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 5

Hippocampus leads ventral striatum in cross-structural replay of contextual and reward information

Carien S Lansink, Pieter M Goltstein, Jan Lankelma, Bruce L McNaughton and Cyriel MA Pennartz.

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Abstract

Learning associations between spatial locations and rewards is fundamental to foraging and other goal directed behaviors. This type of memory requires the integrity of the hippocampus and ventral striatum, but its underlying mechanisms remain unknown. In joint multi-neuron recordings from these areas, hippocampal-striatal ensembles reactivated together during sleep. This process was especially strong in pairs of which the hippocampal cell processed spatial information and ventral striatal firing correlated to reward. Moreover, reactivation was dominated by cell pairs maintaining a preferred order in which the hippocampal cell fired prior to the striatal neuron. Our results suggest a plausible mechanism for consolidating place-reward associations and are consistent with a central tenet of consolidation theory, namely that the hippocampus leads and orchestrates reactivation in its projection areas.

Introduction

Successful foraging requires that animals maintain a representation of a multitude of reward properties including the location at which a reward can be found. Forming a place-reward association is thought to depend critically on the communication between the hippocampal formation and the ventral striatum (VS). Cells in the hippocampus proper (HC; O'Keefe and Dostrovsky, 1971; O'Keefe and Conway, 1978) and adjacent subiculum (Barnes et al., 1990; Sharp and Green, 1994) show location-specific firing (i.e. 'place fields') and these structures are crucial for spatial and contextual learning (O'Keefe and Nadel, 1978; Morris et al., 1982; Barnes, 1988; Kim and Fanselow, 1992; McNaughton et al., 1996). Neurons in the VS fire in relation to rewards, as they are expected or actually delivered, as well as cues predictive of reward (Apicella et al., 1991b; Schultz et al., 1992; Setlow et al., 2003; Roitman et al., 2005). The VS is thought to utilize information of reward-predicting cues and contexts to guide goal directed behavior (Schultz et al., 1992; Pennartz et al., 1994; Robbins and Everitt, 1996; Cardinal et al., 2002). Although the hippocampal formation projects directly to the VS, and this connection has been implicated in contextual conditioning (Ito et al., 2008), it is unknown how neural representations of contextual and motivational information are integrated and stored to enable the learning of place-reward associations.

In several brain areas, neuronal patterns evoked during behavior are reactivated during subsequent sleep (Pavlides and Winson, 1989; Wilson and McNaughton, 1994; Hoffman and McNaughton, 2002; Pennartz et al., 2004; Ji and Wilson, 2007). Through modification of synaptic connections, this reactivation has been theorized to constitute an important step in memory consolidation (Marr, 1971; Buzsaki, 1989; McClelland et al., 1995; McNaughton et al., 2003). Joint reactivation of HC and VS may enable the formation of a memory

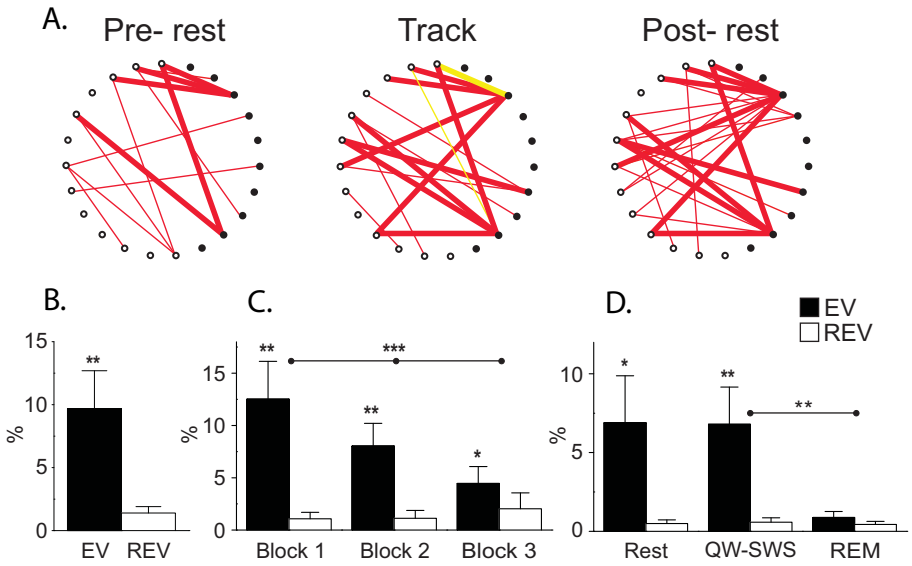


Figure 5.1

*Coherent cross-structural reactivation in the hippocampal-ventral striatal circuitry. A: Diagrams representing firing pattern correlations for pairs of simultaneously recorded hippocampal and ventral striatal units during periods of active behavior and rest in a single session. Individual neurons are represented as dots around the perimeter of a circle (filled dots: hippocampal CA1 units, n = 10; open dots: ventral striatal units, n=13). Lines indicate a significant firing correlation between two neurons (red: positive, yellow: negative correlations). A pattern of correlations emerges during track running and is reinstated in post-behavioral rest whereas it was largely absent in rest preceding behavior. B: The Explained Variance (EV) was significantly larger than the control value (REV) when compared across sessions (**p<0.01) Error-bars represent SEM. C: Temporal dynamics of joint reactivation were examined in three twenty-minute blocks of concatenated rest. Reactivation occurs at least up to an hour of rest after the experience but decays gradually (***p<0.002). D: Reactivation was prominent in periods of quiet wakefulness-slow wave sleep (QW-SWS; **p<0.01), but was not observed in REM sleep. Between-condition statistics hold for EV values and the difference between (EV-REV).*

trace comprising both contextual and motivational components. In this study, we recorded activity from neuronal ensembles in the rat HC and VS simultaneously during wake and sleep episodes to examine whether the HC and VS reactivate coherently and to reveal the temporal dynamics of this process. First, during active behavior much of the dynamics of hippocampal processing is governed by the theta rhythm and therefore we studied whether neural activity modulation by this rhythm is correlated to reactivation. A second foremost question in this field, not yet addressed in previous studies (Qin et al., 1997; Hoffman and

McNaughton, 2002; Ji and Wilson, 2007), is whether cross-structural replay depends on the type of behavioral information coded by cell assemblies. To address this question, we studied whether the expression of place fields and reward-related neural responses is associated with reactivation. Thirdly, theories of consolidation (Marr, 1971; Buzsaki, 1989; McClelland et al., 1995; McNaughton et al., 2003) posit that replay is initiated and orchestrated by the HC, which prompted us to examine whether hippocampal activity leads the VS during reactivation.

Results

Four rats were implanted with a tetrode-drive allowing joint HC-VS recordings of spike trains of multiple neurons and local field potentials in each area (see Materials and Methods in Supporting information (SI)). Daily recording sessions were composed of an episode of reward searching behavior flanked by two episodes of rest, which rats spent on a 'nest' next to the track. The task was to traverse a triangular track repeatedly in one direction. On each lap, one of three reward wells was baited with a drop of one of three corresponding reward types; i.e. sucrose solution, vanilla desert or chocolate mousse. First, we assessed reactivation of neuronal patterns using an Explained Variance (EV) method based on the spike correlations of cell pairs across all simultaneously recorded neurons (Kleinbaum et al., 1998; Kudrimoti et al., 1999; Pennartz et al., 2004). The Explained Variance reflects the extent to which the variance in the distribution of spike correlations during post-behavioral rest is statistically accounted for by the correlation pattern found during track running, factoring out the correlations present in pre-behavioral rest. Joint HC-VS reactivation was examined during rest periods in which the rat was immobile, using only spike correlations between pairs composed of one HC and one VS neuron.

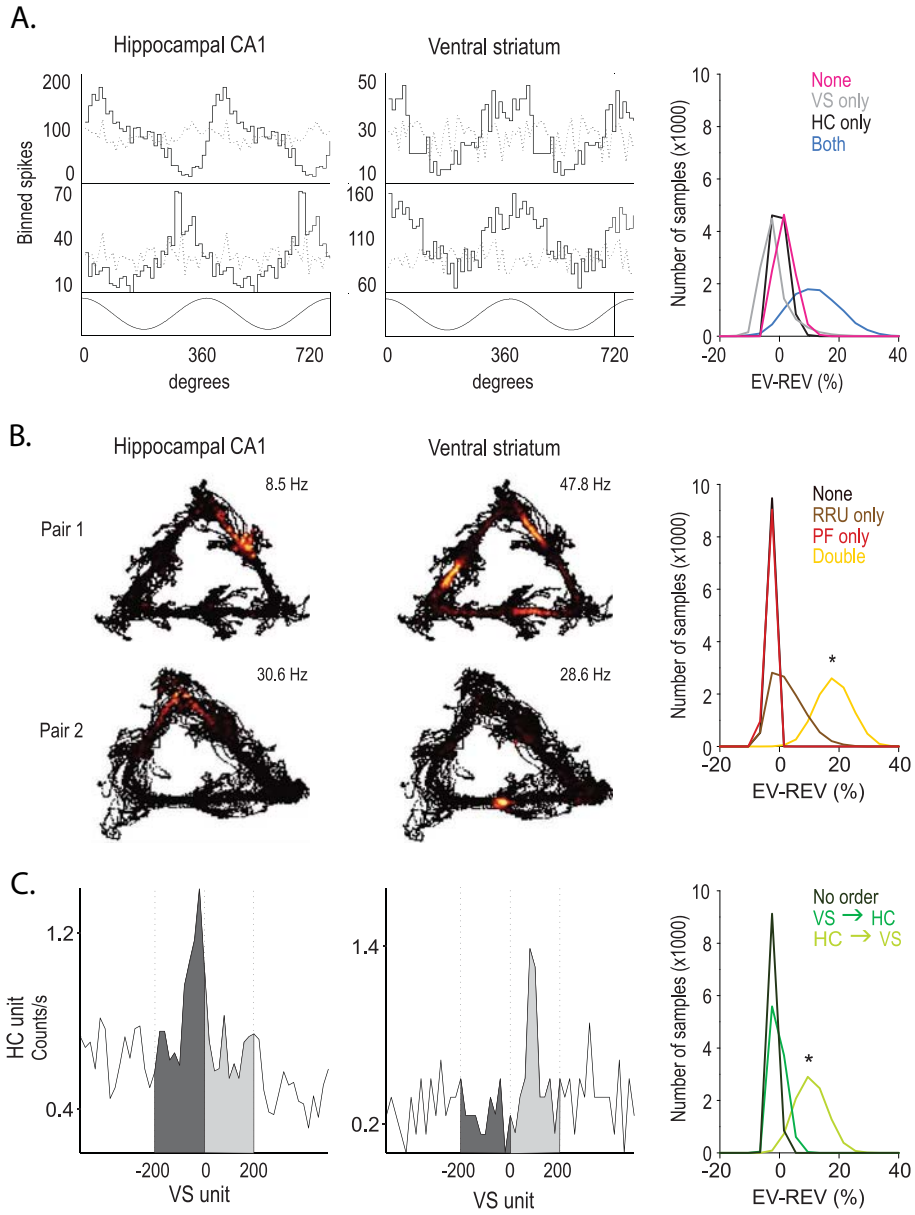
We found coherent, cross-regional reactivation between ensembles of the HC and VS as expressed by an EV of 9.7 $\pm$ 3.0% which was significantly higher than the control measure, the Reverse Explained Variance (REV: 1.4 $\pm$ 0.5%, $p < 0.01$, $n = 21$ sessions; Fig. 5.1A,B; SI). Analysis of the temporal dynamics of reactivation in 20-minute blocks of concatenated rest revealed a gradually decaying reactivation which was significant for at least one hour of post-behavioral rest (Block 1 EV: 13.3 $\pm$ 3.7%, REV: 1.1 $\pm$ 0.6%; Block 2 EV: 8.0 $\pm$ 2.2%, REV: 1.1 $\pm$ 0.8 %; Block 3 EV: 4.5 $\pm$ 1.6%, REV: 2.0 $\pm$ 1.5%; $n = 15$ sessions; Friedman $p < 0.002$; Fig. 5.1C). Within periods of rest, reactivation was prominent especially during quiet wakefulness-slow wave sleep episodes (EV: 6.8 $\pm$ 2.4%, REV: 0.6 $\pm$ 0.3%; $p < 0.01$; n= 13 sessions) but it was not significant for Rapid Eye Movement (REM) sleep (EV: 0.9 $\pm$ 0.4%, REV: 0.4 $\pm$ 0.2 %; n.s. Fig. 5.1D). The lack of pattern recurrence during REM sleep

was not attributable to its relative short duration and its late occurrence after sleep onset compared to quiet wakefulness-slow wave sleep (Supporting Fig. 5.2).

The existence of joint HC-VS reactivation raises the question which physiological and behavioral factors are associated with the strength of this process. We examined three not mutually exclusive factors, first by computing the degree to which cells in each pair fired together, and then by pooling all of these correlation values per episode across sessions and animals. We next formed subgroups of cell pairs by partitioning the complete set of correlation values on the basis of the factor under scrutiny. Reactivation values were computed for these subgroups and statistical significance was assessed by applying a bootstrapping procedure with re-sampling of pooled correlation values (SI; Sokal and Rohlf, 1995; Pennartz et al., 2004; cf. Hoffman and McNaughton, 2002).

The two-stage model of memory trace formation posits that theta oscillations are crucial for encoding information in the HC (Buzsaki, 1989). The hippocampal theta rhythm may also have a role in governing the temporal organization of activity in target structures to ensure efficient communication (cf. Jones and Wilson, 2005a; DeCoteau et al., 2007). Thus, our first hypothesis holds that HC-VS reactivation will only be strong when information is cross-structurally aligned by a common temporal framing, the theta oscillation, creating windows of near-synchronous firing.

During track running, robust theta oscillations were observed in the HC and VS. In both areas, cells were recorded that fired in close relation to the local theta oscillation ($n = 121$ out of 263, 46.0% in HC, and $n = 20$ out of 243, 8.2% in VS). Ventral striatal cells that were modulated by the local theta rhythm generally also showed modulation by hippocampal theta oscillations (n=20, 8.2%). When the peak of the theta oscillation recorded near the hippocampal fissure was taken as synchronizing time point, CA1 cells fired at an average angle of 199.9 ± 6.1 degrees (range: 43.9 – 356.4) and VS cells fired at a slightly later phase (217.1 ± 26.0 degrees, range: 4.5 - 336.3; n.s.). Cell pairs were divided on the basis of modulation by the hippocampal theta rhythm, resulting in four subgroups: 'Both cells' (n=140), 'HC only' (n=1273), 'VS only' (n=81) and 'None modulated' (n=1422). Reactivation was observed for the 'Both-cells' group, the 'HC only' group and the 'none' group (Fig. 5.2A and Supporting Table 5.2). Comparison of the reactivation strength between subgroups did not reveal significant differences. Similar results were found when the local field potentials in the VS were used to determine theta modulation of VS cells. Thus, theta modulation of both members of a cell pair was not preferentially associated with strong cross-structural reactivation.



Our second hypothesis departed from the assumption that spike patterns are not reactivated equally, but are re-processed especially when they convey behaviorally relevant information. For the HC-VS system we predicted that cells expressing spatial (HC) and reward-related information (VS) should be preferentially reactivated. Location-specific firing was found for 102 out of 263 (38.8%) hippocampal cells (SI). Place fields were distributed uniformly across the track, there was no indication that place fields occurred

◀ Figure 5.2

*Reactivation of subgroups composed according to three firing-pattern characteristics. A: Modulation by theta oscillations. Four examples show binned spike counts (upper panels, solid lines) in relation to the hippocampal theta rhythm (bottom panels) for two successive theta cycles. Randomization of spike intervals abolished the relation between firing pattern and theta rhythm (dashed lines). Distributions of EV and REV values for each subgroup, obtained with bootstrapping, showed significant reactivation in all but the 'VS only' subgroups, which was of similar strength across groups (right hand panel). B: Processing of place and reward information. The left panel shows the spatial distribution of local firing rates of two hippocampal-ventral striatal pairs. The rat's trajectory is shown in black and the firing rate of the neurons is color coded ranging from low rates in dark red to their individual maxima (top right corners) in yellow and white. The 'Double Correlates' group reactivated significantly stronger than other groups (*p<0.05), which did not show reactivation. C: Firing order was defined by the difference in area between the light- and dark-shaded regions of cross-correlograms. Reactivation was observed in all groups, but the HC → VS group reactivated more strongly than the other two (*p<0.05).*

more frequently near reward sites or corner passages. In contrast, a subset of VS neurons fired in close temporal relationship with reward site visits (41 out of 243 cells, 16.8%; SI). Reward-related responses were generally increments in firing rate and could be generated at one, two or all three reward sites. Furthermore, they were often sensitive to either the presence or absence of reward. In line with previous studies on the VS (Roitman et al., 2005; Tran et al., 2005), we will apply the term ´reward-related´ to all VS units showing significant responses time-locked to reward site visits.

Depending on the expression of place fields and reward-related correlates, cell pairs were grouped in four categories, 'Double Correlates' (n=192), 'Place Field only' (n=941); 'Reward-related Correlate' only (n=287), or 'No Correlates' (n=1496). The group 'Double Correlates' group showed very strong reactivation (EV: 22.9%, REV: 0.1%). The strength was significantly higher than in any other of these four subgroups, which did not yield significant reactivation (Fig. 5.2B and Supporting Table 5.1).

A long standing assumption in theories of memory consolidation holds that the HC initiates and orchestrates reactivation in its projection areas (Marr, 1971; Buzsaki, 1989; McClelland et al., 1995; Pennartz et al., 2002; McNaughton et al., 2003). This implies that reactivation should be strong when a particular firing order is maintained: during replay a hippocampal cell should fire predominantly in advance of a VS cell. During behavior, VS firing may also precede HC firing, but this order should not be associated with strong reactivation. The HC → VS order would also be consistent with the unidirectionality of the projection from

HC to VS (Kelley and Domesick, 1982; Groenewegen et al., 1987). Despite the finding that sleep reactivation occurs in a 'forward' direction, meaning that the order of firing during sleep is similar to the order during the preceding behavior (Skaggs and McNaughton, 1996; Nadasdy et al., 1999; Lee and Wilson, 2002; Euston et al., 2007b), this critical assumption has yet to be confirmed or refuted. Hence, our third hypothesis holds that reactivation is strong when the information flow is organized according to a leading role of the HC.

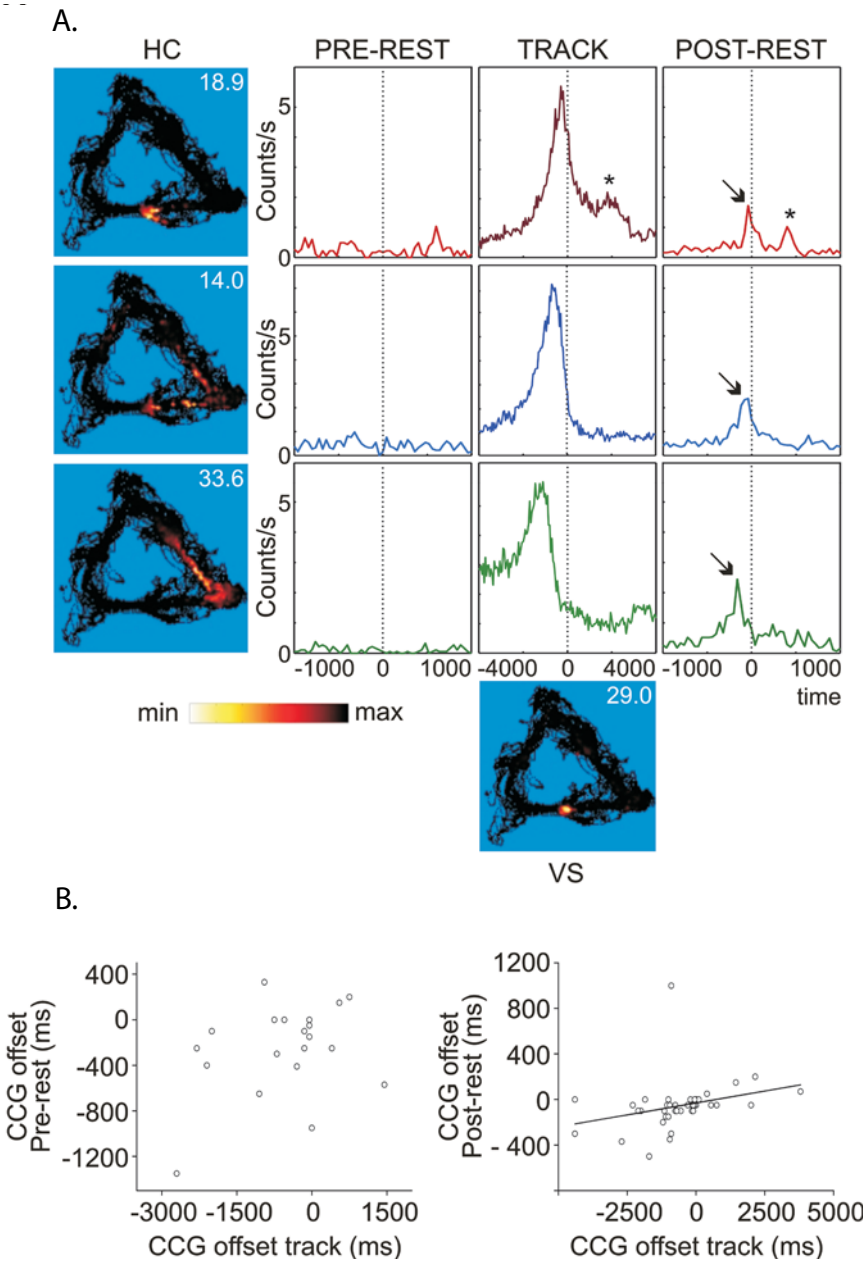
The firing order of each cell pair was assessed by computing cross-correlograms (Aertsen et al., 1989; Eggermont, 1992) and determining which order of firing was most prevalent using a 'temporal bias' measure (SI; Skaggs and McNaughton, 1996). Three subgroups were formed, i.e. HC → VS pairs (n=608), VS → HC pairs (n = 796) and 'No Clear Order' which included pairs that did not show a preferred firing order (n=1512). The HC → VS group reactivated most strongly (EV: 15.2%, REV 0.0%). Reactivation was also observed for the other groups although the observed strengths were significantly lower than for the HC → VS group. (Fig. 5.2C; Supporting Table 5.2).

Variations in reactivation measured across all of the subgroups partitioned according to each of the three factors could not be attributed to differences in varying numbers of cell pairs or differences in correlation strengths (SI). Altogether, these results indicate that maintenance of the HC → VS firing order and expressing a combination of a place field and a reward correlate are important indicators for strong reactivation. However, since a reactivating cell pair may display multiple characteristics at the same time (e.g. behavioral correlates and a particular firing order), we used a multi-linear regression model to test whether the contribution of each cell pair to the session EV value was dependent on firing order, theta modulation, behavioral correlates or any combination of these characteristics. First, the relative contribution of each cell pair to the session reactivation was estimated by excluding a pair from the simultaneously recorded population and recomputing the reactivation values. The difference between the session EV minus the EV after pair exclusion represents the estimated contribution of that pair to the session EV. Multi-linear regression showed that both the expression of a double correlate and the HC → VS firing order were significant factors in explaining the contribution to the session EV ($p<0.02$ and $p<0.002$, respectively; theta modulation was not significant, $p = 0.6$). The combination of firing order and expression of a double correlate predicted the pair's contribution better than either one alone ($p < 0.0002$). This analysis confirms the importance of the HC → VS firing order and expressing combined place and reward information during track running for subsequent reactivation.

We next explored whether HC-VS cell pairs fire in the same order during reactivation as during behavior and whether replay is accelerated relative to active behavior. For each pair of neurons that showed a place field and a reward-related correlate we constructed three cross-correlograms, one for each task-episode ($n = 192$, Fig. 5.3A). We compared the time-offsets during active behavior and rest for pairs that showed significant peaks in the cross-correlograms of track running and in at least one of the rest episodes ($n = 53$, 27.6%). The time-offsets of the peaks during track running were positively correlated to those in post-behavioral rest ($R^2 = 0.09$, $p < 0.05$; $n = 47$) but not to those of pre-behavioral rest (Fig. 5.3B; $n = 26$). Thus, the recurrent firing patterns reflected the preceding experience rather than intrinsic network dynamics during rest in general. Spatial overlap between the firing fields of a cell pair appeared not to be a prerequisite for concurrent firing during subsequent sleep, as 29.8% of the cell pairs that showed peaks in the cross-correlograms for track running and post-behavioral rest exhibited non-overlapping firing fields. The peak offset in the cross-correlograms of track running ranged from -4.5 to 3.8 s and was correlated to the spatial distance between the firing fields ($R^2 = 0.27$, $p < 0.001$).

To determine whether the order of firing on the track was preserved or reversed in the subsequent rest episode the offset sign (+/-) relative to 0 was considered. Peaks during track running and post-behavioral rest were consistently found with the same offset sign ($43/47 = 89\%$, sign test, $p < 0.0001$) which demonstrates that replay took place in a forward direction. In combination with the strong reactivation of HC → VS pairs, the preservation of firing order suggests that replay should be dominated by activity patterns in which HC firing largely precedes VS firing, both during track running and post-behavioral rest. Indeed, in a vast majority of cases, the hippocampal cell fired preferentially before the striatal cell during both periods ($36/43 = 83.7\%$), indicating that HC-VS circuitry maintains a rather strict organization of firing order (sign test, $p < 0.0001$). Thus, not only is the firing order preserved from the behavior to ensuing sleep, but apparently the HC also takes the lead in replay and the VS follows. An additional analysis on all cell pairs with significant cross-correlogram peaks yielded similar results and confirmed that the preferential firing order during reactivation was not attributable to a lack of VS → HC correlations during track running (SI).

Replay may occur at a different time scale than applicable during behavior (Skaggs and McNaughton, 1996; Nadasdy et al., 1999; Lee and Wilson, 2002; Euston et al., 2007b). We examined whether joint HC-VS firing patterns were replayed on an accelerated time scale. Peak times in the post-behavioral rest cross-correlogram occurred significantly closer to 0 than during track running (track: 525.5 ± 201.9 ms, post-behavioral rest: 53.2 ± 28.5 ms;



$p<0.01$; $n = 47$), showing a ~10 fold compression (Fig. 5.3). Replayed patterns appeared compressed and not merely truncated as the shape of the cross-correlogram peaks with offsets of up to several seconds during behavior were fully re-expressed during sleep, including their offset from zero (Fig. 5.3A).

◀ **Figure 5.3**

A: Cross-correlograms for three pairs of simultaneously recorded neurons showing the temporal relation of firing during pre-behavioral rest, track running and post-behavioral rest. Hippocampal activity is synchronized on ventral striatal firing (time = 0) with a bin resolution of 20 ms. Spatially distributed firing patterns of the neurons are shown in the blue squares (see also Fig. 5.2B). During track running the three pairs of neurons show correlated firing with peaks at different offsets relative to time zero. This correlated firing was absent in pre-behavioral rest but reinstated during post-behavioral rest. In the topmost example a secondary peak during track running is recurring in post-behavioral rest (*). The relative time-offsets of peaks were preserved from track running to post-behavioral rest. B: Scatter plots of the temporal offsets of the peaks in the cross-correlograms (CCG) during track running and pre-behavioral rest (left) and post-behavioral rest (right). The peak offsets during track running were correlated to the peak-offsets during post-behavioral rest ($R^2 = 0.09$, $p < 0.05$) but not to pre-behavioral rest. The peak offsets during post-behavioral rest were significantly reduced compared to track running, indicating accelerated reactivation.

Discussion

Altogether, our results demonstrate coherent reactivation between the HC and a subcortical structure and identify two major factors governing cross-structural reactivation in the hippocampal-striatal system, suggesting a plausible mechanism for consolidation of associative place-reward information. The first factor that significantly correlated to strong HC-VS reactivation bears on the dependence of reactivation on the coding of behaviorally relevant information. Cell pairs that exhibited a double correlate, one place field plus one reward-related correlate, showed the strongest reactivation amongst all four subgroups. In addition, the contribution to reactivation by individual pairs depended specifically on such a co-expression of behavioral correlates. The near-synchronous reiteration of spatial and motivational information during sleep may serve to integrate these types of information and support the learning of place-reward associations. Such associations are essential to predict and localize desired food and liquids within a known environment and are therefore fundamental to foraging behavior and learned behaviors such as conditioned place preference (Carr and White, 1983; Kim and Fanselow, 1992; Parkinson et al., 1999a; Ito et al., 2006; Ito et al., 2008).

The second significant factor in joint HC-VS replay is the preferred firing order of hippocampal and VS cells, consistent across track running and subsequent sleep. The HC → VS firing order during track running was associated with a significantly elevated reactivation as compared to other temporal relationships, and the cell pair contributions to reactivation depended on this specific firing order. This organization of firing order obeys the direction of the anatomical projection (Kelley and Domesick,

1982; Groenewegen et al., 1987) and presents necessary, although not sufficient, evidence for a central tenet of consolidation theory, proposing the hippocampus to initiate reactivation in its target structures, as predicted by Marr (1971) and subsequent theorists (Buzsaki, 1989; McClelland et al., 1995; McNaughton, 1998; Pennartz et al., 2002).

An important finding is that the joint reactivation is compressed by a factor 10 compared to the behavioral time scale of neuronal activation. Thus, several seconds of 'real-time' information during behavior are brought together in a time frame of hundreds of milliseconds during sleep. This further supports the plausibility of a mechanism for the associative storage of place and reward information by way of synaptic weight changes in the HC-VS system. If a hippocampal cell, coding place, fires consistently and briefly in advance of a VS cell signaling reward (Fig. 5.3), spike timing dependent plasticity can be induced in their connection (Markram et al., 1997; Bi and Poo, 1999; Abbott and Nelson, 2000; c.f. Pennartz et al., 1993; Kombian and Malenka, 1994).

Cross-correlogram analysis revealed that joint reactivation is not restricted to neuronal pairs that exhibit overlapping firing fields; peaks that were separated by up to about 4.5 seconds during behavior were found to recur during post-behavioral rest. In a scenario where a series of place fields is followed by a reward-related correlate, this indicates that value information during slow wave sleep is not only paired with locations nearby but also with more remote stages of the path leading to the reward site. Formation of reward-predicting representations should, by definition, obey the temporal order of predictor-reward events, a requirement which is met by the preferential HC → VS firing order during replay. In principle then, the characteristics of hippocampal-striatal replay are suitable for mediating the 'backwards' association between reinforcements and cues and contexts situated progressively earlier in time. This temporally backwards referral is a key feature of conditioning theory and models of reinforcement learning (Rescorla and Wagner, 1972; Pennartz, 1995; Schultz et al., 1997; Sutton and Barto, 1998).

Although the causal role of ensemble reactivation in memory consolidation remains to be proven, the temporally ordered cross-structural replay of spatial and motivational information during sleep illuminates a plausible 'off-line' mechanism by which information processed in different parts of the brain can be integrated to enable the composition and strengthening of memory traces comprising various attributes of a single event.

Supporting Information

Materials and Methods

Subjects, Behavioral Paradigm and Recordings

Four male Wistar rats (375-425 g, Harlan, the Netherlands) were individually housed under a 12/12h alternating light-dark cycle with light onset at 8:00 AM. All experiments were conducted in the animal's inactive period. During training and recording periods, rats had access to water during a two hour period following the experimental session whereas food was available *ad libitum*.

Prior to surgery, rats learned to shuttle back and forth on a linear track (185 cm long x 10 cm wide, 40 cm elevated from the floor). Rewards (sucrose solution, 10%, vanilla desert or chocolate mousse) were provided on the track ends according to a partial reinforcement schedule. Recording sessions consisted of a rest period (pre-behavioral rest, 20-60 min) followed by a phase of reward searching behavior on a triangular track (20 min) and concluded with a second period of rest (post-behavioral rest, 60-120 min). All rats were unfamiliar with this track when recording sessions commenced. Rats were required to traverse the track repeatedly in one direction stopping only at reward wells positioned in the center of each arm to check for the presence of a reward. Each type of reward mentioned above was assigned to one of the three reward wells and the reward-location combination remained fixed throughout the recordings. Each lap, one reward was delivered to its corresponding cup according to a pseudorandom schedule. Rats rested on a towel in a wide flowerpot located next to the track.

Rats were chronically implanted with a micro-drive containing 14 individually movable tetrode drivers above the right hemisphere of the brain (Lansink et al., 2007). Five tetrodes were directed to the dorsal hippocampal CA1 area (4.0 mm posterior and 2.5 mm lateral to Bregma) and seven to the VS (1.8 mm anterior and 1.4 mm lateral to Bregma; Paxinos and Watson, 1986). Reference electrodes were placed in the corpus callosum dorsal to the hippocampus, and near the hippocampal fissure. A skull screw inserted in the caudal part of the parietal skull bone served as ground.

Recordings were conducted using a 64 channel Cheetah recording system (Neuralynx, Bozeman, MT, USA). Activity of individual neurons was sampled during one ms windows (32 kHz, amplifier gain 5000, filter settings: 600-6000 Hz), whenever the voltage signal exceeded a manually preset voltage threshold. Local field potentials (LFP) were continuously sampled at 1690 Hz and band-pass filtered between 1 and 475 Hz. The rat's headstage was equipped with an array of light-emitting diodes which allowed to track the

position of the rat and to indicate body movements during periods of rest (60 frames/ seconds, resolution ~2.5 mm/pixel).

Data Analysis

Spike sorting Groups of spikes belonging to a single unit were discriminated from other clusters and noise events on the same tetrode on the basis of waveform properties across the four channels of a tetrode using standard automated and manual clustering methods (Bubbleclust by P. Lipa, University of Arizona, AZ, Tucson U.S.A. and MClust by A.D. Redish, University of Minnesota, Minneapolis, MN, U.S.A. respectively). Clusters that were selected for analysis exhibited less than 0.1% of spike intervals within a 2 ms period in their inter-spike interval histograms and emitted at least 20 spikes in each task episode. Putative interneurons were discriminated from principal cells on the basis of average firing rate (>8Hz) and waveform characteristics such as small peak- to-valley width and the valley shape and were not included in the analysis.

Resting state and sleep phase identification. Pre- and post-behavioral rest episodes included all periods of at least 20s in which the rat was in the flower pot and remained motionless. Episodes of movements were generally short and were excluded from analysis of neural activity during resting periods. Within these periods of rest, episodes of SWS and REM sleep were identified. SWS periods were characterized by the presence of large irregular activity and the occurrence of sharp wave-ripple complexes in the LFP of the CA1 pyramidal layer. Ripples were detected each time the squared amplitude of the filtered LFP trace (100-300 Hz) crossed a threshold of 3.5 SD for at least 25 ms. Short intervals of quiet wakefulness may have been included in SWS episodes as these two phases share principal LFP characteristics. Therefore, these combined states are referred to as quiet wakefulness-slow wave sleep (QW-SWS). REM sleep periods were indicated by an elevated ratio (>0.4) of spectral density in the theta band (6-10 Hz) to the overall power of the LFP trace recorded near the hippocampal fissure. Their borders were refined upon visual inspection of the trace. Only SWS and REM sleep episodes lasting more than 20 s were included in the analysis. In summary, analysis of reactivation was performed for resting periods, which mainly consisted of QW-SWS and REM sleep phases, while a minor part was made up by unclassified, transitional periods.

Quantification of reactivation The assessment of covariation in firing rates and the quantification of reactivation with the Explained Variance method was previously described in detail (Kleinbaum et al., 1998; Kudrimoti et al., 1999; Pennartz et al., 2004; Tatsuno et al., 2006). Briefly, the temporal correlation between the firing patterns of two neurons was

expressed in a Pearson's correlation coefficient which was computed for all concurrently recorded cell pairs using binned spike trains (50 ms bin size) of each rest/task episode. Pairs in this study always consisted of one hippocampal and one ventral striatal cell; intra-area pairs were not taken into account. Thus each recording session yielded three matrices of Pearson's correlation coefficients corresponding to pre-behavioral rest, track running and post-behavioral rest. The similarity of the matrices between each combination of two episodes was determined by computing a separate correlation value. These matrix-based correlation values were used to determine which proportion of the variance in the post-behavioral correlation pattern can be explained by the pattern established during track-running while controlling for any correlations that were present before the track running experience (i.e. in the pre-behavioral rest). This quantity is expressed in the Explained Variance (EV) measure:

$$EV = r^2_{Track,R2|R1} = \left(\frac{r_{Track,R2} - r_{Track,R1} r_{R2,R1}}{\sqrt{(1 - r^2_{Track,R1})(1 - r^2_{R2,R1})}} \right)^2 \quad \text{(EQ 5.1)}$$

in which R1 and R2 represent the pre-and post-behavioral rest episode respectively and for example $r_{Track,R2}$ equals the correlation coefficient between the track and rest 2 pattern. EV equals the square of the partial correlation coefficient and is bounded between 0 and 1. The within-control measure, i.e. Reverse Explained Variance (REV), was derived by exchanging R1 and R2 in the previous equation, thereby switching the temporal order of episodes. EV and REV values were computed for all recorded sessions that contained at least 5 well-isolated active neurons from each area and for 20 minute time blocks of concatenated periods of quiet rest and sleep, i.e. periods of active behavior were excluded. Sessions that showed reactivation (EV $>$ REV) in the first 20 minute rest block after track running were used to assess decay of reactivation ($n = 15$). Sleep phase dependent reactivation was computed for sessions that showed more than 4 minutes of REM and QW-SWS in each rest episode ($n = 13$) Differences between EV and REV values were statistically tested for significance with the Wilcoxon´s matched-pairs signed rank test.

Reactivation analysis for subgroups of cell pairs For assessing reactivation in subgroups of cell pairs (e.g. 'both' and 'none' modulated in the section on theta modulation) all Pearson's correlation coefficients for cell pairs within each subgroup were pooled across all sessions and all rats before the EV and REV value were computed. Estimates of the mean

and variance of these values were derived using a bootstrapping procedure in which random samples were taken (n=10000) from the observed set of Pearson's correlation coefficients (Sokal and Rohlf, 1995; cf. Hoffman and McNaughton, 2002). During re-sampling the triplets of correlation coefficients belonging to the three rest/active episodes were kept together and the created samples were of the same size as the original subset. The re-sampling procedure included replacement, thus samples may have contained multiple copies of one triplet while others were omitted. For each sample, reactivation measures were computed resulting in distributions of EV and REV and the difference (EV-REV) values for each subset. Differences in reactivation strength between two subgroups were statistically assessed using the (EV-REV) values, although EV values alone yielded generally similar results. Individual (EV-REV) values drawn from the two distributions were compared in pairs and we determined which parent-distribution contributed the larger value. Distributions were classified as significantly different from each other when the largest value was contributed by the same parent-distribution in more than 95% of the comparisons.

Theta-modulated firing To determine whether the firing pattern of a cell was modulated by the theta rhythm recorded from HC or VS, LFP traces recorded from the hippocampal fissure and the VS were filtered using a Chebyshev type-1 band pass filter between 6 and 10 Hz. Binned spikes (10 degrees/bin) were plotted relative to the theta peaks of two successive theta periods. The spike distribution was considered non-uniform when Rayleigh's score was $< 1x10^{-5}$. The phase angle of the spikes was determined by computing the Hilbert transform of the filtered theta signal. If the firing pattern of a cell is truly modulated by the theta oscillation, randomization of the spike trains should disrupt the phase relationships between the spikes and the LFP and thus result in a uniform firing distribution relative to the theta oscillation. Firing of a unit was considered as being modulated by the theta rhythm when shuffling of the spikes abolished the non-uniformity of the spike firing distribution as assessed with the Rayleigh score.

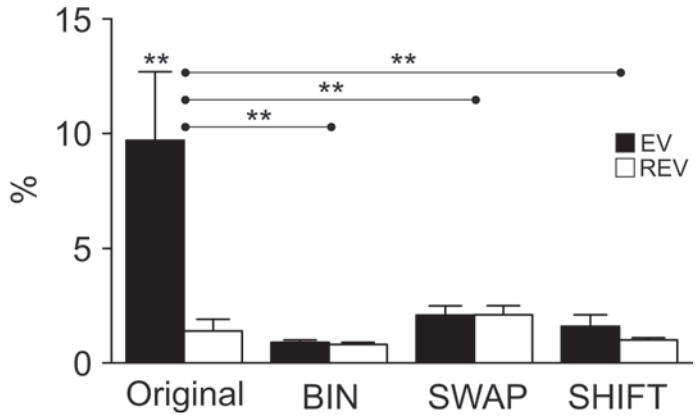
Identification of place fields The spatial selectivity of firing of hippocampal pyramidal cells was assessed by computing the information conveyed per spike. Instantaneous firing rates were computed for bins of 50 ms. The spatial position of the rat's head was determined by creating a one-dimensional representation of the track and using a resolution of 2.3 cm. Mutual information was computed between the binned spike trains and the position and corrected for finite sampling size (Skaggs et al., 1992; Panzeri and Treves, 1996). A cell was considered to express a place field if its firing rate during track running was at least 0.3 Hz and if it carried at least 0.25 bits/spike of spatial information.

Identification of reward-related firing patterns Because rewards were applied at discrete moments in time, the method used to identify place fields could not be applied to assess reward-related firing patterns. These patterns were identified with peri-event time histograms which were constructed for the rewarded and non-rewarded condition for each reward site. The histograms were synchronized on crossings of off-line installed 'virtual photobeams' positioned at the points where the rat was just reaching the reward sites. Reward-related responses were assessed within a period of 1s before and 1s after arrival at a reward site using a bin resolution of 250 ms and were statistically evaluated with Wilcoxon´s matched-pairs signed rank test ($p < 0.01$). Spike counts in the eight bins comprising the reward period were each compared to three separate control bins taken from the corner passage opposite to the well under scrutiny within the same lap. A bin of the reward period was only considered significantly different when the rank test indicated significance from each of the three control bins. A response was qualified as reward-related when one or more bins in the reward period were significantly different from the control bins. In addition we verified that the control bins did not show marked deviations from overall base line firing using peri-event time histograms and plots of the spatial distribution of firing rates. Differences between the responses at different reward sites were assessed with a Kruskal-Wallis test ($p<0.05$) followed by a Mann-Whitney's U-test (MWU; $p<0.05$), while rewarded versus non-rewarded conditions were compared using MWU ($p<0.05$).

Regression analysis Multi-linear regression was used to determine whether the contribution of the cell pairs to the Explained Variance per session was dependent on firing characteristics of the neuronal pairs; i.e. modulation by the theta oscillation, expression of place and/or reward-related firing patterns, order of firing and any combination of these factors. Each partition was used as regressor and the cell pairs were assigned a value according to the subgroup they belonged to (e.g. 'Double Correlate': 4, 'Place field only': 3, 'Reward-related correlate': 2 and 'No correlates': 1). Thus the values of the regressors varied between 1 and the number of subgroups in the partition. Similar results were obtained when a value was assigned to the number of correlates that a pair expressed (e.g. 'Double correlate': 2: 'Place field only' and 'Reward-related correlate only': 1 and 'No correlates': 0)

Cross-correlograms and Temporal Bias for determination of firing order The temporal relationship between firing patterns of HC place cells and VS reward-related units was examined by construction of cross-correlograms according to Perkel (1967) and Eggermont (1992). Spikes were binned into 10 or 50 ms intervals and the cross-correlation was examined across at least three time windows; viz. [-500, 500] ms, [-2000, 2000] ms and [-5000, 5000] ms. The firing order of pairs of HC and VS cells was assessed with the

temporal bias method (Skaggs and McNaughton, 1996) using cross-correlograms with the spikes of the striatal cell serving as reference. The ordinate expressed spike counts per second, which was integrated across intervals of 200 ms before (I) and after (II) zero. The difference between II minus I divided by the sum of the spike counts determined the temporal bias score. If this score was negative, the hippocampus was determined to fire preferentially before the VS cell. If this score was positive the preferred firing order was in the opposite direction. The classification 'No Clear Order' was assigned when the scores were approximately equal or when the cross-correlogram did not have a clear single maximum.

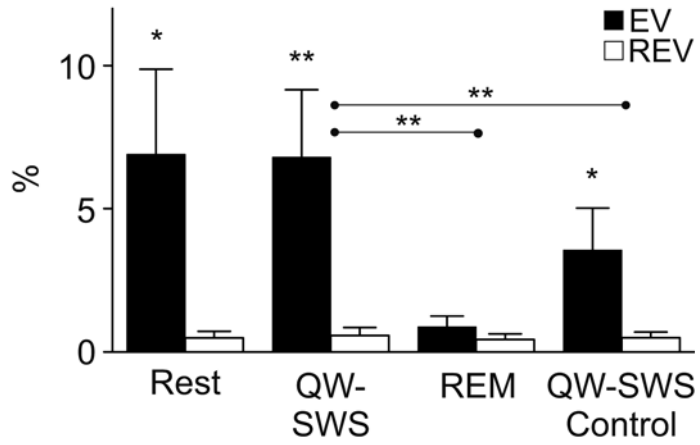


Supporting Figure 5.1

*Cross-structural reactivation was observed across 21 sessions (Original: Wilcoxon´s matched-pairs signed rank test, **p < 0.01). To test whether the reactivation was cell- and time specific, spike train vectors of the track running episode were randomized according to three different protocols. Shuffling of the time bins within the spike train vector of each cell (BIN), exchanging cell identities (SWAP) and disruption of the temporal alignment of the spike train vectors (SHIFT) each abolished reactivation. Residual EV and the difference (EV-REV) after randomization were significantly reduced compared to the original values (** p < 0.01).*

To estimate the significance of peaks in the cross-correlograms, we followed Eggermont's procedure (1992). The mean expected number of joint spike counts m and the levels of $\mu \pm 3$*SD (corresponding to p=0.0013; SD, standard deviation) were computed to provide indications for non-random excursions of spike counts above or below the expected range. Each cross-correlogram was then subjected five times to a spike

shuffling substraction procedure (Perkel et al., 1967; Aertsen et al., 1989). Peaks were accepted as significant only when they exceeded the +3*SD threshold above the mean in each of the 5 repetitions.



Supporting Figure 5.2

*Reactivation occurred during periods of QW-SWS (** p<0.01) but was absent in REM sleep. The lack of reactivation in REM sleep could not be attributed to the total REM sleep time or to the relatively late occurrence of REM sleep periods after sleep onset, because post-REM segments of QW-SWS showed significant reactivation (QW-SWS control, *p<0.05). Reactivation in the overall QW-SWS phase was significantly larger than in REM sleep or post-REM sleep segments of QW-SWS (** p<0.01).*

In addition to cross-correlograms, a ranked-order sequence analysis of multi-neuron spike trains has been applied to hippocampal and neocortical datasets to demonstrate temporally structured replay (Lee and Wilson, 2002; Diba and Buzsaki, 2007; Ji and Wilson, 2007). Typically, templates consisting of ranked-order sequences are extracted from the period of active behavior, while sequential replay is indicated when a partial or complete 'match' is found between the template and a multi-neuron spike sequence in a putative reactivation period, be it in an awake or sleeping state. Although this method appears to work well for hippocampal datasets, it is considered less suitable for studying HC-VS replay in the current task, first because reward-related neurons in the VS often showed multiple foci of intense firing on the behavioral track, which does not result in an unambigous sequence when ordered together with firing of hippocampal place cells. Second, the probabilistic reward-search task we applied did not result in strong behavioral regularity, because in some trials a reward was present at a given site but not in other trials. If a VS cell

was responsive to reward at that same site, it fired in close relation to activity of an hippocampal cell having a nearby place field in some trials but not others, again leading to variability in the elicited spike sequences. Because correctly replayed multi-neuron spike sequences appear to be quite sparse even under conditions of strong behavioral regularity (Lee and Wilson, 2002; Diba and Buzsaki, 2007; Ji and Wilson, 2007), they are probably very hard to detect against a background of other, similar sequences arising from differently structured trials within the same session. In contrast, cross-correlograms provide a lumped measure of temporally related firing of two cells and may therefore be less sensitive to variations in behavior and concomitant VS firing.

Histology Following termination of an experiment, the tetrode endpoints were marked by a small lesion resulting from passing a 25 μA current for 10 s through one of the leads of each channel. The next day, rats were transcardially perfused with 0.9% saline solution followed by 4.0% paraformaldehyde in phosphate-buffered saline (0.1 M, pH 7.4). Coronal brain sections (40 μm) were cut on a Vibratome and Nissl-stained for verification of tetrode tracks and endpoints. Recordings of hippocampal CA1 neurons were made from 103 locations between 2.6 mm and 4.8 mm posterior and between 1.2 mm to 2.8 mm lateral to Bregma compared to an atlas of the rat brain (Paxinos and Watson, 1986). Ventral striatal tetrodes were situated approximately between 2.2 and 1.2 mm anterior to Bregma and between 1.6 and 3.0 mm laterally. From a total of 140 recording sites, 58% was estimated to be situated in the core region and 42% in the shell region of the ventral striatum. Note that the recordings from one session may contain both core and shell units. Six sessions were most likely composed of HC-VS core cell pairs only. Reactivation was observed among these sessions.

Supporting Results

Behavior on the triangle track

On the track, rats ran in one direction and paused shortly at each reward site to check for reward availability. As the rats gained experience on the track, the average number of laps completed in one session increased (15.3 ± 4.1 in the first session to 62.3 ± 6.2 in the tenth, linear regression $R^2 = 0.53$, $p<0.0001$). The time between two reward site visits was significantly longer when the rat consumed a reward than when the well was not baited (16.54 ± 0.38 and 7.65 ± 0.25 s, Wilcoxon´s matched-pairs signed rank test, $p<0.0001$). The travel-time between wells did not decrease when a few consecutive wells were empty.

Reactivation strength of individual sessions expressed as the difference between (EV-REV) was highly variable but on average increased across sessions and was correlated to the

progression through the sessions (linear regression $R^2 = 0.20$, $p<0.05$) and the number of laps completed on the track ($R^2 = 0.46$, $p<0.001$). These positive correlations do not confirm or contradict a role for reactivation in learning and memory consolidation per se. The increasing strength of reactivation with experience on the track might reflect a learning process underlying improving task performance. Alternatively, the same result may be explained by an enhancement of reactivation detectability along with task familiarity (Jackson et al, 2006). As the behavior of the rats becomes more regular and repetitive with training, neuronal patterns may well be more consistently repeated.

Randomized controls for verification of cross-structural reactivation

To examine whether the observed cross-structural reactivation was due to cell- and time-specific firing correlations, EV and REV values were recalculated after the binned spike trains of the track-running episode had been randomized in three different ways (Louie and Wilson, 2001). In the BIN condition, time bins were randomly exchanged within the spike train vector of each cell. In the SWAP condition, the spike train vectors were randomly reassigned to different cells. In the SHIFT condition entire spike train vectors were individually shifted with a maximum of 10 s forward or backwards. The shift was circular so that bins that were removed from one end of the vector are reinserted at the other end. In all three control conditions, these randomization procedures reduced the difference between EV and REV values to insignificant levels (Supporting Fig. 5.1; BIN: EV: 0.9 ± 0.1%, REV 0.8 ± 0.1%; SWAP: EV: 2.1 ± 0.4%, REV 2.4 ± 0.4%; SHIFT: EV: 1.6 ± 0.5 %, REV: 1.0 ± 0.1%; Wilcoxon´s matched-pairs signed rank test, n.s.). In all conditions, reactivation measures were significantly decreased compared to the reactivation observed with the original spike train vectors ($p < 0.01$).

Control procedure verifying absence of reactivation in REM sleep

In pre- and post behavioral rest episodes, rats spent significantly more time in QW-SWS than in REM sleep (pre-QW-SWS: 19.3 ± 2.4 min, pre-REM sleep: 9.0 ± 0.8 min, post-QW-SWS 37.0 ± 2.9 min, post-REM: 11.6 ±1.0 min, $p<0.01$). Compared to quiet wakefulness-slow wave sleep, episodes of REM sleep were short and occurred at relative remote times after sleep onset. In combination with a decaying reactivation, these factors may provide an explanation for the absence of reactivation during REM sleep. To examine this, reactivation was computed over segments of quiet wakefulness-slow wave sleep that were of identical length as the REM epochs but occurred at later time points in the rest period. Significant reactivation was observed in these quiet wakefulness-slow wave sleep segments (EV: 3.6 ± 1.4%, REV: 0.5 ± 0.2%; $p< 0.05$; Supporting Fig. 5.2), indicating that the lack of pattern recurrence during REM sleep is most likely unrelated to its short duration and its late occurrence after sleep onset.

Hippocampal cells emitted on average significantly more spikes per cell during the QW-SWS segments than in REM sleep during the post- but not the pre-behavioral rest phase (Supporting Table 5.1, *$p<0.05$). In contrast, striatal cells were more active during REM sleep episodes than during the SWS sleep segments in both rest phases (**$p<0.001$).

Supporting Table 5.1: Mean spike counts per cell during REM sleep and QW-SWS segments

	REM sleep	QW-SWS control segments
Hippocampal CAI		
pre-behavioral rest	199.1 ± 92.1	252.6 ± 70.3
post-behavioral rest	231.9 ± 40.4	305.9 ± 71.5*
Ventral Striatum		
pre-behavioral rest	179.8 ± 24.6	102.7 ± 16.8**
post-behavioral rest	232.6 ± 32.9	145.1 ± 19.7**

Behavioral correlates of ventral striatal firing patterns

Out of 243 recorded ventral striatal units, 41 (16.8%) showed significant firing rate changes in close association with reward site arrivals. Although these changes were by and large firing rate increments (40, 97.6%), response patterns were heterogeneous. Responses were found when rats were approaching reward sites (9, 21.9 %), after arrival at reward sites (12, 29.3%) or spanning both phases (20, 48.8 %). A majority of responses signaled reward presence (25, 61.0%) whereas only three (7.3%) neurons responded when the wells were found unbaited. Four (9.8%) other neurons showed firing rate changes in both conditions with different response magnitudes. Firing rate changes in the remaining group of neurons (9, 21.9%) only reached significance only when the reward presence and absence condition where lumped together. Responses could be generated in relation to one (10, 24.4%), two (6, 14.6%) or all three reward sites (3, 7.3%). When a neuron responded to several reward sites, differences in magnitude were observed between the individual responses (5 out of 9 neurons, 55.6%). Changes in the firing rate of about half of the responding neurons (22, 53.7%) reached significance only when the three reward sites were pooled.

A large majority of responsive neurons fired differentially for reward presence or absence, for the various reward sites or in both conditions (34, 82.9%), which renders the possibility that their firing was purely spatially modulated highly unlikely (cf. Lavoie and Mizumori, 1994; Shibata et al., 2001). For two neurons which increased their firing rates to a single reward site irrespective of the presence or absence of a reward this option cannot be excluded. Reactivation analysis yielded similar results when these two neurons were removed from the data set.

Control procedures for reactivation of subgroups

In principle, differences in numbers of cell pairs and/or correlation distributions may account for the differences in the observed reactivation between the subgroups according to the 'firing order' and 'expression of correlates' partitions. To examine these possible confounds the bootstrapped reactivation distribution (EV-REV) of the subgroup with the highest reactivation was tested against the (EV-REV) distributions obtained from 10000 samples of each of the other subgroups in that partition, matched for the total number of cell pairs and showing a comparable distribution of correlation strengths (MWU: $p > 0.05$). In the 'firing order' and 'expression of correlates' partitions, the sampled subgroups all showed significantly less reactivation than the HC → VS or the 'double correlate' groups (this was tested as explained in the Methods section 'Reactivation analysis for subgroups of cell pairs'; $p<0.001$).

Further analysis of maintained firing order from active behavior to post-behavioral rest

As pointed out in the main text, the large majority of HC-VS pairs with double correlates, which maintained their firing order from track running to post-behavioral rest showed hippocampal firing preferentially before ventral striatal firing. Although this result indicates a predominance of HC → VS pairs in replay, it might be the case that this effect arises from a lack of clear cross-correlations in which the VS precedes the HC during track running. To examine this possible confound, we analyzed all cell pairs exhibiting significant peaks in their cross-correlograms of the track running period ($n = 222$; see Materials and methods for assessment of significant peaks). In most of these cell pairs the hippocampal cell fired preferentially before the ventral striatal cell during track running ($n=153$, 69%). Activity of the ventral striatal cell preceded that of the hippocampal cell in 54 pairs (24%) and in the remaining 15 pairs (7%) the peak was found at 0 offset. Thus, although during behavior more highly cross-correlated HC → VS pairs were found than VS → HC pairs, there was no shortage of pairs in which the VS neuron fired before the HC neuron did. As in the subgroup of cell pairs exhibiting double correlates, the time-offsets of the significant peaks during track running were positively correlated to those in post-behavioral rest

($R^2 = 0.09$, $p < 0.005$, $n = 84$) but not to those of pre-behavioral rest ($n = 64$), which confirms the experience dependence of replay in this larger population of cells. Similarly, most of the cell pairs that showed significant peaks during track running and in post-behavioral rest maintained their firing order (72/84; 86%).

Cell pairs exhibiting a HC → VS firing order during track running showed a recurring peak with the same offset sign during post-behavioral rest in 58 out of 153 cases (38%). This fraction was almost twice as high as for pairs that showed a VS → HC order (11/54, 20%; Fisher exact $p < 0.02$). Similar results were obtained when the analysis was performed more selectively, viz. when only cell pairs were taken into account having cross-correlograms with significant peaks and containing more than 500 counts (within [-2000,2000] ms, binsize 50 ms) during track running. In conclusion, during behavior we found more HC VS pairs with well-articulated cross-correlations than VS → HC pairs, but even if this difference is taken into account, the probability that a cross-correlation with the same order recurs in the HC VS remained significantly higher than for the VS → HC group.

Acknowledgements

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Supporting Table 5.2: Mean reactivation values of subgroups composed on the basis of three physiological and behavioral factors.

	Mean EV (%)	Mean REV (%)	EV-REV (%)
Firing modulated by HC theta rhythm			
Both	17.3*	0.0	17.3
HC only	4.5*	0.0	4.5
VS only	1.6	0.0	1.6
none	6.5*	0.1	6.4
Expression of behavioral correlates			
Double correlates	22.9*	0.1	22.8 ♓ † §
Placefield only	0.9	0.0	0.9 ♓
Reward-related correlate only	8.9	1.3	7.6 †
No correlates	0.5	0.1	0.4 §
Order of firing			
HC → VS	15.2*	0.0	15.2 ¶ °
VS → HC	4.2*	0.2	4.0 ¶
No order	2.1*	0.0	2.1 °

Overview of reactivation values for the different subgroups of cell pairs partitioned on the basis of modulation by the theta rhythm, the expression of behavioral correlates and the order of firing between the cells from hippocampus (HC) and ventral striatum (VS). Occurrence of reactivation within subgroups was statistically assessed as explained in the Methods section 'Reactivation analysis for subgroups of cell pairs' ($p<0.05$). The difference in reactivation between subgroups of the same partition was tested for statistical significance with the same procedure using the difference (EV-REV) as measure for reactivation. Reactivation in the 'Double Correlate' group was significantly stronger than in any of the other subgroups within the partition 'Expression of behavioral correlates' (♓ † § $p < 0.05$). The cell pairs in the HC → VS subgroup showed stronger reactivation than the other two subgroups in the partition 'Order of firing' (¶ ° $p < 0.05$).*

CHAPTER 6

Summary of results
General discussion
Concluding remarks