lesions of the amygdala nor orbitofrontal cortex caused deficits in the acquisition of the initial stimulus-reward association, but after reversal orbitofrontal or amygdala lesions caused distinct impairments. Rats with lesions of the basolateral amygdala were impaired on the first reversal, showing no deficit in acquiring the reversal back to the original contingencies. Rats with lesions of the orbitofrontal cortex, however, were impaired on both types of reversals. This is indicative of a somewhat independent role of the orbitofrontal cortex during reversal learning (except for the first reversal), but both structures seem to have a role in the use of associative information during discrimination learning.

In addition to reversal learning and devaluation paradigms, evidence that supports a role of the orbitofrontal cortex in the integration of reinforcer value in decision-making processes comes from studies applying delayed, uncertain or probabilistic reward paradigms. For example, humans with orbitofrontal lesions are impaired in performing the Iowa Gambling Task (Bechara et al., 1997, 1999; Ptak
et al., 2000). In this task, subjects must choose a card from a deck associated with rewards and penalties of different sizes. 'Bad', high-risk decks are associated with a large reward but occasionally also with large penalties, and ultimately lead to an overall loss, whereas 'good', low-risk decks yield low rewards and no penalties, leading to an overall gain. Normal subjects initially choose high-risk decks, but bias their choice behavior towards the low-risk decks later on. Orbitofrontal patients fail to change their choice behavior towards the low-risk decks, despite the fact that they have an accurate account of the task and of the correct strategy. Apparently, the more delayed and probabilistic penalties are not used to guide choice behavior by these patients (Bechara et al., 1997, 1999; Ptak et al., 2000). In addition, damage to the orbitofrontal cortex impairs performance on a task in which subjects are required to choose between two possible outcomes and to bet on their choice: patients with orbitofrontal damage decide slowly and fail to choose the optimal, most likely outcome (Rogers et al., 1999a). Recent imaging studies provide further support for a role of the orbitofrontal cortex in decision-making under uncertainty (Ernst et al., 2004; Hsu et al., 2005). For example, using PET imaging, the choice between small, likely rewards and large, unlikely rewards was shown to increase blood flow in orbital areas (Rogers et al., 1999b). In addition, visual stimuli associated with higher uncertainty elicit increasing activations in orbital areas, which covaries with risk-seeking and risk aversion activity within this area (Tobler et al., 2007).

Behavioral lesion studies in rats indicate involvement of the orbitofrontal cortex in the evaluation of reward under probabilistic or uncertain conditions. Using behavioral tasks in which delay discounting or probability discounting paradigms are applied (respectively the free-choice between immediate small rewards and delayed but larger rewards, or between a certain small reward and an uncertain larger reward with fixed probability of delivery), it was demonstrated that the orbitofrontal cortex modulates the threshold at which animals switch to preferring immediate or reliable but smaller rewards over delayed or unreliable but larger rewards (Kheramin et al., 2003; Mobini et al., 2002). According to these studies, orbitofrontal lesioned rats tend to be more risk-aversive than normal rats when making choices, which is in contrast with the finding in humans that orbitofrontal damage leads to more 'risky' behavior. However, Winstanley et al. (2004) reported that rats with orbitofrontal lesions increasingly prefer the large, delayed reward in a delay discounting paradigm. A possible explanation for the discrepancy between these rat studies might be related to whether the orbitofrontal lesion is made before or after training in the behavioral task, since in the study by Winstanley et al. animals were trained before the orbitofrontal cortex was damaged and retested afterwards, whereas Mobini et al. trained and tested postoperatively. A more recent study, which uses an alternative probability discounting task that mimics the fundamental aspects of the gambling tasks as used in humans better than the previously used paradigms for rats,
demonstrated that rats with orbitofrontal lesions made after training indeed preferred the larger but uncertain reward, with stronger risk-taking behavior as demonstrated by human experimental data (Pais-Vieira et al., 2007).

The main findings of these behavioral lesioning studies involving delay- or probability discounting paradigms, an increased choice of risky options in a gambling task and an increased choice of a large uncertain or delayed reward after orbitofrontal damage, probably result from an impaired integration of the representation of the incentive value of the reinforcer with the representation of consequences of responding for that particular reinforcer (Winstanley et al., 2004). Hence the ‘punishment’ (loss of money in the gambling task or highly uncertain or delayed reward) is failing to induce a devaluation of the response outcome and to alter choice behavior.

To summarize, behavioral studies in rats and primates indicate that the orbitofrontal cortex is involved in the flexible guidance of goal-directed behavior, a function that requires the integration of representations of the incentive value of stimuli with schemes for appropriate behavioral responses.

*Orbitofrontal cortical functions: neurophysiology of reinforcement expectancy*

Associations that link initially neutral cues with the incentive value of reinforcers provide a framework for constructing expectancy in goal-directed behavior. Expectations of likely outcomes reflect judgments about chances of receiving a future reinforcer using associative information that is acquired during learning. This predictive information is updated continuously, providing an internalized representation of possible future outcomes that can be used to respond in an appropriate manner when changes in the environment ask for adaptations in behavior. For example, Montague and Berns (2002) developed a computational model (the predictor-valuation model, or PVM) of decision-making, in which the orbitofrontal cortex integrates information regarding rewards and punishments and their predictors, and supports the conversion of disparate types of future rewards into a common neural currency, i.e. a common scale used to compare the valuation of future behavioral acts or stimuli, on which decisions can be based. In another computational model, proposed by Pennartz (1997), expectations for future outcomes are compared with actual outcomes, a process that involves the glutamatergic projections neurons of the orbitofrontal cortex and basolateral amygdala. Subsequently, these projection neurons instruct sensorimotor regions, such as the striatum and pre- and supplementary motor cortical areas, to optimize stimulus-response mapping (Pennartz et al., 2000).

That animals use expectations about future outcomes was already demonstrated in a behavioral study by Tinklepaugh published in 1928. In this study, monkeys perform a delayed-response task for different kinds of food reward. In
particular trials, the monkey is shown her favorite food (banana) placed in one of the
food cups in front of her. During the delay, in which the animal must maintain the
information about the location of the banana, the experimenter replaces the banana
for a less favorite reward, a piece of lettuce. Discovering the lettuce instead of the
banana, the monkey, to use the author’s terminology, ‘becomes angry’: she does not
touch the lettuce but instead looks around for the banana, and sometimes shrieks to
the observers in apparent anger. However, when lettuce is used as cue, so that the
monkey expects the lettuce after the delay, she does not become angry but responds
normally and eats the lettuce. Apparently, the monkey only becomes angry when the
reward is different than expected.

Human brain imaging studies have demonstrated the involvement of the
orbitofrontal cortex in expectancy-related signaling (Breiter et al., 2001; Gottfried
et al., 2003; O’Doherty et al., 2002). Furthermore, Nobre et al. (1999) showed using
PET imaging that when learned and expected stimulus-reward associations were
violated, the orbitofrontal cortex is activated. In addition, firing characteristics of
orbitofrontal neurons also indicate that predictive information concerning outcomes
is present within the orbitofrontal cortex. Neurons in the orbitofrontal cortex do not
only respond to sensory features of predictive stimuli, but their firing patterns during
sampling of these stimuli also reflect the expected outcomes (Rosenkilde et al., 1981;
Thorpe et al., 1983). For example, manipulating the aversiveness of a syringe alters
the response of orbitofrontal neurons: when the syringe provides glucose to the
animal, no response to the sight of the syringe is observed, but when the same
syringe is used to deliver aversive hypertonic saline, neurons do become responsive to
the sight of the syringe. Other findings support the occurrence of cue-selective firing
within the orbitofrontal cortex that reflects predicted outcomes. Schoenbaum and
Eichenbaum (1995a) reported that in an 8-odor olfactory discrimination task, in
which four odors are associated with reward and four with no reward, the large
majority of neurons discriminates during cue sampling between rewarded and non-
rewarded odors, whereas only a minority discriminates between the different, reward-
associated odors. Similar findings were obtained in primates performing an odor
discrimination task, in which they learn to discriminate eight rewarded odors from
two odors associated with an aversive saline solution. Also in this task firing activity
of orbitofrontal neurons reflected, besides odor identity, the reinforcement
association of the odors (Critchley and Rolls, 1996). In addition, several studies in
rats and primates report switching of cue-selective firing of orbitofrontal neurons
during reversal learning (Rolls et al., 1996; Schoenbaum et al., 1999; Thorpe et al.,
1983).

Expectations for upcoming reinforcers have not only been found to be coded
during cue sampling. Studies in rats and primates that incorporated a delay before a
particular reinforcer is delivered demonstrated that neural firing in the orbitofrontal
cortex during this delay reflects expectations for different response outcomes, showing differential firing activity during the anticipation of various types or amounts of reinforcers, or reflects relative reward preference (Hikosaka and Watanabe, 2000; Ichihara-Takeda and Funahashi, 2006; Padoa-Schioppa and Assad, 2006; Roesch and Olson, 2004; Roesch et al., 2006; Schoenbaum et al., 1998; Tremblay and Schultz, 1999; Wallis and Miller, 2003). This differential activity appears to represent the expectation of the animal for the likely outcome. However, such activity was also demonstrated in other areas, including the basolateral amygdala (Schoenbaum et al., 1998) and other prefrontal regions (Wallis and Miller, 2003). Interestingly, expectancy-related activity in these areas seems to develop only after this activity has appeared in the orbitofrontal cortex (Roesch and Schoenbaum, in press): outcome-expectant activity was found to appear first in the orbitofrontal cortex and later in the basolateral amygdala. Furthermore, outcome-expectant firing within the basolateral amygdala was also demonstrated to depend on input from the orbitofrontal cortex. In contrast, during cue-sampling, selective activity seems to develop first in the basolateral amygdala, and development of cue-selective firing in OFC seems to be dependent on input from the basolateral amygdala (Saddoris et al., 2005; Schoenbaum et al., 1999, 2003a). As proposed by Roesch and Schoenbaum, the basolateral amygdala takes the lead to activate the network when external cues are present, as is the case during the cue period, whereas in the absence of external cues, for example during the delay-period, the orbitofrontal cortex is dominant in activating the network. Hence, the asymmetrical flow between orbitofrontal cortex and basolateral amygdala is distinguished primarily by the representational memory demand (Roesch and Schoenbaum, in press).

Besides coding of reward predictive information it was demonstrated that the orbitofrontal cortex is also involved in odor-place associations (Lipton et al., 1999). More recent, a role of the orbitofrontal cortex in the representations of spatial goals was demonstrated as well (Feierstein et al., 2006; Roesch et al., 2006). In these studies, the response site was explicitly coupled to differently valued rewards, and orbitofrontal neurons were found to encode the direction of choice or the location of the goal. Moreover, Feierstein et al. (2006) reported that a subset of neurons encoded both the direction and choice outcome. Hence, according to these authors, orbitofrontal neurons, in contrast to a pure valuation signal (Montague and Berns, 2002; Roberts, 2006), encode specific spatial-motor variables for the representation of goal locations, together with the responses needed to obtain that goal.

**Aims and outline of this thesis**

The general aim of this thesis is to examine the coding of predictive information regarding several reward-related parameters (i.e. magnitude and
probability of reward) by single units and ensembles in the orbitofrontal cortex of the rat. Electrophysiological recordings are made while animals perform an olfactory discrimination learning task, in which they are required to learn response outcomes based on the identity of a predictive stimulus. Since outcomes in this task can be appetitive or aversive, animals need to decide whether they execute a behavioral response or not. Examination of neural activity during this task will provide more information about how reward parameters, which can be considered main determinants of behavior, are represented in the brain, and how these neural representations may regulate decision-making processes.

In Chapter 2, we examine the coding of predictive information about reward magnitude by single units in the orbitofrontal cortex. To this end, we perform ensemble recordings during an olfactory discrimination ‘go/no-go’ task, in which five different odor stimuli are predictive for different amounts of a rewarding sucrose solution, no reward or an aversive reinforcer, i.e. quinine. Since the orbitofrontal cortex is known to code predictive information regarding upcoming reinforcers of different quality, we hypothesized that during several task phases, predictive information about reward quantity would be encoded in the orbitofrontal cortex as well.

In Chapter 3, we elaborate on the encoding of reward magnitude in the orbitofrontal cortex by examining how information about this reward parameter is represented by orbitofrontal ensembles. We use different reconstruction methods to reconstruct whether activity of orbitofrontal ensembles represents predictive information about reward magnitude, and to examine in more detail how this activity develops as the task progresses, during which task phases and on which time scales.

In Chapter 4, we extend our experiments to another reward parameter, the probability of reward. Using a similar paradigm as employed in the previous two chapters, we examine the neural coding of reward probability by both single units and ensembles in the orbitofrontal cortex. As the orbitofrontal cortex is indicated to be involved in decision-making under uncertainty, we hypothesized that neural activity in the orbitofrontal cortex represents information about reward probability. Of special interest in this chapter is the question whether orbitofrontal ensembles, active during an odor-reward probability task, encode the uncertainty or probability of a rewarding outcome.

To add to new developments in the field of ensemble recordings, we have developed the combidrive, as described in Chapter 5. The combidrive combines ensemble recordings with the technique of microdialysis, and might bring closer understanding of the interaction between neurotransmitters and cell populations during ongoing behavior. The general aim of this study was to develop an instrument for studying effects of local drug infusions on ensemble firing patterns during
ongoing behavior and cognitive processing. Specifically, we validated the instrument by comparing how three inactivating compounds (i.e. lidocaine, tetrodotoxin and muscimol) affected the capacity of frontal-cortical ensembles to sustain firing.

In the General Discussion contained in Chapter 6, the results of this thesis are discussed in the context of current ideas on the functional role of the orbitofrontal cortex, especially in relation to reversal learning.