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The ventral striatum in goal-directed behavior and sleep: intrinsic network dynamics, motivational information and relation with the hippocampus

Lansink, C.S.

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CHAPTER 1

Introduction



Our brain's ability to acquire and retain new information for recollection later on in time is invaluable for successful navigation through life. Memories serve as a compass for making decisions for the future; provide a database on ways to conduct complex actions and color one's personal life; however, defining what a memory actually is appears not very easy. Great variation exists for example in the time that memory is retained and in the type of information that is involved. Individual memories can contain little information or consist of a large amount of details bound together. Scientists have classified the memory spectrum according to various principles. For long-term memory, declarative or explicit memory is distinguished from non-declarative or implicit memories (Tulving, 1985; Squire and Zola, 1996; Milner et al., 1998). Declarative memories pertain to facts and events that people consciously can recollect and make reports about, such as autobiographical memories and 'world' knowledge. On the other hand, non-declarative or implicit memories are those that can be formed outside conscious and linguistic control. The main sub-class of implicit memory is procedural learning, which comprises the development and adjustment of motor skills and habits. Although researchers now acknowledge that memory is dependent on the integrity of multiple interconnected brain structures, some level of anatomical distinction can be justified as the circuitries underlying different types of learning consist of different subsets of brain structures. The formation and retrieval of declarative memories require at least an intact medial temporal lobe, whereas implicit memories rely more on phylogenetically older brain regions such as the striatum and other parts of the basal ganglia.

Irrespective of the type of memory, extensive research has been conducted towards understanding of the mechanisms by which the brain stores information for the long term. Memory consolidation in the strict sense refers to the process, that follows the initial registration of information, by which the memory trace is stabilized and becomes increasingly resistant to interference by newer information or other disturbances. This processing takes place across extended periods of time; it occurs beyond awareness and does not necessarily involve additional behavioral training or exposure to original stimuli (McGaugh, 2000). A broader view also includes process like *memory association*, by which new information is embedded in existing knowledge, *memory reconsolidation*, the renewed consolidation that takes place following recall of an existing memory and possibly *active erasure of memory* (Walker and Stickgold, 2004). Even higher order processes, such as extracting the gist of information and generalization of rules across contexts, may be included in memory consolidation.

The consolidation of memory traces on the cellular level is thought to depend on strengthening of synaptic connectivity between those neurons of which the activity patterns

represent the learned information (Hebb, 1949). Such strengthening may be initiated at the moment a scene, event or experience evokes a unique neuronal activity pattern and is thought to continue through reactivation of this pattern both as a result of re-exposure to the environmental cues during for example repeated training on a task, or, in the absence of such cues, during periods of rest and sleep (Marr, 1971; Buzsaki, 1989; McClelland et al., 1995; McNaughton et al., 2003).

Spontaneous reactivation of neuronal patterns during periods of rest and sleep is central in two studies that are presented in this thesis. We confined our research to two connected brain areas, the hippocampus and the ventral striatum, and focused on the type of information that is reactivated, the coordination of reactivation between the two areas, and the basic dynamics of the reactivation process. In addition, we studied the functional connectivity of different cell types in the ventral striatum. First, a general introduction will provide an overview of relevant literature to explain the rationale of the conducted research.

Hippocampal involvement in declarative memory formation

The involvement of the medial temporal lobe, including the hippocampal formation, in forming and maintaining declarative memories became dramatically clear when this region was bilaterally damaged in a group of patients that underwent medical treatment (Scoville and Milner, 1957). The most famous case, patient H.M., received a temporal lobe resection to relieve severe epileptic attacks. As a consequence of the surgical intervention, H.M. lost the ability to form new memories of facts and events. In addition to this *anterograde* amnesia, H.M. could not recall events that preceded the surgery by several years, whereas more remote memories were left largely intact. This *retrograde* amnesia thus showed a temporal gradient, also known as the Ribot gradient. These lesions were extensive, including the amygdaloid complex and uncus in addition to the hippocampal formation. That declarative memory formation is specifically dependent on the hippocampus was later demonstrated by another patient, R.B., who lost the CA1 region of the hippocampus bilaterally because of ischemia (Zola-Morgan et al., 1986). His amnesia of declarative information was profound, albeit not as severe as in the patients that lost the entire temporal lobe bilaterally (Squire, 1992).

Several theories attempt to explain the involvement of the hippocampus in the consolidation of long term declarative memories and differ, besides on this point, in their view on the necessity of the hippocampus for the consolidation of different types of declarative memory, i.e. episodic memory (memory for one's past) and semantic

memory (memory for general knowledge, or 'decontextualized' memory). Among influential theories is the 'standard' theory of declarative memory consolidation. This theory posits that the hippocampus, together with other areas of the medial temporal lobe, is crucial for all forms of declarative memory for a limited period of time (Squire, 1986; Squire et al., 2004). Ultimately, all memories will be consolidated in the neocortex and will thus become resistant to subsequent medial temporal lobe damage. Alternatively, the cognitive map theory holds that the hippocampus is necessary for the creation of a 'world based' spatial representation of an environment which provides the context in which episodic events are embedded (O'Keefe and Nadel, 1978; Burgess et al., 2002). According to this view, episodic but not semantic memory is mediated by the hippocampus because spatial context is part of episodic memory whereas semantic memory is essentially free of personal context. On this account, episodic and spatial memories will be affected upon trauma to the hippocampus regardless of the time-point in the past at which the memory was formed, whereas semantic memory will be spared. The 'multiple trace' theory posits that episodic memories exhibit a life-long dependency on the hippocampus (Nadel and Moscovitch, 1997). This theory states that the memory gains strength each time an episodic memory is retrieved and re-encoded, leading to the formation of multiple traces mediated by ensembles of hippocampal-neocortical neurons. The temporal extent and severity of retrograde amnesia is therefore related to the extent of the hippocampal damage. In contrast, semantic memories are dependent on the hippocampus only during a limited period after which they are supported by neocortical areas.

Evidence from patient studies is now complemented with studies using brain imaging techniques such as functional magnetic resonance imaging (fMRI). This non-invasive technique used predominantly with human subjects allows the measurement of changes in blood-oxygen-level-dependent (BOLD) or hemodynamic responses which are associated with changes in electrical activity of groups of neurons at localized spots in the brain. Studies using fMRI showed an enhanced activation of the medial temporal lobes as well as the frontal cortices during the encoding phase of learning words (Wagner et al., 1998), objects (Martin et al., 1997), faces (Haxby et al., 1996) or visual scenes (Brewer et al., 1998). In the latter case, the level of activation predicted whether an item was forgotten or recalled as well as the subjective confidence rating of remembered items. Differential roles of the hippocampus and neocortex were demonstrated in a study in which subjects had to memorize pictures and were tested for recall in an fMRI scanner immediately, a day, a month and three months after the learning episode (Takashima et al., 2006). Over time, the hippocampus showed decreasing activity whereas the medial prefrontal cortex showed increasing activity when the memory was retrieved.

Experimental animal studies require a different definition of declarative and episodic memory, because animals are not able to make overt reports about what they remember. It has been argued that episodic-like memories should contain at least a “what”, a “where” and a “when” component (Tulving, 1972; Clayton et al., 2003). Using the food-caching and retrieval behaviors of scrub jays, it was shown that indeed these birds can retain information on what type of food was stored at which location and can estimate how long ago each food item was stored (Clayton and Dickinson, 1998). Likewise, rats were able to associate particular flavored foods with locations on a radial arm maze and furthermore to predict the availability of the foods according to the length of the time interval between two trials on the maze (Babb and Crystal, 2006). Whereas in human episodic memory the “when” component includes the ability to “mentally travel in time” to locate a past event, it was shown that the “when” component in the animal studies is most likely based on the ability to estimate how long ago food was hidden or encountered (Roberts et al., 2008), indicating a qualitative difference between human episodic and animal episodic-like memory.

Behavioral studies on the formation of declarative-like memories in primates and rodents are less stringent on the above mentioned criteria, and rather require animals to represent complex relations among stimuli and/or to form memories that integrate object, spatial and/ or temporal information. A widely used task in this respect is the Morris water maze. In this task, rodents are placed in a large tank filled with an opaque water solution and have to escape to a submerged platform. Over a series of trials, rodents learn to use distal cues to predict where the platform will be and improve their escape time dramatically. Rodents whose hippocampus is disabled are severely impaired in learning this task (Morris et al., 1982; See BOX 1 for a brief description of the anatomy of the rat hippocampal region). These subjects are not impaired when the escape platform is visible, which represents a version of the task that does not require the integration of contextual information to escape quickly. In general, intervention in hippocampal functioning of rodents and non-human primates (Zola-Morgan and Squire, 1986; Zola-Morgan and Squire, 1990), either by lesion (Morris et al., 1982; Kim and Fanselow, 1992; Clark et al., 2002; Fortin et al., 2002), pharmacology (Riedel et al., 1999) or genetic manipulation (Tsien et al., 1996; Huerta et al., 2000; Rondi-Reig et al., 2001) results in a profound loss of both spatial and non-spatial relational memory (but see e.g. Zamanillo et al, 1999). A recent study showed that the dependence of relational memories on the hippocampus may change as a consequence of prior knowledge (Tse et al., 2007). The memory for novel flavor-place associations appeared to rely on the hippocampus during a time-limited period of at least several weeks. The authors hypothesized that over the course of learning these associations, the neocortex may consolidate the memories by developing a ‘schema’,

i.e. an abstract representation of the task contingencies. Activation of this schema upon re-entry of the testing arena would then facilitate the learning of new pairs. Indeed, they found that subsequent new pairs were learned after one presentation only and became rapidly (< 48 h) independent of the hippocampus.

BOX 1: Brief overview of the anatomy of the rat hippocampus

In the rat brain, the hippocampal formation is a prominent curved structure that extends from the dorso-septal pole to ventral-temporal sites (Fig. 1.1). In a cross section of this longitudinal axis, several hippocampal areas are recognized; the dentate gyrus, the three areas of the *Cornu Ammonis* (CA1-3) that constitute the hippocampus proper and the subiculum. The hippocampus is characterized by a strict laminar organization, clear layers of cell bodies and dendrites can be distinguished. The principal cells, granule cells in the dentate gyrus and pyramidal cells in Ammon's horn, use the excitatory transmitter glutamate. In addition, the hippocampal formation hosts several classes of interneurons. By using the inhibitory transmitter GABA (i.e. gamma-aminobutyric acid), interneurons are coordinating the activity of the principal cells (Klausberger et al., 2003). The hippocampal formation is surrounded by the parahippocampal region, consisting of the entorhinal, perirhinal and postrhinal cortices and the pre- and parasubiculum. Information from the neocortex flowing towards the hippocampus is conveyed mainly through connections within the parahippocampal region. From the entorhinal cortex, it is then partially transmitted through the trisynaptic pathway, from the dentate gyrus to the CA3 area before reaching the CA1 area, or directly to the CA1 area and/or subiculum. Subcortical projections that are considered to have a modulatory influence on excitability such as the cholinergic or dopaminergic systems, involving the lateral septum and ventral tegmental area respectively, reach the hippocampus directly. Efferent projections originate in the CA1 and subiculum and innervate the parahippocampal region which projects to sensory and associational areas of the neocortex, as well as subcortical areas by means of the fornix. For detailed reviews on the anatomy of the hippocampal formation see (Amaral and Witter, 1995; Witter et al., 2000).

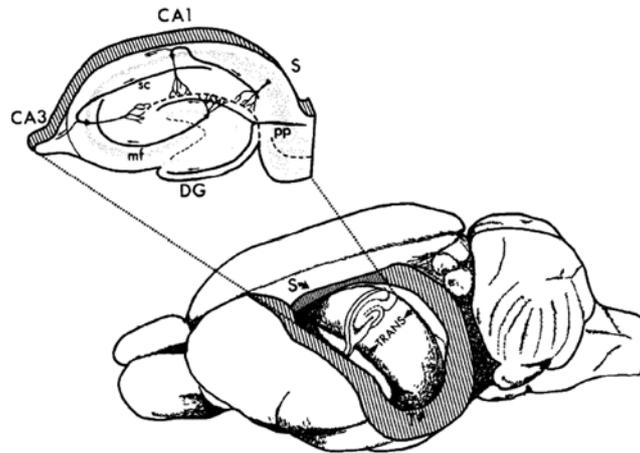


Figure 1.1

*Schematic representation of the rat brain. Parts of the neocortex have been removed to expose the underlying hippocampus. The curved hippocampus extends from the septal pole (S) to the ventro-caudal temporal cortex (T; i.e. longitudinal axis). Top left: A cross section of the hippocampus along the short or transverse axis (TRANS) showing the subfields of the Cornu Ammonis (CA1-3) and dentate gyrus (DG). Abbreviations: mf, mossy fibers; pp, perforant path; SC, Schaffer collaterals. Adapted from Amaral and Witter (1989) *Neuroscience* 31: pp. 573*

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The role of the hippocampus in memory consolidation in rodents has been primarily tested in tasks with a spatial component. Therefore, it is still a matter of debate whether spatial memory just represents an aspect of general hippocampus-dependent memory or whether it is a distinct form of memory in itself (Squire, 1992; Eichenbaum et al., 1999; Wood et al., 1999; Leutgeb et al., 2005).

Behavioral correlates of neural firing patterns of the rat hippocampus

The focus on the role of the hippocampus in spatial memory originated in studies from the early 1970's when O'Keefe and colleagues demonstrated that dorsal hippocampal CA1 neurons in the rat fired action potentials only in a specific location of the environment: the place field (O'Keefe and Dostrovsky, 1971; O'Keefe, 1976). An ensemble of hippocampal neurons will generally display a stable, uniformly distributed pattern of place fields in an environment, and this phenomenon has been proposed to serve as the neural representation of a cognitive map or spatial reference frame (O'Keefe and Nadel, 1978; Gothard et al., 1996). Such a representation of physical space was thought to be a key component of the rat's navigational system, as well as to provide the contextual framework

contributing to declarative memories. Extensive research has shown, however, that hippocampal firing cannot entirely be explained by purely spatial parameters. For example, place fields changed location when a salient cue was displaced in an environment (Muller and Kubie, 1987; Bostock et al., 1991), place fields showed directionality when the animal was required to run through a narrow path (McNaughton et al., 1983b; Wiener et al., 1989; Markus et al., 1995) and the field size depended on the abundance of landmarks along the track (Battaglia et al., 2004a). Furthermore, a pattern of place fields was shown to destabilize upon changing the environmental cues inside or outside the testing arena (Gothard et al., 1996; Knierim, 2002), or by altering the task contingencies (Wiener et al., 1989; Markus et al., 1995; Wood et al., 1999) when the geometrical coordinates of the maze were kept constant. Place fields may thus appear or disappear, shift position and change firing properties according to the current environmental contingencies, a process that is called remapping (Bostock et al., 1991; Kentros et al., 1998) or orthogonalization (Marr, 1969; Leutgeb et al., 2004). Remapping has been shown to occur in two distinct forms depending on the environmental changes (Leutgeb et al., 2005). If the local features of a behavioral enclosure (e.g. the shape or color of wall cues) were modified while the place of the enclosure was kept constant, hippocampal CA3 and CA1 neurons changed their maximal firing rate while the location of the place field remained the same, which is called *rate remapping*. In contrast, *global remapping* occurred when the behavioral enclosure was transferred to a new room or environment, which disrupted the distributions of location and maximal rate of the place field pattern. Rate remapping may serve the ability to represent different experiences within the same environment while global remapping may afford the possibility of representing different environments.

Hippocampal place fields may thus be considered to represent conjunctions of cues, including allocentric-environmental as well as egocentric cues that together define a place or situation in the environment (McClelland et al., 1995). Other researchers, however, have emphasized that the hippocampus is responsive also to purely non-spatial variables (Eichenbaum et al., 1999). In a delayed non-matching to sample task for rats it was demonstrated that hippocampal pyramidal cells changed their firing rate during approach of the sample and reward location, odor and/or trial type in addition to spatial location. Moreover, in this task, the influence of non-spatial variables appeared at least as strong as that of the location of the animals (Wood et al., 1999).

Dominant oscillatory patterns in the rat hippocampus

Depending on the sleep/wake state of the rat, the hippocampal network expresses different oscillatory profiles, reflecting the overall activity patterns of large volumes of cells. During

so called 'exploratory behaviors' such as running through or exploring an environment and during rapid-eye movement (REM) or paradoxical sleep, the hippocampus is dominated by an approximately sinusoid oscillation with a frequency between 4-12 Hz (Vanderwolf, 1969; Leung et al., 1982; Buzsaki and Eidelberg, 1983; Buzsaki et al., 1986). This "theta" oscillation or "rhythmic slow activity" is partly attributable to the pacemaker properties of the septum/ diagonal band of Broca, while additional synchronization may be accounted for by intrinsic network properties of the (para-)hippocampal region (Buzsaki, 2002). Both the amplitude and the phase of theta waves were shown to change as a function of depth of recording (i.e., the successive passage of several layers) with the phase difference between the cell-body or pyramidal layer of area CA1 and the fissure (i.e. the border between the CA1 and dentate gyrus), being 180 degrees (Buzsaki and Eidelberg, 1983; Buzsaki et al., 1986). Often, a faster gamma oscillation (30-100 Hz) is associated with theta waves (Bragin et al., 1995; Csicsvari et al., 2003). Theta oscillations are absent during 'consummatory' behaviors such as drinking, eating and during grooming as well as during quiet rest periods and slow wave sleep (SWS). In contrast, the hippocampus shows a desynchronized local field potential (LFP) in this state, referred to as 'large irregular activity'. During this type of activity, 'sharp waves' can be observed in CA1, occurring as a result of synchronous bursting of CA3 pyramidal cells (O'Keefe and Nadel, 1978; Buzsaki, 1986). In conjunction with sharp waves, fast field oscillations are present in the CA1 pyramidal layer and adjacent regions (Buzsaki et al., 1992; Ylinen et al., 1995; Chrobak and Buzsaki, 1996). These ripples consist of several sinusoid waves with a 150-200 Hz frequency. Pyramidal cells and interneurons generally fire action potentials during ripples while they are virtually silent during the periods in between.

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The ventral striatum in reward-related learning

One of the main target areas of hippocampal and subicular projections is the subcortical ventral striatum (Kelley and Domesick, 1982; Groenewegen et al., 1987; Brog et al., 1993), which mostly consists of the nucleus accumbens (see BOX 2 for a brief introduction of the anatomy of the ventral striatum). Besides hippocampal information, the ventral striatum receives extensive input from various cortico-limbic structures such as the prefrontal cortex, amygdaloid complex and the thalamus (Kelley et al., 1982; Berendse and Groenewegen, 1990; Groenewegen et al., 1990; Berendse et al., 1992). Output projections from the ventral striatum reach brain areas related to motor behavior such as the ventral pallidum, the substantia nigra, the ventral tegmental area and the lateral hypothalamus (e.g. Groenewegen and Russchen, 1984).

BOX 2: Brief overview of the anatomy and connectivity of the rat ventral striatum

The ventral striatum is located in the rostro-ventral part of the basal forebrain. Together with its dorsal counterpart it constitutes the input structure of the basal ganglia, which further consists of the globus pallidus and ventral pallidum, the subthalamic nucleus, and the substantia nigra pars compacta and pars reticulata. The dorsal and ventral striatum may be perceived as a continuum in the sense that no clear boundary can be drawn between the two regions (Fig 1.2; Voorn et al., 2004). The major structure of the ventral striatum is the nucleus accumbens (Heimer and Wilson, 1975), which can be divided on histochemical grounds into a core and a surrounding shell region (Voorn et al., 1989; Zahm and Brog, 1992). The ventromedial parts of the caudate-putamen and the bridges of the olfactory tubercle are usually taken to be part of the ventral striatum. The ventral striatum is characterized by strong inputs from limbic and cortical structures, such as the hippocampal formation (dorsal and ventral CA1 and subiculum), entorhinal cortex, basolateral amygdala, prefrontal cortex and anterior cingulate cortex and also from the midline thalamic nuclei (Fig. 1.2; Kelley and Domesick, 1982; Kelley et al., 1982; Groenewegen et al., 1987; Berendse and Groenewegen, 1990; Groenewegen et al., 1990; Berendse et al., 1992; Brog et al., 1993). Neuromodulatory transmitters are provided by the dopaminergic ventral tegmental area and the serotonergic median raphe nucleus (Haglund et al., 1979), as well as by cholinergic interneurons of the striatum (e.g. Kawaguchi et al., 1995). Striatal output pathways target cells in the ventral pallidum, the ventral tegmental area, the substantia nigra pars compacta and pars reticulata and the lateral hypothalamus (Groenewegen and Russchen, 1984). Via relay stations such as the ventral pallidum, subthalamic nucleus and substantia nigra pars reticulata, striatal output reaches the mediodorsal, ventromedial and ventrolateral thalamus, which in turn project to the premotor and prefrontal cortices (Zahm and Brog, 1992).

Inputs from the cortico-limbic areas form glutamatergic synapses on the dendritic spines and shafts of the principal cells of the ventral striatum, the medium-sized spiny neurons (MSNs), which constitute about 90-95% of the cell population (Chang and Kitai, 1985; Gerfen and Wilson, 1996). GABAergic striatal output is transmitted via axons of the MSNs, which, besides projecting outside the ventral striatum,

maintain a relatively extended pattern of axon collaterals within this area, providing a short-range regulation of the excitability of neighboring cells or ensembles (Wilson and Groves, 1980; Bolam et al., 1983; Czubayko and Plenz, 2002; Tunstall et al., 2002; Taverna et al., 2004).

Interneurons, of which the fast spiking interneurons (FSI), low-threshold spike interneurons and large, aspiny cholinergic interneurons are most prevalent (Wilson et al., 1990; Kawaguchi, 1993), comprise the other 5-10% of striatal neurons. FSIs constitute a class of GABAergic interneurons that can fire action potentials at high frequencies (Kawaguchi et al., 1995; Koos and Tepper, 1999; Berke et al., 2004; Taverna et al., 2007) and express the calcium-buffering protein parvalbumin (Cowan et al., 1990). These cells receive glutamatergic inputs from cortical areas and have aspiny dendrites and axon collaterals that spread to relatively large areas of the striatum but do not project outside the striatum (Cowan et al., 1990; Kita et al., 1990; Kawaguchi, 1993; Kawaguchi et al., 1995). Because of the latter two properties, FSIs are thought to provide a feed-forward inhibition of MSNs, thereby shunting glutamatergic inputs and simultaneously regulating persistent synaptic changes in glutamatergic inputs onto MSNs (Pennartz and Kitai, 1991; Pennartz et al., 1993; Pennartz et al., 1994; Thomas et al., 2000). *In vitro*, striatal FSIs have been shown to exert an inhibitory influence on MSNs mediated by GABA_A receptors (Koos and Tepper, 1999; Koos et al., 2004; Taverna et al., 2007).

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A classic view considers the ventral striatum as a neuronal “interface” between cognitive and emotional brain systems and systems generating behavioral output; i.e. the ‘limbic-motor interface’ (Mogenson et al., 1980). Physiological and pharmacological studies indicated, however, that the overall activity of the medium sized spiny neurons in the ventral striatum is not linearly correlated to the level of locomotor activity (see Pennartz et al, 1994 for a review). Furthermore, evidence from histochemical and connectional studies showed that the output of the ventral striatum also, though indirectly, reaches centers in the mid- and forebrain such as prefrontal cortical areas, suggesting a role for the ventral striatum in non-motor functions (e.g. Groenewegen and Russchen, 1984; Zahm and Heimer, 1993). Subsequent evidence from behavioral studies showed that the amygdala-ventral striatal projection conveyed information on environmental cues that predicted reinforcement, indicating that the output of the ventral striatum is influenced by reinforcement (Robbins et al., 1989). Considering the complexity of the input and output

topography, the heterogeneity of the cellular composition and the numerous behavioral functions in which the ventral striatum participates, Pennartz et al. (1994) proposed that within the ventral striatum, distinct networks of cells may form functional ensembles which act in parallel and in this way maintain a broad range of functions.

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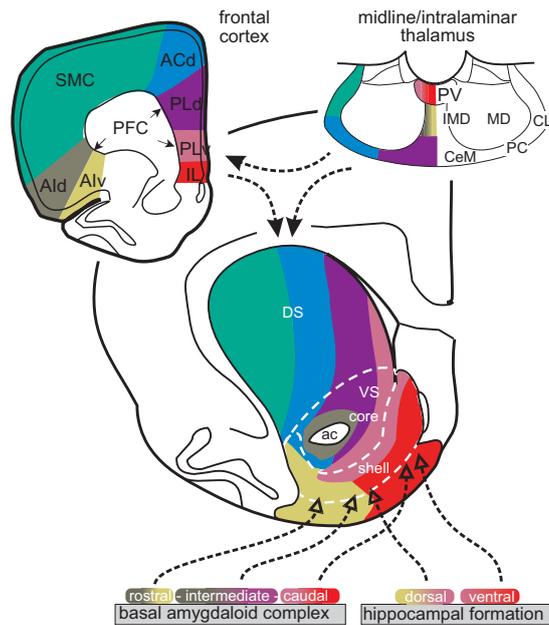


Figure 1.2

*Schematic representation of a coronal section through the brain at the level of the striatum (middle panel) showing the dorsal (DS) and ventral (VS) part of the striatum. The ventral striatum consists of a core and a surrounding shell region. The topographical arrangement of striatal afferents originating in the frontal cortex (upper left), midline and intralaminar thalamic nuclei (upper right), basal amygdaloid complex (lower left) and hippocampal formation (lower right) is color coded. Corticolimbic areas and their corresponding striatal projection zones are shown in the same color. Note that the hippocampal formation (in particular the subiculum and the CA1 region) projects to the ventral striatum, specifically to the medial, ventral and rostral shell, as well as to the immediately adjacent parts of the core. Abbreviations: ac, anterior commissure; ACd, dorsal anterior cingulate cortex; Ald, dorsal agranular insular cortex; Alv, ventral agranular insular cortex; CeM, central medial thalamic nucleus; CL, central lateral thalamic nucleus; IL, infralimbic cortex; IMD, intermediodorsal thalamic nucleus; MD, mediodorsal thalamic nucleus; PC, paracentral thalamic nucleus; PFC, prefrontal cortex; PLd, dorsal prelimbic cortex; PLv, ventral prelimbic cortex; PV, paraventricular thalamic nucleus; SMC, sensorimotor cortex. Adapted from Voorn et al. (2004) *Trends Neurosci* 27: pp. 470*

In this view, a specific pattern of glutamatergic and dopaminergic inputs recruits the associated functional ensemble in the ventral striatum. The selected ensemble generates a characteristic output pattern that, when transferred to target structures, may elicit behavioral effects that are linked to this ensemble. Currently, ventral striatal functioning is summarized as driving or invigorating goal directed behavior on the basis of the motivational value of environmental cues and contexts (Pennartz et al., 1994; Robbins and Everitt, 1996; Cardinal et al., 2002).

The role of the ventral striatum in different types of reinforcement-dependent learning has been extensively studied over the past decades. The acquisition and performance of both Pavlovian and operant conditioning appear to depend on an intact ventral striatum. In Pavlovian or classical conditioning tasks, paired presentations of a neutral cue or context and a reinforcer induce appetitive responses to that cue or context in the case of a positive reinforcer (i.e. reward) or aversive reactions to avoid punishment in the case of a negative reinforcer. Selective lesions of the accumbens core (Parkinson et al., 1999b), antagonism of NMDA or dopamine receptors in the nucleus accumbens during task performance (Di Ciano et al., 2001) or dopamine depletion of the nucleus accumbens (Parkinson et al., 2002) severely impaired appetitive Pavlovian conditioning. In instrumental or operant conditioning, subjects are required to make a response, for example a nose-poke or a lever press, in order to obtain a reinforcer. The learned association between a response and the outcome evokes increased responding. Rats with manipulated accumbens (core) functioning, because of a lesion or NMDA or dopamine receptor antagonism, were retarded in learning this association, and showed a depression of overall response rates (Balleine and Killcross, 1994; Kelley et al., 1997; Sokolowski and Salamone, 1998; Smith-Roe and Kelley, 2000; Corbit et al., 2001).

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In addition, when the nucleus accumbens was disabled by a lesion or dopamine antagonist infusion, rats were impaired in tasks that require the use of spatially distributed environmental cues in order to reach a goal, such as the Morris water maze (Sutherland and Rodriguez, 1989; Ploeger et al., 1994). A study that used a radial arm maze task, in which the rats had to remember and revisit the maze arms in which they previously found food, reported retarded learning and performance after transient inactivation of the nucleus accumbens by infusions of the local anesthetic lidocaine (Seamans and Phillips, 1994). Noteworthy is, that in all studies thus far, rats with a disrupted nucleus accumbens performed like controls in hippocampal-independent versions of the water maze task and radial arm maze task, which excludes the possibility that the observed impairments were due to factors unrelated to the task, such as motor behavior.

Studies in which pharmacological agents were injected into the nucleus accumbens shortly after training on a task disclosed a role for the ventral striatum in the consolidation of newly acquired information. An inhibitory avoidance task is an aversive conditioning task in which a single pairing of an environment with a foot shock inhibits rats to re-enter the environment on subsequent testing. Rats that received an intra-accumbens injection of tetrodotoxin following this experience, showed shorter latencies than controls on the re-entry of the compartment where they were previously shocked (Lorenzini et al., 1995).

In a Pavlovian autoshaping paradigm, classically conditioned appetitive responding on the day following task acquisition was impaired after post-training administration of a D1 dopamine receptor antagonist or a NMDA receptor antagonist (Dalley et al., 2005). It was also shown that blocking protein synthesis in the ventral striatum shortly following training on an instrumental conditioning task impaired the retention of the memory of the task (Hernandez et al., 2002). Immediate post-training infusions of a selective D2 dopamine receptor antagonist or an NMDA receptor antagonist impaired retention on the spatial, but not on the visible, platform version of the Morris water maze task (Setlow and McGaugh, 1998; Sargolini et al., 2003). In these studies, the agents had no effect on consolidation when they were applied a few hours after training, indicating the specific dependence of early consolidation on glutamate and dopamine, which provide signals to the intracellular machinery that promotes the synthesis of proteins.

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The above mentioned studies show that, besides the glutamatergic inputs that arise in cortico-limbic structures, the functioning of the ventral striatum critically depends on dopamine release originating from activity in the ventral tegmental area. Dopamine, in general, plays a major role in controlling and regulating cognitive and behavioral functions in rodents, primates and humans (Robbins and Koob, 1980; Taylor and Robbins, 1986; Schultz, 1997; Spanagel and Weiss, 1999). Dysfunction of dopaminergic transmission is associated with various pathologies ranging from Parkinson's disease to schizophrenia, mania and depression (Hornykiewicz, 1962; Stevens, 1973; Randrup and Munkvad, 1974; Willner, 1983; Swerdlow et al., 1987; Laruelle et al., 2003; Nestler and Carlezon, 2006). Moreover, the addictive properties of several drugs of abuse arise to a major extent via the mesolimbic dopaminergic system (Kalivas and Volkow, 2005; Everitt and Robbins, 2005). The role of dopamine in the striatum and its impact on reward-related learning is controversial (Pennartz, 1995; Spanagel and Weiss, 1999). An influential theory holds that dopamine neurons in the ventral tegmental area signal the deviation or 'error' between the temporal prediction and the actual occurrence of a reward (Schultz et al., 1997; Schultz, 2007). Dopamine neurons in the ventral tegmental area and the substantia nigra

have been shown to respond only to primary rewards when these occur unexpectedly. When a reward can be predicted by the animal, the response is transferred to the earliest conditioned, reward-predicting stimulus. Likewise, when a predicted reward is omitted, dopamine neurons show a depression in their firing activity at the time the reward would have been obtained.

An alternative hypothesis holds that the basal ganglia may subserve a selection mechanism between available behavioral strategies (Redgrave et al., 1999). In this view, the dopamine signal facilitates the re-allocation of cognitive and behavioral processing capacities towards any unexpected event of significance, including reward. When converging inputs compete for limited cognitive or motor resources, dopamine in the ventral striatum will filter out irrelevant patterns or switch between existing patterns of incoming information (Pennartz et al., 1994; Taverna and Pennartz, 2008). This switching hypothesis thus proposes a more general role for dopamine in associative learning.

The role of the ventral striatum in reward-related processing is further emphasized by chronic recordings of single unit activity in the rodent ventral striatum and the primate striatum. Single units signal reinforcers by increments and decrements in firing rate time-locked to the receipt of primary reinforcers of both positive and negative valence. These transient modulations in firing rate can occur during the consumption phase following reward delivery (Apicella et al., 1991b; Roitman et al., 2005) or prior to the delivery of a reward signaling the expectancy or anticipation of that reinforcer (Schultz et al., 1992; Setlow et al., 2003). Within trials, the latter responses may gradually gain strength until the reinforcer becomes available. Moreover, responses are also found to cues predicting the availability of reinforcers (Apicella et al., 1992; Setlow et al., 2003; Roitman et al., 2005). Such representations have been shown to develop with learning; responses first occur to the primary reinforcement but subsequently, over the course of learning, they are thought to be transferred back to the time of cue presentation (Roitman et al., 2005). In addition, rodent studies using operant conditioning paradigms showed brief firing rate increments and decrements prior to, during and after the behavioral response next to firing rate changes attributable to primary natural rewards or drugs of abuse (Carelli and Deadwyler, 1994; Chang et al., 1997; Janak et al., 1999; Carelli, 2002). Primate neurons fired differentially in relation to the availability of a reward (Hollerman et al., 1998) and to the type (Hassani et al., 2001) magnitude (Cromwell and Schultz, 2003), and temporal proximity (Bowman et al., 1996; Shidara et al., 1998) of predicted reinforcers. These studies excluded the possibility that the expectancy-related responses were due to particular motor behaviors, general arousal, or to the sensory properties of the stimuli that were used. Studies in

rodents also reported neuronal correlates related to location and locomotion (Lavoie and Mizumori, 1994; Shibata et al., 2001).

Recent fMRI studies have supported the postulated role of the ventral striatum in processing and predicting reinforcers. The human ventral striatum was activated as evaluated by BOLD signal contrasts during cue presentation and reward delivery in an appetitive classical conditioning task (O'Doherty et al., 2006). The level of BOLD activation appeared to be dependent on the individual preferences of the available juice tastes. The most and the least preferred juices induced the highest activations whereas intermediate juices evoked less activation. Other studies have shown predictive activations of the ventral striatum during appetitive learning for juice and money rewards, as well as during learning with aversive stimuli such as pain (Knutson et al., 2001; Jensen et al., 2003; O'Doherty et al., 2004; Seymour et al., 2004).

Synaptic plasticity

Strong evidence indicates that the storage of information in neural networks takes place through alterations in synaptic connectivity induced by firing patterns during learning and consolidation, which is called plasticity. In 1949, Donald Hebb postulated that, when the pre-synaptic cell fires closely in advance of the post-synaptic cell, the synapse between the two cells will be strengthened (Hebb, 1949). Hebb envisioned this mechanism to underlie the formation of cell assemblies; i.e. networks of neurons that are functionally interrelated. Electrical stimulation patterns were shown to induce both long term potentiation (LTP; Bliss and Gardner-Medwin, 1973; Bliss and Lomo, 1973; Andersen et al., 1977) and reduction (long term depression, LTD; Lynch et al., 1977) of synaptic responses *in vitro* and *in vivo*. LTP could be induced by stimulation patterns resembling physiological activity (Larson and Lynch, 1986; King et al., 1999a) and was shown to be long-lasting (Abraham, 2003) which are two fundamental features ascribed to cellular memory formation. Learning-associated LTP was found to occur in the lateral amygdala of rats when they were conditioned to an electrical shock (Rogan et al., 1997) and in the hippocampus after the rats mastered an inhibitory avoidance test (Whitlock et al., 2006). In spike timing dependent plasticity (STDP) the time and order in which spikes are emitted is critical for induction of an enhancement or depression in synaptic efficacy (Levy and Steward, 1983; Markram et al., 1997; Bi and Poo, 1999; Abbott and Nelson, 2000; Dan and Poo, 2004). If firing of the presynaptic cell was followed within 40 ms by activity of the postsynaptic cell, synaptic transmission between the two was enhanced. In contrast, when the postsynaptic spike preceded presynaptic activity by at most 40 ms, synaptic efficacy was decreased.

If plasticity represents synaptic mechanisms for establishing memories in the hippocampus and elsewhere, then changes in evoked plasticity should affect the formation of memories. Blocking NMDA receptors in the hippocampus impaired the formation of spatial memories to solve the water maze task (Morris et al., 1986). Furthermore, repeated induction of LTP to asymptotic levels in the hippocampus, thereby saturating the synapses, blocked the ability of rats to learn the location of the hidden platform in the water maze (McNaughton et al., 1986; Castro et al., 1989; Barnes et al., 1994). Recently it was shown that manipulations that reversed the maintenance of LTP also produced persistent loss of spatial memories (Pastalkova et al., 2006). Additional evidence was provided by the use of genetically modified mice, lacking a gene for a protein that is involved in the cascade of events leading to the induction of LTP. Mice lacking the R1 subunit of the NMDA receptor in the hippocampal CA1 neurons showed normal basal synaptic transmission but LTP, induced through stimulation of the Schaffer collaterals, was disrupted (Tsien et al., 1996). These mice were also dramatically impaired in the formation of spatial memories. Furthermore, knock-out mice lacking the gene for CAM kinase II, or those that expressed a form of this protein resistant to autophosphorylation, did not show LTP, and were impaired in spatial learning (Silva et al., 1992a; Silva et al., 1992b; Giese et al., 1998; Lisman et al., 2002). Mice lacking the A subunits of the AMPA type glutamate receptor did not show LTP but could nonetheless learn the Morris water maze task (Zamanillo et al., 1999), indicating that there may be other ways to form memory traces on the cellular level in addition to the currently known mechanisms.

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Most of the research on synaptic plasticity has been dedicated to the hippocampus but LTP and LTD have also been observed in other brain areas such as the neocortex, amygdala and the ventral striatum (Artola and Singer, 1987; Pennartz et al., 1993; Kombian and Malenka, 1994; Rogan et al., 1997). In the ventral striatum, LTP of excitatory transmission on medium sized spiny neurons may constitute an important mechanism underlying acquisition and storage of reward-related information (Pennartz et al., 1993; Pennartz et al., 1994).

Memory consolidation during sleep

Anchoring information in long term memory may take a considerable amount of time. It has long been hypothesized that consolidation processes continue during periods of rest and sleep, when the brain is 'off line'; i.e. not engaged in the processing of external stimuli, and considerable research has aimed to disclose the role of sleep in memory consolidation. Early rodent studies focused on the role of REM sleep, and deprived subjects of this sleep phase between training and testing on a behavioral task. Typically,

rats were sleeping on a pedestal surrounded by water. As muscle tone decreased at the time of REM sleep onset rats would fall in the water and wake up. The body of evidence obtained with this method failed to confirm or refute a clear link between REM sleep and memory. This can be explained by the fact that, besides REM sleep deprivation, the method also caused stress, loss of SWS, isolation and fatigue, which are factors that could have influenced the results (see Vertes and Eastman, 2000; Siegel, 2001).

Currently, evidence from human studies indicate that memory can indeed benefit from a period of sleep. This relation was most convincingly shown for procedural memory tasks in which sleep dependent consolidation is expressed by an increase in performance that is unrelated to time *per se*. A set of complementary studies showed that learning on a visual discrimination task improved significantly after the first night of sleep following training (Karni et al., 1994; Stickgold et al., 2000a) whereas a 4 to 12 hour period of waking following acquisition did not induce this increment (Stickgold et al., 2000a; Stickgold et al., 2000b). In these studies, some of the subjects were deprived of sleep on the night following learning but had a recovery period of two days before performance on the task was tested. This protocol prevented fatigue and loss of alertness from influencing the results. Gais and colleagues (2000) deprived subjects during early-night sleep (SWS enriched) or late-night sleep (REM sleep enriched) and found a SWS-dependent increase in performance that was enhanced by subsequent REM sleep. Whereas previously a specific REM sleep dependence was reported (Karni et al., 1994), it was shown more recently that a suppression of SWS by 30% can limit the improvement on the visual discrimination skill (Aeschbach et al., 2008). A study by Stickgold et al. (2000b) verified the findings by Gais et al. by showing that performance benefits were correlated with both early night SWS and late night REM sleep and that the product of these sleep parameters can explain over 80% of the inter-subject variance. Findings of comparable studies using other procedural learning tasks such as the sequential finger tapping task, in which subjects type sequences of numerals as fast and accurately as they can (Fischer et al., 2002; Walker et al., 2002; Walker et al., 2003) or learning tasks based on auditory cues (Fenn et al., 2003; Gaab et al., 2004), yielded results remarkably consistent with those of the visual discrimination task, including apparent contributions by the two different sleep states.

Sleep has also been shown to enhance the retention of declarative memories, often tested with recall and recognition of previously learned items. Several studies by Born and his colleagues have shown an improvement on an associative word-pair task, which depended on an early-night, SWS-enriched, sleep (Plihal and Born, 1997; Gais and Born,

2004). Later studies that included a recovery night after sleep deprivation confirmed these results, and showed that they could not be attributed to fatigue or circadian influences (Drosopoulos et al., 2005; Gais et al., 2006). In addition, factors like task difficulty (Empson and Clarke, 1970; Tilley and Empson, 1978), emotional salience (Wagner et al., 2001; Hu et al., 2006; Wagner et al., 2006) and explicit recollection (Drosopoulos et al., 2005) have been shown to determine the sleep-dependence of retention.

Interestingly, recent studies show that sleep may also promote the formation of novel, higher-order associations. A night of sleep, following training on a numeric sequence problem solving task, was shown to trigger insight into a hidden rule that enhanced performance on the task the next morning (Wagner et al., 2004). Likewise, sleep has been shown to facilitate the formation of inference-based novel relationships between previously learned items (Ellenbogen et al., 2007).

Reactivation of neuronal activity patterns during ‘off-line’ periods

Based on the early ideas of Marr (1971), it is currently thought that memory consolidation benefits from spontaneous replay of neuronal patterns representing behavioral experiences during periods of rest and sleep; i.e. when the brain is not occupied with the processing of environmental stimuli. Reactivation may strengthen synaptic connections activated during the preceding behavior and may link pieces of information stored in distributed form throughout the brain. The first hint towards the occurrence of experience-specific reactivation of neurons was provided by a study in which the activity of two hippocampal neurons was monitored during periods of wakefulness and sleep in each recording session (Pavlidis and Winson, 1989). During recordings, the experimenters confined a rat to the place field of one neuron, which induced robust firing of that neuron. The other neuron, that exhibited a place field elsewhere in the environment, remained virtually silent. During subsequent sleeping periods, the cell that was active during the awake period displayed an elevated firing rate and increased burst firing compared to the inactive neuron. This effect was not observed during periods of wakefulness after place field exposure. This study showed that the level of activity of a hippocampal neuron during an awake experience can influence its excitability in a subsequent sleep period. To demonstrate experience-specific reactivation however, the temporal relationships between patterns of multiple neurons, reflecting their co-activation during the preceding behavior, requires consideration. If reactivation occurs, cells that were near simultaneously active during the behavioral experience should tend to be co-active in subsequent sleep and cells that are activated independently of one another during running should not be coactive during sleep afterwards (but see our results in *chapter 5*).

Wilson and McNaughton (1994) recorded the activity of relatively large ensembles (up to ~100 cells) of hippocampal neurons during behavioral episodes flanked by periods of sleep. In each of these periods they assessed the co-activation of pairs of neurons by computing a correlation of the spike trains binned in small time intervals (100 ms). When firing of cell pairs within the ensemble was correlated during the behavior, mainly because their place fields overlapped, it was also correlated during subsequent SWS, but not necessarily during the sleep preceding the behavior. Just as significant is the finding that cells that were not active together during the behavior, because of non-overlapping place fields or inactivity, remained uncorrelated during sleep.

30 | Subsequently, Kudrimoti et al. (1999) formulated an analysis to quantify reactivation, based on partial regression methods, that represents the similarities in firing pattern correlations for all pairs of simultaneously recorded cells. The *Explained Variance* reflects the extent to which the variance in the distribution of cell pair firing-rate correlations during rest and sleep following track running can be accounted for statistically by the pattern of correlations induced during the running itself. In addition, the analysis controlled for the distribution of correlations that was present in the rest and sleep periods before track running (i.e. the baseline period; before the activation of neurons in the behavioral task). Using this method, the authors showed that hippocampal reactivation was significantly stronger during sharp wave-ripples compared to the intervals between these events (see also Wilson and McNaughton, 1994). This finding suggested that reactivation may depend on the occurrence of sharp-wave ripples rather than on SWS *per se*. In addition, no indications for the occurrence of reactivation were found during REM sleep. The reactivation strength decayed generally within ~30 minutes to undetectable levels although the authors note that neural patterns were incidentally re-expressed for up to 24 hours after the behavioral experience. Rats in this study were sequentially exposed to familiar and novel spatial tasks interleaved with periods of rest and sleep. This paradigm demonstrated that reactivation occurred both after running in a well-known environment as well as following a novel experience. Moreover, neural patterns of these two separate events recurred in the same SWS episode, indicating that multiple traces are processed in close temporal proximity to one another.

If the reactivated trace represents an experience that evolved in time, the trace should not only contain information on the activated ensemble but also on the order in which the individual cells fired. Indeed, hippocampal cell pairs with overlapping place fields that showed a particular firing order during running on a triangular track preserved temporal asymmetry in subsequent sleep (Skaggs and McNaughton, 1996). During sleep, the

temporal offset between the firing of the two neurons appeared compressed about 10-20 fold while the relative magnitude of the offset was maintained. Later studies showed that also in larger ensembles of hippocampal neurons the order of spike timing is preserved in the sleep period after a behavioral experience (Nadasdy et al., 1999; Lee and Wilson, 2002). Both studies also indicated a time compression of the reactivated trace. In this context the term replay is used to indicate the re-expression of neural activity patterns. In contrast to 'reactivation', 'replay' assumes recurrence of the same or a similar temporal order of neuronal firing as evoked during the behavioral experience.

Most studies emphasize the dependence of replay on quiet resting states and SWS but firing pattern similarity between track running and REM sleep has also been shown (Louie and Wilson, 2001). Interestingly, the firing patterns observed during running on a track were similar to those observed during REM sleep episodes *before* running on the task. It should be noted, however, that REM periods following track running were sparse. Hence, under-sampling might explain the lack of observed reactivation. Nonetheless, the question remains whether the effect represents lingering recurrence of activity patterns induced during the track running episode on the day before, or whether some pre-activation process occurs, based on the expectations of the upcoming track running. The former option is supported by evidence showing that the plasticity-associated immediate early gene *zif-268* was up-regulated in REM sleep periods following a behavioral experience (Ribeiro et al., 1999; Ribeiro and Nicolelis, 2004). This elevation is found to be more prominent in remote than in early REM sleep episodes.

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Although pioneering work already indicated that reactivation might be occurring during sharp waves-ripples rather than during SWS as a whole (Kudrimoti et al., 1999), the idea that replay thus could take place during short pauses between running periods and/or reward consumption in an arena has only recently been tested. Indeed, ensemble firing patterns during task performance were reliably reactivated during sharp-wave ripples on the task (O'Neill et al., 2006). The similarity of the firing patterns during behavior and awake sharp wave-ripples and the emission rate of sharp wave-ripples were found to increase with experience and regularity of the behavior (Jackson et al., 2006). While during rest and sleep associated reactivation the firing order induced during behavior ('forward replay') is preserved, sequences of neural activity were found to be replayed in reversed order during the behavioral episode when rats were pausing after running down a linear track (Foster and Wilson, 2006). This conclusion was subsequently refined by the observation that replay occurred in forward order when rats were about to start running the next lap on the track, while if they just had finished the lap and had not turned around yet, the order

of firing was reversed (Diba and Buzsaki, 2007). Taken together, these results suggest that the 'internal rehearsal' and consolidation processes already starts during the behavioral experience and continues during rest and sleep following the behavior.

Many theories on consolidation of declarative memories assume an interaction between the hippocampus and the neocortex (O'Keefe and Nadel, 1978; Squire, 1986; Nadel and Moscovitch, 1997; Squire et al., 2004). In line with this view, hippocampal and neocortical activity patterns might become aligned during a behavioral event and subsequent reactivation of the hippocampal pattern might orchestrate reactivation of the associated neocortical pattern leading to the stabilization of the memory trace (Marr, 1971; Buzsaki, 1989; McClelland et al., 1995; McNaughton et al., 2003). If this were the case, reactivation is expected to occur in the neocortex as well as between hippocampal and cortical ensembles. Studies in rodents and primates have indeed shown neocortical and joint hippocampal-neocortical reactivation (Qin et al., 1997; Tatsuno et al., 2006; Battaglia et al., 2007; Euston et al., 2007b; Ji and Wilson, 2007). In addition, cross-regional reactivation was also found for different neocortical areas (Hoffman and McNaughton, 2002). During sleep, the parietal and the prefrontal cortices showed forward reactivation, which in the latter structure was compressed by a factor ~ 7 (Euston et al., 2007b). Two studies provided evidence of joint hippocampal-cortical reactivation. A coordinating role of the hippocampus in reactivation in its target areas would be revealed by a firing order in which the hippocampal cells are consistently activated before the neocortical neurons. However, the studies did not present evidence for a preferred firing order (Qin et al., 1997; Ji and Wilson, 2007).

The main focus of reactivation research has been on the hippocampus and neocortex as these structures have been shown to be important for declarative memory consolidation; however, the ventral striatum also displays recurring neural activity patterns during sleep (Pennartz et al., 2002; Pennartz et al., 2004). These findings imply that reactivation is a widespread phenomenon that also occurs in subcortical structures. Reactivation appeared prominent during SWS, and did not decay until at least 40 minutes after track running. Neurons that increased their firing rate during and shortly after the occurrence of sharp wave-ripples reactivated more strongly than others, which suggest that ventral striatal reactivation is influenced by the hippocampus.

The reactivation of neuronal patterns during sleep has been shown to occur in several species: rats, primates, song birds (Dave and Margoliash, 2000) and probably also humans. Using PET scanning, which measures regional cerebral blood flow (rCBF) as indicator of

activity of vast groups of neurons, Peigneux et al. (2004) showed that rCBF was elevated in the human hippocampus in SWS following a route-learning task in a virtual environment. The level of rCBF increase during sleep was correlated to the task performance of the subjects on the next day. In a follow-up fMRI study, the hemodynamic response in the hippocampus appeared also elevated during awake episodes following training on the route-learning task, when subjects were engaged in a control task that did not tax spatial memory (Peigneux et al., 2006). Thus consistent with the rodent literature, reactivation may occur at least at a global level in the human brain both in periods of wake and periods of sleep. Imaging studies addressing reactivation also indicated REM sleep as potential substrate. When a hippocampus-independent serial reaction time task was used, REM sleep associated elevations in rCBF were found in areas located in the occipital and pre-motor cortices. (Maquet et al., 2000; Peigneux et al., 2003).

Outline of this thesis

Cognitive operations, including reactivation, are likely mediated by large networks of connected neurons distributed throughout the brain. To understand the neural underpinnings of these processes, scientists have recognized the need for methods that allow examination of neural processing on the network level rather than on the level of single cells. This project focused on the interplay within and between cell groups of different phenotype in the ventral striatum. Furthermore, we aimed to examine the process of reactivation in the ventral striatum as well as in the hippocampal-striatal circuitry. Our experimental approach included recordings made simultaneously from neural ensembles in the dorsal hippocampus and ventral striatum in freely moving rats. These areas are anatomically and functionally strongly connected, but are widely separated in the brain. To be able to conduct these multi-area recordings we developed a device, the 'split-hyperdrive' containing 14 individually adjustable electrode drivers. Chronic implantation of the drive allowed stable recordings of ensembles of up to 25 hippocampal and striatal cells during several weeks, under low restraint and low stress conditions for the rat. Recordings were made while the rats were running along a triangular track and during periods of rest and sleep. This experimental method and its validation are described in **chapter 2**.

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In the ventral striatum, a relatively small group of fast-spiking interneurons are thought to exert inhibitory control over the activity of the principal cell population, the medium-sized spiny neurons, thereby possibly influencing processes such as synaptic plasticity. Evidence on this interaction has been provided by *in vitro* electrophysiological studies, leaving open the question on how firing patterns of the fast spiking interneurons manifest themselves in the awake brain while the animal is engaged in behaviors such as running and reward consumption. **Chapter 3** deals with the question of whether the fast spiking interneurons exhibit changes in firing rates linked to rewards and other behavioral events in a way that could be similar or dissimilar to the principal cells. Furthermore, the temporal relationships between the firing of putative fast-spiking interneurons and medium-sized spiny neurons are addressed, to shed light on the interactions between these two important cell groups.

The evidence that the ventral striatum is involved in the consolidation of memories and its prominent role in the mesolimbic reward processing system of the brain triggered the question of whether the ventral striatum would specifically reactivate information related to rewards. If this were true, it would indicate that memory traces not only consist of feature- and spatial/contextual information, but also contain an emotional or motivational

component. Given that striatal reactivation occurred during quiet wakefulness and SWS, it appeared also interesting to test whether it is most prominent during sharp wave-ripple complexes, as in the hippocampus, or whether reactivation is not modulated during these events. Moreover, the question arises whether reactivation also occurs during REM sleep, when the hippocampal local field potential is devoid of sharp wave-ripples and shows a pattern that is more related to an active awake state. **Chapter 4** describes our study on the relation between striatal reactivation and reward-related processing, including the dynamics of the reactivation process in the striatum.

Successful foraging behavior as well as the mastering of a range of laboratory tasks depends on a representation of a type of food and the location where the food can be found, information that is processed in the hippocampal-ventral striatal circuitry. A coordinated reactivation between the two areas may contribute to consolidation of spatial/contextual and motivational information during and after foraging behavior. Moreover, based on the key role of the hippocampus in declarative memory consolidation, it has been hypothesized that the hippocampus initiates and coordinates reactivation in its target areas. This would imply the existence of an organized interaction between the hippocampus and ventral striatum during periods of reactivation. A guiding role of the hippocampus would be indicated when activity in hippocampal networks consistently precedes activity in striatal networks during a behavioral experience and reactivation during subsequent rest. The investigation of cross-structural replay is described in **chapter 5**. In this study, we also examined factors that may govern joint reactivation as detected by near-synchronous firing in the hippocampal-striatal circuitry during the behavioral experience, such as firing-rate modulation by the theta oscillation, the temporal order of firing, and the expression of place and reward-related firing patterns. Furthermore, the temporal dynamics of firing patterns between hippocampus and ventral striatum are addressed here. In **chapter 6** our results are summarized and discussed in relation to the current literature and future possibilities for research.

