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

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# Summary of results

Communication between neurons is the driving force of all processing in the brain, from encoding sensory stimuli to mediating complex cognitive operations and facilitating output of specific behavioral patterns. To gain insight into multi-faceted processes such as memory consolidation and reactivation, it is of importance to study the temporal relation of firing patterns of groups of neurons, whether they are organized in relatively small neural circuits within a brain area or in large networks distributed through several distinct parts of the brain. In the studies described in this thesis, we simultaneously monitored the activity of multiple neurons in the hippocampal area CA1 and ventral striatum of the rat brain when the animals were awake and during periods of sleep. We focused on the following questions i) how are firing patterns of different classes of ventral striatal (i.e. principal cells and interneurons) neurons temporally related to the activity of neurons belonging to the same or to another class? *(chapter 3)*, ii) is motivationally relevant information preferentially reactivated in the ventral striatum? *(chapter 4)* and iii) do the hippocampus and ventral striatum reactivate coherently during sleep? *(chapter 5).*

To record neural activity simultaneously from the dorsal hippocampus and the ventral striatum, two areas which are widely separated in the rodent brain, we designed a 'split-hyperdrive', described in *chapter 2*. With 5 tetrodes placed in the hippocampus and 7 in the ventral striatum we were able to record ensembles of up to 20 cells per area for a few weeks following implantation. During recordings, rats were running along a triangular track searching for sweet, semi-liquid rewards or resting in a nest, indicating that they where neither restrained nor observably stressed by the (implanted) recording equipment. Among the principal cells that we recorded from hippocampal area CA1 where those that showed place fields (38%), i.e. firing patterns selective for a particular location on the track. These place fields were distributed uniformly across the track, not accumulating at reward locations or corner turns. In contrast, a subset of putative medium-sized spiny neurons (pMSN) of the ventral striatum was selectively responsive in relation to reward locations (17%, *chapter 4*). Firing rates of these neurons generally increased when rats approached or paused at a reward site. Moreover, differential responses were observed towards the various reward sites, or to reward availability or in both of these conditions.

In *chapter 3* we examined the firing properties of another class of striatal neuron, the so called fast spiking (FS) neurons. FS neurons most probably represent a particular type of interneuron that is thought to regulate the activity of the medium sized spiny neuron population by a feedforward inhibitory mechanism. In contrast to pMSNs, FS neurons

generally expressed a high baseline firing rate (pMSNs: 0.23 ± 0.05 Hz and FS units: 21.5 ± 4.0 Hz during active behavior) and showed a firing rate decrement when rats encountered and consumed a reward. During reward site approach some FS units started to gradually decrease their firing rate, whereas others showed a ramping increase in firing rate during this phase. Analysis of the temporal dynamics of the firing of FS units and pMSNs revealed a depression of activity in a large subset of pMSNs around the time of FS unit firing, which is basically in line with *in vitro* evidence indicating a GABAergic connection between FS interneurons and MSNs in the ventral striatum (Taverna et al., 2007). The current findings also go beyond this *in vitro* evidence in that they suggest that FS interneurons form a network for 'early-inhibition' of MSNs, so that many MSNs already decrease their firing rate before an arbitrary recorded FS unit has fired. Other pairs of the two classes showed concurrent firing, presumably because of common glutamatergic input. The temporal relation of activity of pairs of FS neurons or pMSNs could also be examined when two of these cells were simultaneously recorded on different tetrodes. The observed relationships between FS units were by and large instances of near-synchronous activity. Previous *in vitro* evidence of electrotonic coupling between these neurons indicated such a relationship (Koos and Tepper, 1999; Taverna and Pennartz, 2008). In some cases the co-activation was followed by a trough in the cross-correlogram. *In vitro*, also pairs of MSNs were previously found to be connected via unidirectional inhibitory synapses (Czubayko and Plenz, 2002; Tunstall et al., 2002; Koos et al., 2004; Taverna et al., 2004; Venance et al., 2004). *In vivo*, a subset of pMSNs showed a decrease in firing upon activity of another pMSN but two pMSNs could also be concurrently active.

Spontaneous reactivation of neuronal activity patterns representing a scene or event during periods of rest and sleep is widely thought to contribute to memory consolidation by strengthening synaptic connections activated during the preceding behavior (Marr, 1971; Buzsaki, 1989; McClelland et al., 1995; McNaughton et al., 2003). Consistent with current concepts on declarative memory consolidation, reactivation was primarily studied in the hippocampus (Pavlides and Winson, 1989; Wilson and McNaughton, 1994; Kudrimoti et al., 1999; Lee and Wilson, 2002) and neocortex (Qin et al., 1997; Hoffman and McNaughton, 2002; Battaglia et al., 2007; Euston et al., 2007b; Ji and Wilson, 2007). Reactivation theories have proposed that the recurrence of memory traces in the hippocampus may initiate a process of reactivation in associated neocortical ensembles leading to the strengthening of the cortical memory trace. The observation of reactivating ensembles in the subcortical ventral striatum indicated that this process also extends outside the hippocampal-neocortical circuitry (Pennartz et al., 2004). In our study on the ventral striatum (chapter 4), we showed an elevated level of reactivation of neurons

exhibiting a reward-related correlate as compared to neurons that did not respond to reward sites, indicating that motivationally relevant information is specifically reactivated in the ventral striatum. Moreover, this reactivation was especially strong for those spike patterns that occurred nearby reward sites, while spike trains occurring during corner passages were less reactivated. These findings indicate that memory traces are likely to contain a motivational component in addition to object and contextual information. We furthermore showed that reactivation in the ventral striatum is a sparse phenomenon, because only few cells within the recorded ensemble accounted for the observed reward-related reactivation. Concerning the general reactivation process in the ventral striatum, we revealed that reactivation is unlikely to occur during REM sleep periods in the first hour after the behavioral experience, but instead mainly depends on periods of slow wave sleep. Reactivation appeared particularly strong during small time windows (~200 ms) during and following emission of sharp wave-ripple complexes in the hippocampus. We also verified the somewhat unexpected finding of a previous study (Pennartz et al., 2004) that reactivation in the ventral striatum does not decay until at least forty minutes of sleep after the track running experience.

The finding of ripple associated reactivation in the ventral striatum extended the existing evidence of a hippocampal influence on reactivation in its subcortical target. The study in *chapter 5* showed for the first time the existence of coherent reactivation across ensembles of the hippocampus and ventral striatum. Analysis indicated two firing pattern characteristics during active behavior that were associated with strong reactivation during subsequent sleep. The first was the co-expression of a place field in the hippocampus and a reward-related correlate in the ventral striatum and the second was the maintenance of a firing order during active behavior in which the hippocampal neuron fired preferentially before the ventral striatal neuron. Whether or not hippocampal and ventral striatal neurons showed firing modulation by the hippocampal theta rhythm seemed less indicative for cross-structural reactivation as examined by subgroup comparisons and multi-linear regression analysis. The temporal dynamics of joint reactivation of spatial/contextual and reward-related information showed that hippocampal neurons fired preferentially before the ventral striatal neurons during track running and during the post-behavioral rest episode whereas such order was not consistently observed in the pre-behavioral rest phase. This preservation of firing order from track running to subsequent sleep indicates a forward reactivation in which the hippocampus orchestrates the ventral striatum. The time lag of firing of two neurons during track running and during subsequent sleep was correlated. Interestingly, the timescale of co-activation during the post-behavioral rest was about a factor 10 accelerated compared to track running, suggesting that a compressed version of the behavioral neuronal pattern was reinstated.

# General discussion

### Selection of a functional ensemble in the ventral striatum

The ventral striatum is an important node of the limbic-corticostriatal system and has functions in signaling and predicting reinforcers (Schultz et al., 1992; Setlow et al., 2003; Roitman et al., 2005), controlling consummatory behaviors (Stratford and Kelley, 1999) and is thought to invigorate goal directed behavior on the basis of the motivational value of cues and contexts (Pennartz et al., 1994; Cardinal et al., 2002). The ventral striatum is proposed to consist of distinct networks of neurons that form functional ensembles which act in parallel and in this way maintain a broad range of functions (Pennartz et al., 1994). Although at this point in time ventral striatal ensembles have been postulated but not explicitly demonstrated, they offer a parsimonious way of interpreting the diverse and heterogeneous roles of the ventral striatum in directing behavior, and a number of arguments has been raised in support of the theory (Pennartz et al., 1994; Badiani et al., 1999; see also our results on highly similar, strongly reactivating cell pairs in *chapter 4*). The striatal complex integrates incoming information of various glutamatergic and dopaminergic origin, after which relevant information for guiding behavior may be selected through the activation of a specific functional ensemble. Output of the selected functional ensemble will be transferred to target areas such as motor-related structures where it could initiate or modify an appropriate behavioral action program, or to the prefrontal cortex, where the output may contribute e.g. to the formation of predictions of reward associated to cues and contexts. Inappropriate or redundant inputs not leading to sufficiently coherent excitation of a ventral striatal ensemble will fail to gain control over behavior. The ventral striatum may thus enhance the contrast between ensembles as they respond to diffuse neuronal inputs (Groves, 1983; Pennartz et al., 1994).

Two intra-striatal inhibitory mechanisms are thought to sculpt the recruitment of a functional ensemble through regulating its activity level relative to other ensembles. The first is a feedforward inhibition mediated by FSIs onto MSNs (Kita et al., 1990; Pennartz and Kitai, 1991; Kita, 1996; Plenz and Kitai, 1998; Koos and Tepper, 1999; Koos et al., 2004). Glutamatergic drive excites particular MSNs by way of monosynaptic contacts and in addition silences the same or other MSNs by activating FSIs (Kita et al., 1990; Pennartz and Kitai, 1991; Bennett and Bolam, 1994; Plenz and Kitai, 1998) which subsequently exert inhibitory influence on MSNs. The monosynaptic projection from FSIs onto MSNs was established in vitro using dual cell patch clamp techniques in brain slices (Koos and Tepper, 1999; Taverna et al., 2007). In chapter 3 of this thesis we present in vivo evidence of depressed pMSN activity associated with FS unit firing. Whereas a feedforward inhibition

from FS neurons on MSNs would predict the FS neuron to fire preferentially before MSN firing drops, our data generally showed a different pattern; i.e. in most cases the decrease in firing of the pMSN began before FS unit fired. Pairs of FS units generally showed a broad peak of concurrent firing, indicating that activity of one FS unit is often preceded by activity of another FS unit. This rough synchronization of activity can result from FS units forming networks through electrotonic coupling (Koos and Tepper, 1999; Taverna and Pennartz, 2008). Together these results suggest an organization of feedforward inhibition in which limbic-cortical input to one locus of the FS network transmits excitation to other FS units that subsequently exert a broad inhibitory influence on MSNs situated at other loci of the same system (*chapter 3* Fig 3.5). A release of FSI-mediated inhibition would subsequently gate the activity of pMSNs (Koos and Tepper, 1999). This process may well be reflected in the opposite polarity of the neuronal responses of FS units and pMSNs associated with reward consumption; when a reward was consumed, FS units generally decreased their firing rates whereas pMSNs often showed an increase in firing rate (*chapter 3*, Fig 3.4). It remains unclear how a release of FSI-mediated inhibition is accomplished. Candidate mechanisms include a transient reduction of glutamatergic inputs, FS mediated inhibition onto other FS units via GABAergic collaterals (Taverna and Pennartz, 2008) or GABAergic projections onto FS units originating in the ventral pallidum (Bevan et al., 1998). Our data contained one FS-FS pair (14%) that showed a firing rate decrement of one member associated with activity of the other providing *in vivo* evidence that concurrent activation and inhibition can occur in different groups of FS units.

The second mechanism for ensemble recruitment is proposed to mediate an inhibitory control by way of MSN axon collaterals that synapse on dendritic sites of fellow MSNs (Czubayko and Plenz, 2002; Tunstall et al., 2002; Koos et al., 2004; Taverna et al., 2004; Venance et al., 2004). Unidirectional inhibitory GABAergic connectivity between MSNs was shown to exist at a rate of about 34% in *in vitro* dual cell patch clamp recordings (Taverna et al., 2004). Whereas FS units synapse on the MSN cell body (Kita et al., 1990; Kita, 1993; Bennett and Bolam, 1994), MSN-MSN synaptic contacts were found to be predominantly located on the distal dendrites (Wilson and Groves, 1980). On the basis of individual synapses, MSN to MSN inhibition is therefore considered less powerful than FSI to MSN inhibition in influencing the spiking of a MSN. Nonetheless, the abundance of MSN-MSN synapses compared to FS-MSN synapses suggest that overall the MSN-MSN inhibition may contribute significantly in regulating the excitability of MSNs. As our data showed (*chapter 3*, Fig 3.3), depressions in firing activity of a pMSN can occur at the moment that other pMSNs are activated, which could be interpreted as the result of the combined inhibitory drive from FSIs and MSNs on the MSN under scrutiny.

Thus, feedforward inhibition exerted by an interconnected network of FS units on MSNs and feedback inhibition within the MSN population indicates that the ventral striatum contains important network mechanisms to dynamically activate or deactivate a particular neuronal ensemble at the expense of others.

**The ventral striatum is implicated in post-experiential processing of information**

Traditionally the ventral striatum is viewed as a 'limbic-motor' interface – a view that refers to a function as a computational device where motivation is translated into action (Mogenson et al., 1980). Accumulating evidence however also shows the significance of the ventral striatum in the consolidation of memories. Several studies have shown that interference with protein synthesis, glutamate and/or dopamine receptor function in the ventral striatum shortly following training on spatial (Setlow and McGaugh, 1998; Sargolini et al., 2003), Pavlovian approach (Dalley et al., 2005) and instrumental (Hernandez et al., 2002) learning tasks drastically impaired the performance on the next day. The acquisition and consolidation of task-related information likely depends on long-term changes in synaptic efficacy that occur at the synapses of the glutamatergic afferents onto MSNs (Pennartz et al., 1993; Kombian and Malenka, 1994) as interference in the receptor- and signal-transduction cascade that underlies LTP induction prevents consolidation of an instrumental learning task (Baldwin et al., 2002b). The spontaneous reactivation of ventral striatal neuronal patterns during periods of sleep immediately following a behavioral experience (Pennartz et al., 2004; *chapter 4*) confirms the participation of this area in post-training information processing, and provides a potential neurophysiological mechanism for striatal involvement in the consolidation of memories. Thus, not only is the ventral striatum involved in the integration of various streams of cortical and limbic information to invigorate the appropriate behavioral output pattern, it also contributes to cognitive functions such as memory consolidation of which reactivation may be an important step.

**Distributed reactivation of distinct components of a memory trace**

One of the major novel results presented in this thesis is that the ventral striatum specifically and preferentially reactivates reward-related information. This was demonstrated by the stronger reactivation of neurons that expressed a transient change in firing rate related to the arrival at reward ports compared to neurons that did not show such a response pattern *(chapter 4)*. Furthermore, when we studied reward-related neurons generating spike trains that were either temporally associated with reward sites or with other locations on the track, the former ones were much more strongly reactivated than spike trains occurring at other locations on the track. We hypothesize that the ventral striatum supports consolidation by endowing reactivation processes with motivational value. The

enhanced reactivation of reward-related information in the ventral striatum indicates that this structure re-processes a different type of information than for example found in the dorsal hippocampus. As hippocampal reactivation has been studied in populations of dorsal CA1 neurons exhibiting place fields, it is reasonable to assume that this process pertains primarily to spatial and contextual information (Wilson and McNaughton, 1994; Kudrimoti et al., 1999; Lee and Wilson, 2002; Foster and Wilson, 2006). This may point to an important organizational principle of the reactivation process; i.e. multiple brain areas are participating in the reactivation of a single event and each area contributes a distinct aspect to the reactivated trace. Replay therefore may be conceived as a distributed process in which some structures reactivate information about the physical-sensory properties (e.g. neocortex; Ji and Wilson, 2007) of an object and others about its spatiotemporal context and motivational value.

The ventral striatum participates in a larger network of cooperating brain structures that are involved in processing reward-related information and together control motivated behavior (Cardinal et al., 2002). Therefore, it is unlikely that the reactivation of motivationally relevant information depends on a single structure like the ventral striatum only. Neurons in different areas of the limbic-corticostriatal network process various aspects of rewards, ranging from the detection and perception of rewards, trough the expectation of imminent rewards, to the use of information about predicted rewards for the control of goal directed behavior (Schultz, 2000). Neurons responding to food or liquid rewards are found in a number of areas that project to the ventral striatum, such as the amygdala (Nishijo et al., 1988; Pratt and Mizumori, 1998; Tye and Janak, 2007), the medial prefrontal cortex (i.e. infralimbic and prelimbic cortices; Mulder et al., 2003), the anterior cingulate cortex (Niki and Watanabe, 1979; Shidara and Richmond, 2002) and the dopaminergic neurons in the ventral tegmental area (Schultz, 1986; Ljungberg et al., 1992). Although the orbitofrontal cortex innervates the dorsal striatum more densely than the ventral part (Haber et al., 1995; Groenewegen et al., 1997), it most likely participates in the limbic-corticostriatal network, as it contains neurons responsive to reward (Schoenbaum et al., 1998; Tremblay and Schultz, 1999; van Duuren et al., 2007) and is amply interconnected with e.g. the basolateral amygdala, medial prefrontal cortex and the mediodorsal thalamic nucleus (Groenewegen and Uylings, 2000). The ventral striatum receives information on reinforcers and the associated cues and contexts through projections from the prefrontal cortex, amygdala and hippocampus. Subsequently, reward-related information processed by striatal neurons may re-enter the cortico-limbic system via a route through the ventral pallidum and mediodorsal nucleus of the thalamus to the prefrontal cortex (Zahm and Brog, 1992; Groenewegen et al., 1997) or via the dopamine release by ventral tegmental neurons that is regulated by striatal output (Lisman and Grace, 2005).

Reactivation was recently observed in another area participating in the network for emotional and motivational learning, viz. the medial prefrontal cortex (Tatsuno et al., 2006; Battaglia et al., 2007; Euston et al., 2007b). Although the nature of the replayed information was not directly addressed in these studies in rats, Euston et al. showed that during the task, consisting of running a spatial pattern in a circular arena for food or medial forebrain stimulation reward, neurons fired differentially in anticipation of and during receipt of rewards (Euston et al., 2007a). Thus, in addition to the ventral striatum, other brain areas might reactivate aspects of emotional and motivational information. The answers to the questions as to which exact type of motivational information is reactivated in the ventral striatum and in other structures involved in emotional and motivational control, and how these areas interact during reactivation, should be found in further research into these topics.

**Organization of a distributed reactivation process**

Reactivation across multiple brain structures such as hippocampus and ventral striatum implies the existence of a mechanism facilitating the coherent re-processing of pieces of information belonging to the same event and at the same time preventing the formation of erroneous associations with other events. Synchronized cross-structural replay (Qin et al., 1997; Hoffman and McNaughton, 2002; Ji and Wilson, 2007) may subserve such a function. In this thesis we showed for the first time that hippocampal and ventral striatal ensembles reactivate coherently *(chapter 5)*. We identified two characteristics of hippocampal-ventral striatal firing that were associated with subsequent reactivation and that could explain the contribution of individual cell pairs to the reactivation observed in a recording session. First, cell pairs co-expressing a place field and a reward-related correlate reactivated more strongly than pairs of which only one or none of the neurons showed such a response. Second, cell pairs of which the firing of the hippocampal cell preceded that of the ventral striatal cell during track running reactivated more strongly than pairs showing the opposite pattern or pairs not showing a clear firing order. A third characteristic that we analyzed, the modulation of firing patterns by the hippocampal theta oscillation, was not indicative for strong reactivation.

The first factor indicates that the combination of spatial/contextual (hippocampus) and reward-related (ventral striatum) information was specifically reactivated in the rest period following an episode of food searching behavior. Joint reactivation of hippocampal and ventral striatal ensembles may provide a unique opportunity to integrate neuronal representations of contextual and motivational information to enable the learning of place-reward associations. Such associations support the ability to predict and localize desired

foods and liquids while foraging. The significance of the hippocampal-ventral striatal circuitry in forming context-reinforcer associations has been demonstrated with laboratory tasks in which successful performance is dependent on such association. Rats with selective lesions of the hippocampus or ventral striatum were impaired in spatial learning tasks (Morris et al., 1982; Annett et al., 1989; Sutherland and Rodriguez, 1989) and in Pavlovian conditioning in which context is paired with a reinforcer (Kim and Fanselow, 1992; Parkinson et al., 1999a; Fanselow, 2000; Levita et al., 2002; Ito et al., 2006; Ito et al., 2008). Thus, learning combined place and reinforcer information will lead to situations in which environments become predictive of a reinforcer. The power of such an association is demonstrated by humans addicted to drugs of abuse that experience intense craving for drugs when they visit places where they have obtained or used the drug before (O'Brien et al., 1992).

144

The second factor indicative for strong reactivation was a firing order in which the hippocampal cell fired before the striatal cell. Naturally, we also found many pairs of which the ventral striatal cell fired in advance of the hippocampal cell during track running but apparently these pairs contributed less or not to reactivation. Subsequent analysis of the temporal relation between hippocampal and ventral striatal firing showed that in pairs expressing a double correlate generally the hippocampal cell fired before the striatal cell during track running *and* during reactivation in the subsequent rest period, indicating a forward replay. To our knowledge, this is the first evidence supporting a temporally leading role of the hippocampus in reactivation together with any other brain structure. Earlier studies of dual reactivation in the hippocampus and neocortex have not shown such a relationship (Qin et al., 1997; Ji and Wilson, 2007). This organization of firing order obeys the direction of the anatomical hippocampal-striatal projection (Kelley and Domesick, 1982; Groenewegen et al., 1987) and complies with a central tenet of consolidation theory, proposing the hippocampus to initiate reactivation in its target structures (Marr, 1971; Buzsaki, 1989; McClelland et al., 1995; McNaughton et al., 2003).

Because hippocampal reactivation is strongest during sharp wave-ripples (Kudrimoti et al., 1999; Diba and Buzsaki, 2007) these events are thought to be the LFP "carrier" for replay between the hippocampus and its targets. Cross-regional reactivation between the hippocampus and ventral striatum indeed showed a tendency of being stronger following sharp wave-ripple emission than during the intervals but remained sub-threshold for statistical significance presumably because of the limited number of sessions analyzed (chapter 5). Nonetheless, several other indications of sharp wave-ripple associated reactivation in the ventral striatum are in support of a coordinating role of the hippocampus.

Reactivation in the ventral striatum appeared particularly strong during ~200 ms following the emission of sharp wave-ripples *(chapter 4)*. Moreover, spike patterns of cells responding to reward-related elements which strongly contributed to reactivation became temporally more aligned with sharp wave-ripple episodes during post-behavioral rest relative to pre-behavioral rest. An earlier study showed that ventral striatal reactivation was mediated by cells that change their firing rates (i.e. increments, decrements or a combination) upon the emission of hippocampal sharp wave-ripples (Pennartz et al., 2004). However, as yet a causal function of sharp wave-ripples in orchestrating replay outside the hippocampus remains to be demonstrated.

The proposition that the hippocampus orchestrates cross-structural and ventral striatal reactivation by way of ripple associated burst activity, implies that such excitatory input from the hippocampus should direct or regulate ventral striatal firing patterns during sleep. The questions then arise as to whether the hippocampal burst activity is capable of doing so, and how the hippocampal drive onto the ventral striatum relates to inputs from other cortico-limbic structures that could potentially influence or even initiate reactivation in the ventral striatum. An alternative structure that may be able to drive the ventral striatum into a state of reactivation is the prefrontal cortex.

145

Electrophysiological studies in anesthetized rats showed that membrane potentials of MSNs fluctuate between two defined levels (Wilson and Groves, 1981; Yim and Mogenson, 1988). In the resting state condition, the membrane potential of MSNs is hyperpolarized and the probability of spike emission was low. Such 'down-states' alternated with periods in which excitatory input brought the membrane potential to a relatively depolarized state in which action potentials can be generated, i.e. 'up-states'. Such a pattern of bi-stable membrane potentials was also observed in dorsal striatal MSNs during periods of SWS (Mahon et al., 2006). Populations of ventral striatal neurons were shown to shift synchronously from down- to up-states and vice versa (Goto and O'Donnell, 2001b). Periods of enhanced MSN activity alternated with relatively silent periods which could lead to a slow oscillation (0.5 - 3 Hz) in the local field potential (Leung and Yim, 1993). Down- to upstate transitions in the ventral striatum appeared to be correlated to the occurrence of sharp waves and ripples in the ventral hippocampus (Goto and O'Donnell, 2001a). This correlation was stronger than the correlation between up- to down state transitions in the same structure and bouts of activity occurring in the prefrontal cortex. The activity pattern in cortical areas is also organized according to up- and down states under anesthesia (Steriade et al., 1993; Cowan and Wilson, 1994) and in SWS (Steriade et al., 2001; Timofeev et al., 2001). These results indicate that patterns of activity in the hippocampus and ventral striatum are

temporally more precisely aligned than those of the prefrontal cortex and ventral striatum. As mentioned above, neurons in the ventral striatum have to be excited to the up-state before spikes can be elicited. In anesthetized rats, electrical stimulation of the fornix, the projection pathway from the hippocampus, was sufficient to induce down- to up-state transitions in the ventral striatum (O'Donnell and Grace, 1995). Fornix transection on the other hand arrested the bi-stability of the membrane potentials of MSNs. Electrical stimulation of prefrontal or amygdala afferents to the ventral striatum only evoked spiking of MSNs when the membrane potentials were in an up-state at the time of stimulus arrival but could not induce down- to up state transitions. This evidence indicates that the hippocampus may determine the start of an up-state and therewith activity in the ventral striatal neurons whereas the prefrontal and amygdala may influence the firing patterns during an up-state. On this account, the hippocampus is proposed to 'gate' limbic corticostriatal throughput.

The relation between cortical and limbic inputs to the ventral striatum was further investigated by paired stimulation of two pathways in anesthetized rats (Goto and O'Donnell, 2002). When a stimulation-evoked excitatory response of the prefrontal cortex propagated to the ventral striatum shortly after an input to the ventral striatum evoked by stimulation of the fornix, amygdala or thalamus, the postsynaptic potential recorded from MSNs was enhanced compared to the potential evoked by a single prefrontal cortical stimulation. In the opposite case, in which the arrival of the prefrontal input preceded that from hippocampus, the postsynaptic potential was dampened. These results illustrate again that prefrontal cortical input is most effective when it is preceded by another excitatory input. On the other hand, hippocampal inputs may be shunted when preceded by prefrontal cortical input. Lastly, the electrophysiological evidence is supported by anatomical evidence on the distribution of excitatory synapses on MSNs in the ventral striatum. Whereas prefrontal cortical and hippocampal afferents synapse predominantly on the distal dendrites of MSNs, a fraction of the hippocampal (but not prefrontal afferents) form synapses on more proximal sites relative to the cell body, indicating a stronger control on MSN excitability (Meredith et al., 1990). Taken together, this evidence does support a scenario in which the initiation of cross-structural and ventral striatal reactivation is under control of the hippocampus whereas during ongoing reactivation the process might be influenced by other sources such as the prefrontal cortex. However, these data need to be interpreted with caution as they were mostly obtained from anesthetized rats and have not been replicated in awake subjects. Potential effects of anesthesia on spike firing patterns for example in relation to up- and down states have not been identified.

**Accelerated cross-structural replay**

As hippocampal reactivation occurs predominantly during ripples, the relative short duration of these events (~100-200 ms) poses a time limitation on the temporal extent to which information can be replayed on each occasion. A similar time constraint on reactivation may be effective in the medial prefrontal cortex and the ventral striatum provided that reactivation occurs during periods of enhanced neuronal activity, i.e. up-states. Reactivation may assume several modes of operation. First, stretches of neural activity representing the behavioral experience may be replayed in 'real time' and become truncated at the moment the sharp wave-ripple event has ended. In this case, very short time periods of the experience are replayed. Alternatively, as the brain is not constrained by behavioral demands during sleep, reactivation may occur at the speed determined by the brain's cellular and transmission dynamics (cf. Euston et al., 2007b). In this case, neural activity patterns would be time-compressed during replay compared to the behavioral experience, implicating that a period in the range of seconds of the maze experience can be replayed during a time window of a few hundreds of milliseconds during sleep. As a consequence, time lags between neural firing are shortened considerably, which is beneficial for associative processes such as spike timing dependent plasticity (Levy and Steward, 1983; Markram et al., 1997; Bi and Poo, 1999; Abbott and Nelson, 2000). Our cross-region reactivation results support the latter hypothesis because the cross-correlograms showed the time lags between the firing of neurons to be significantly shorter for the post-behavioral rest episode than for the track running *(chapter 5)*. Also the widths of the peaks in the cross-correlograms of post-behavioral rest were significantly smaller than those of track running. In addition, even neurons that exhibited non-overlapping firing fields on the track showed near-synchronous firing during sleep and the overall shape of the cross-correlograms were preserved despite the compression. The compression of neural firing patterns during replay was previously suggested to occur in the hippocampus (Nadasdy et al., 1999; Lee and Wilson, 2002) and medial prefrontal cortex (Euston et al., 2007b). We observed a compression factor of about 10 times which is comparable to the factor ~7 reported for medial prefrontal cortex but considerably lower than the factor 20 that was reported for hippocampus (Lee and Wilson, 2002). Whereas our results, consistent with the medial prefrontal cortex, support the idea of compressed reactivation, it was recently proposed that reactivating hippocampal spike sequences may already be accelerated during behavior (Euston et al., 2007b). Namely, hippocampal cells show the tendency to fire at earlier phases of the theta cycle as the rat progresses through a place field, a phenomenon known as theta phase precession (O'Keefe and Recce, 1993; Skaggs et al., 1996). As a consequence of theta phase precession, spikes representing adjacent place fields occur in rapid succession within the theta cycle during behavior. In

this way, temporal alignment of activity patterns takes place on a condensed time scale already during behavior and is not further compressed during subsequent sleep.

Euston et al. (2007) suggest that reactivated spike patterns are composed by different mechanisms depending on the brain area; i.e. 'condensation' during behavior in the hippocampus and 'compression' during sleep in the medial prefrontal cortex and other structures such as the ventral striatum. These mechanisms, however, are not necessarily mutually exclusive and may even act in concert. An important aspect of the 'condensation' hypothesis is that it includes only cells pairs that are active in the same theta cycle, and thus show overlapping or adjacent place fields (Euston et al., 2007b). It assumes that only these cell pairs reactivate. Our cross-correlogram results *(chapter 5)* showed cell pairs in the hippocampal-ventral striatal circuitry that exhibited non-overlapping firing fields on the track and were concurrently active during subsequent sleep. Firing patterns of these cells need to be compressed in order to reactivate within the time frame of ripples or up-states, which we observed in our data. Thus, whereas spike pattern 'condensation' may occur on very short segments of an experience (~100-200 ms), additional compression may take place on longer periods (several seconds). To assess whether or not hippocampal spike patterns undergo compression during reactivation, firing relationships between cells that exhibit non-overlapping place fields should be examined. On the other hand, 'condensation' might not be limited to the hippocampus but may also occur in other brain areas that show theta phase precession such as the prefrontal cortex (Jones and Wilson, 2005b).

The assumption that only cells with overlapping firing fields will reactivate together is anchored in the way reactivation is often assessed, on the basis of binned spike train correlations. The pair-wise Pearson's correlation coefficient of binned spike-trains will reach its maximal value of 1 when spikes emitted by two neurons occur consistently in the same time bins, low when spikes occur in different random time bins and negative when spikes of two neurons occur in an anti-correlated manner. Because bins generally contain a rather short time period of 50-100 ms each, only cells that are activated near-synchronously during active behavior because of overlapping firing fields will show a high Pearson's correlation value. In contrast, two cells exhibiting non-overlapping firing fields will be sequentially activated with a constant time-lag that is most likely more than one bin size resulting in a negative correlation value. The temporal relation of firing of these neurons during active behavior will thus be classified as anti-correlated and may as such negatively contribute to the reactivation measure, i.e. Explained Variance *(chapter 4 & 5)*. The spike trains of these neurons may become correlated in the subsequent

sleep period as a result of compression, provided that the same bin size is used for all episodes. The Pearson's correlation value for the post-behavioral rest period will then turn out positive which negatively influences the Explained Variance. Thus ignoring the exact temporal relationship of the firing patterns of two reactivating neurons that expressed non-overlapping firing fields may lead to an underestimation of the true reactivation. Current methods could be improved to take into account the contribution of these cell pairs to reactivation by for example using different bin resolutions for periods of active behavior and sleep according to the compression factor.

**General reactivation dynamics: Dependence on sleep state and temporal decay**
Now that we have investigated hippocampal- *(unpublished observations)*, striatal- *(chapter 4)* and cross-regional *(chapter 5)* reactivation in the same experimental paradigm we can compare its dynamics across the structures on two important aspects; i.e. the dependence on sleep-wake states and the decay across post-behavioral rest.

Reactivation within the hippocampus, the ventral striatum and joint reactivation was found during periods of quiet wakefulness and SWS. Within these periods, striatal and hippocampal reactivation were particularly prominent following short time intervals ($\sim$200ms) following the onset of hippocampal ripples which in previous research was only shown for hippocampal ensembles (Kudrimoti et al., 1999). Joint reactivation of the hippocampus and striatum showed this same trend but did not reach statistical significance presumably because of the limited number of sessions analyzed. Recent studies have reported reactivation of neuronal patterns to occur in ripple epochs during a track running task when rats were pausing at reward sites and, on some occasions, consuming the available reward (Foster and Wilson, 2006; Diba and Buzsaki, 2007). This evidence indicates that reactivation already starts during ongoing behavior and may indeed be solely associated with the occurrence of ripples and thus independent from sleep per se. Whether striatal and joint hippocampal-striatal reactivation also occur during awake states is yet unknown. The partial reinforcement schedule used in our experimental paradigm prompted rats to travel quickly from well to well and consequently they were hardly pausing at reward sites except for consumption of rewards. The low number of ripples occurring during track running did not allow examination of replay during these events.

We did not find an indication for reactivation during REM sleep in any of the three neuronal subsets analyzed. Most electrophysiological reactivation studies in rats have not addressed reactivation in REM sleep specifically because of undersampling; REM sleep episodes are generally rather short and occur at remote times following sleep onset. The

few studies that examined REM sleep associated reactivation yielded equivocal results (Pavlides and Winson, 1989; Kudrimoti et al., 1999; Louie and Wilson, 2001). Human sleep deprivation studies have ascribed a prominent role to REM sleep in procedural memory consolidation (Karni et al., 1994; Gais et al., 2000; Stickgold et al., 2000b). Our evidence is not necessarily in conflict with the evidence derived from these studies with human subjects, because REM sleep might contribute to a different aspect of memory consolidation and might be manifested at different latencies in relation to the experience than reactivation of ensemble firing patterns.

It has been proposed that REM sleep plays a role in separating new from familiar information. In an electrophysiological study, rats were searching for food on a track of which one half was familiar and one half was novel to the rats (Poe et al., 2000). Spikes of hippocampal cells generated in their place field on the familiar part of the track generally coincided with the positive peaks of the theta rhythm in the LFP recorded near the hippocampal fissure. During subsequent REM sleep, cells that had been active on the familiar part of the track fired preferentially on the trough of theta oscillations whereas cells activated on the novel part fired more often on the peaks. Thus, the spikes representing familiar experiences showed a phase shift of ~180 degrees. Moreover, as the novel part of the track became familiar across recording sessions, the cells that were active on this part of the track shifted their firing during REM sleep from the theta peaks to the troughs. Earlier evidence indicated that a short burst of electrical stimuli to hippocampal CA1 inputs, timed to arrive at the theta peak induced LTP, which could subsequently be depotentiated by stimulation during the troughs of theta waves (Huerta and Lisman, 1995; Holscher et al., 1997). Together these data suggest that REM sleep may facilitate the discrimination between old and new information for subsequent consolidation

Others have proposed models for memory consolidation in which SWS and REM sleep have different but interrelated roles. The consolidation of a visual discrimination task in humans seems to require both early-night sleep which is SWS enriched and late-night sleep which contains more REM sleep (Gais et al., 2000; Stickgold et al., 2000b) indicating that REM sleep may become important for consolidation after a substantial period of slow wave sleep. The authors of these studies propose that memory consolidation is initiated during SWS and that subsequent REM sleep may enhance the memory. This notion was shared by Ribeiro et al. (2004) who showed that REM sleep following a novel experience induced up-regulation of a plasticity-associated immediate early gene, viz. zif-268, in the rat hippocampus. Up-regulation of this gene was more prominent in late than in early REM sleep. They suggested that SWS reinstantiates the memory representation

through reactivation of neuronal patterns while subsequent REM sleep then potentiates the memory for subsequent post-sleep recall, through gene induction-mediated synaptic plasticity.

Thus, our results showed that reactivation with characteristics as observed in SWS does not occur during REM sleep episodes in the first hour following a behavioral experience. REM, however, sleep might contribute in other ways to the consolidation of memories.

Existing evidence indicates a discrepancy in the decay times of hippocampal and ventral striatal reactivation. Reactivation in the ventral striatum was found to be of equal strength across the first 40 minutes following behavior (Pennartz et al., 2004), whereas hippocampal reactivation was reported to generally decay in ~ 30 minutes (Kudrimoti et al., 1999; Tatsuno et al., 2006). Our data confirm this pattern; using concatenated periods of sleep we found that hippocampal reactivation decreased to undetectable levels in about 40 minutes (corresponding to a period of ~60 min real time) whereas the striatal ensemble showed no significant decay during this period *(chapter 4)*. Interestingly, joint reactivation was detected across the first hour of concatenated sleep but its strength gradually declined in this period *(chapter 5)*. This cooperation supports the notion that the hippocampus may influence reactivation in the ventral striatum across a considerable time period. The distinct patterns of decay indicate that at some point the ventral striatum may sustain reactivation independently of the hippocampus. Reactivation studies detect the phenomenon generally only during rather short periods following an experience (~ up to one hour). But, if memory consolidation depends on reactivation in a sustained manner, it is predicted to occur during the entire consolidation process; i.e. during periods of days, weeks or even years. This difference in time frames was one of the objectives of Ribeiro et al. (2004) examining reactivation in several brain areas for as long as twenty-four hours following a novel experience. Using a template matching method they indeed observed reactivation for extended time periods. However, replication of this study failed to produce similar results and showed that the long lasting reactivation found before was likely the result of certain parameter settings in the template method analysis (Tatsuno et al., 2006). Another indirect suggestion of extended periods of reactivation was provided by the above mentioned electrophysiological study where reactivation was found during REM sleep periods preceding track running which may reflect the reiteration of the activity pattern of the experience on the previous day (Louie and Wilson, 2001). Thus, reactivation of neuronal patterns is a phenomenon which has a considerable strength immediately following the behavioral experience in which the activity patterns were evoked. Over time the strength of reactivation decays in the hippocampus but less in the ventral striatum and may in both

structures eventually become indistinguishable from neuronal 'noise'. This, however, does not necessarily imply that the process has terminated but it is more likely that it became undetectable with the current methodologies. Further research is required to understand why different decay dynamics exist across areas and how the ventral striatum is able to sustain reactivation without support by hippocampal reactivation.

**General overview of reactivation in hippocampal-ventral striatal ensembles**

Integrating insights obtained by the studies described in this thesis with the current knowledge leads to an interesting though speculative hypothesis on the dynamics and implications of the reactivation process within and between structures.

Because of the auto-associative and plausibly ripple-generating properties of hippocampal area CA3 (Buzsaki, 1986; Ylinen et al., 1995), we assume that spontaneous reactivation arises here, perhaps as a result of decreased neocortical input and activity changes in pontine nuclei (Kirk, 1997; Battaglia et al., 2004b). Propagation of the sharp wave-ripples to the CA1 area excites most likely those networks that were activated in the previous experience as these neurons have a higher probability to spike than others (Pavlides and Winson, 1989). Herewith, a neural representation of the spatial/contextual aspects of the preceding scene or event is reinstated (Wilson and McNaughton, 1993, 1994). Via the subicular complex, massive excitatory drive associated with sharp wave-ripples reaches target sites of the hippocampal formation such as the entorhinal and prefrontal cortex and, via the fibria-fornix bundle, the ventral striatum. Repetitive electrical stimulation of the fimbria-fornix pathway in anesthetized rats was shown to induce postsynaptic potentials and short-term potentiation concurrently in the ventral striatum and prefrontal cortex (Mulder et al., 1997). Likewise, bursts of hippocampal activity may excite neurons in both these areas and initiate reactivation in multiple targets at the same time. Concerning the ventral striatum, synchronized activity of hippocampal cells representing a particular scene may induce a down- to up-state transition in the ensemble of neurons that was previously implicated in the encoding of information on rewards associated with that scene (e.g. O'Donnell and Grace, 1995; Goto and O'Donnell, 2001a). Through the association between neural representations of contextual information and motivational value, the context or scene will become predictive of a particular reinforcer. Once in the up-state, converging hippocampal, amygdaloid, thalamic and prefrontal cortical glutamatergic afferents on ventral striatal neurons may activate a distributed ensemble, capturing relevant aspects of the foregoing behavior. The contrast between the active ensemble and others may be enhanced by intra-striatal feedforward (Pennartz et al., 1991; Koos and Tepper, 1999; Taverna et al., 2007) and feedback mechanisms (Czubayko and Plenz, 2002; Tunstall et

al., 2002; Taverna et al., 2004; see also *chapter 3*) involving MSNs and FS interneurons. Moreover, firing patterns across structures (e.g. hippocampus, prefrontal cortex, amygdala and ventral striatum) may at this stage be synchronized and collectively represent detailed aspects of the foregoing scene or event. As a result of accelerated replay, firing patterns of hippocampal and ventral striatal ensembles are temporally confined to time windows comprising tens to hundreds of milliseconds, which is the condition required for the induction of spike-timing dependent plasticity (Levy and Steward, 1983; Markram et al., 1997; Bi and Poo, 1999; Abbott and Nelson, 2000). Consistent with the unidirectional projection from the hippocampus to the ventral striatum (Kelley and Domesick, 1982; Groenewegen et al., 1987) and the prerequisite for long term enhancement of synaptic efficacy, i.e. that the presynaptic cell has to fire shortly in advance of the postsynaptic cell, those cell pairs of which the hippocampal cell fired closely before the striatal cell reactivate and others hardly do *(chapter 5)*. In this way, ripples may not only transmit reactivated information to hippocampal target structures, but may also facilitate lasting changes in glutamatergic inputs to the ventral striatum (Buzsaki, 1986; King et al., 1999b). Bouts of reactivation in ventral striatal ensembles may last as long as the duration of an upstate (100-1000 ms; cf. O'Donnell and Grace, 1995) and may therefore outlast those in hippocampus as the duration of sharp wave-ripples is generally shorter than striatal up-states.

153

Ventral striatal output predominantly reaches the ventral pallidum, ventral tegmental area (VTA), lateral hypothalamus and substantia nigra pars compacta and reticulata (Groenewegen and Russchen, 1984) and may, via these relay stations, convey motivationally relevant information to several neuronal systems. First, output to the subthalamic nucleus and the substantia nigra pars reticulata may reach the premotor cortex (Zahm and Brog, 1992). By these routes motivational information about cues and contexts could contribute to selection of an appropriate behavioral pattern, depending on the activity patterns in cortico-limbic structures. Second, the ventral striatum and ventral pallidum project to the VTA (Phillipson, 1979; Groenewegen and Russchen, 1984; Groenewegen et al., 1993), where they can influence the activity of dopamine neurons and thereby regulate mesolimbic and mesocortical dopamine release. The VTA has recurrent dopaminergic projections to the ventral striatum but also projects to the hippocampus, amygdala and medial prefrontal cortex (Swanson, 1982; Oades and Halliday, 1987; Van Eden et al., 1987). The ripple-associated 'off-line' retrieval of value information in the ventral striatum *(chapter 4)* may induce dopamine cells to generate a feedback or 'teaching' signal which potentially reaches each of the three projection areas (Floresco et al., 2001; Lisman and Grace, 2005). Furthermore, such signals might underlie the dopamine associated maintenance

of long term potentiation and memory consolidation in the hippocampus (Frey et al., 1990; Gasbarri et al., 1996; Otmakhova and Lisman, 1996; Swanson-Park et al., 1999; Morris et al., 2003; Granado et al., 2008), ventral striatum (Setlow and McGaugh, 1998; Floresco et al., 2001; Dalley et al., 2005; Schotanus and Chergui, 2008; but see Pennartz et al., 1993) and the medial prefrontal cortex (Gurden et al., 1999; Gurden et al., 2000; Baldwin et al., 2002a). Third, via projections mainly from the ventral pallidum to the mediodorsal thalamic nucleus, ventral striatal output may reach the medial prefrontal cortex (Zahm and Brog, 1992) and this way regulate a network of structures involved in the consolidation of reward-dependent learning processes.

# Concluding remarks

Developing the 'split-hyperdrive' placed us in the position to record multi-neuron activity from hippocampus and ventral striatum when rats were performing a behavioral task and during periods of sleep. Our data provide a contribution to the understanding of temporal firing relationships between different classes of neurons in the ventral striatum, which were previously mostly studied *in vitro*. The observed firing patterns support an organization in which a rather broadly synchronized network of interneurons selectively suppresses firing activity of striatal projection neurons. In addition, we showed that FS neurons exhibit reward-related firing responses which supports a function for FS interneurons in fast information processing during reward consumption.

Another major incentive for our study was to gain insight in the process of spontaneous reactivation of neuronal activity patterns during periods of rest and sleep, both in the ventral striatum and in the hippocampal-ventral striatal circuitry. The results described in this thesis hold important implications for how we view these processes. Until recently, the focus of reactivation research has primarily been on the hippocampus and neocortex, defining the current concepts of declarative memory consolidation. The evidence from our lab on the engagement of the ventral striatum in reactivation indicates, however, that reactivation is a widespread phenomenon and illuminates the potential importance of subcortical contributions to memory consolidation, whether declarative, procedural or otherwise. By specifically reactivating reward-related information, the ventral striatum may endow the memory trace with a value component. As this content is distinct from the presumed spatial/contextual information replayed in the hippocampus, we hypothesized reactivation to be a distributed process in which each brain area contributes a specific aspect to the reactivated trace. Binding pieces of information belonging to the same scene or event together may occur though synchronization of firing patterns across brain areas during reactivation. We demonstrated coherent, fast cross-structural reactivation in hippocampal and ventral striatal ensembles in which the firing order was maintained between the experience and the subsequent sleep. Our evidence indeed indicates that within this circuitry a combination of spatial/contextual and motivational information is replayed. We also showed that the hippocampus leads the ventral striatum in joint reactivation which is yet the most direct evidence supporting the long standing assumption that the hippocampus initiates and coordinates reactivation in extra-hippocampal structures. However, whether this finding can be generalized to other hippocampal target structures awaits further investigation.

The interaction between the hippocampus and ventral striatum in reactivation was highlighted in this thesis, but we also emphasized the potential involvement of other areas connected to this axis, for example the prefrontal cortex and the amygdala. The research described in this thesis only marks the start of unraveling the origin of reactivated, motivationally relevant information and the disclosure of the brain areas involved. Future challenges include investigation of the specific contribution of each area of the limbic corticostriatal network to the reactivation of motivationally relevant information, the interaction between the areas in this network during replay and of the question how it is embedded in the overall reactivation and consolidation of a complete memory trace.

Although reactivation possesses attractive properties to be advanced as a suitable substrate for memory consolidation, existing evidence supporting this role is of a correlative nature. The largest challenge currently facing the field of reactivation research therefore is to provide causal evidence on the relation between spontaneous re-instatement of neuronal activity patterns after the event and learning and memory processes.