Mnemonic Information in the Rodent Hippocampus During Wake and Sleep States

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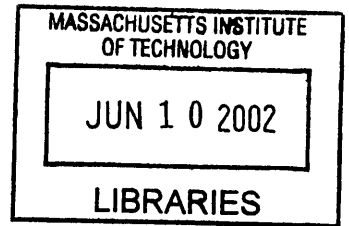Massachusetts Institute of Technology, 1993

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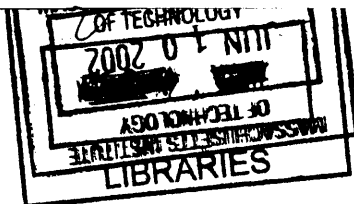MASSACHUSETTS INSTITUTE OF TECHNOLOGY

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Abstract

To investigate the representation of information in the hippocampus during memory processes, we simultaneously monitored the spiking activity of many single neurons in freely behaving rats during spatial locomotor tasks and periods of sleep. The first experiment examined the effect of differential reinforcement on the hippocampal representation of space, as mediated by the spatial receptive fields, or place fields, of hippocampal pyramidal neurons. We show that there is a bias in both place field distribution and population spiking activity towards previously reinforced locations; restriction of analysis to periods of uniform behavior suggests that this inhomogeneity is a mnemonic effect. An inverted bias observed in hippocampal interneurons suggests a broadly distributed coding of this information across the hippocampal network. These results show that information regarding behavioral salience can reach the hippocampus, and becomes incorporated into a broad hippocampal representation of experience.

The second experiment examines the reactivation of such behavioral memory traces in the absence of active behavior or sensory cues, specifically during offline-periods such as sleep. While experience-dependent reactivation occurs during slow-wave sleep, there is no evidence for such activity during REM sleep, despite its association with human dreaming and putative role in memory processing. We report that spatiotemporal patterns of activity - reflecting tens of seconds to minutes of behavioral experience - are reproduced during REM episodes at a roughly equivalent timescale. Furthermore, within such REM episodes behavior-dependent modulation of the subcortically driven theta rhythm is also reproduced. Unlike the short bursts of compressed reactivation seen in slow-wave sleep, these patterns reflect the concerted reactivation of temporally-sequenced firing across multiple neurons over long durations, broadly structured enough to convey information regarding behavioral experience. Such reactivation may represent neural processes underlying memory transfer and consolidation, and provides a basis for the electrophysiological examination of mnemonic content in sleep and dream states.

Contents

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And, to Maria, because without memories there is no living...

1 Introduction

Memory is the residue of experience, the information that remains well after the precipitating events have transpired. In a very broad sense any change left in the wake of an event is stored information: the scar from a wound, your tan from the beach, a pitcher's curveball after hours on the mound. Clearly our common understanding of memory assumes a little more structure, entailing a conscious 'bringing to mind' of information, whether that information is simple and vague, like the feeling you've seen that face somewhere before, or complex and detailed, like the tax code. Understanding the cognitive process of memory requires knowing not just what information is represented but knowing how it is represented, how it is encoded as neural activity within the brain.

The study of the physiological underpinnings of memory has seen several major advances in the past fifty years. Human lesion patients and primate models of amnesia have placed the neuroanatomical substrate of complex memory within structures of the medial temporal lobe, most significantly the hippocampus (Scoville and Milner, 1957; Squire and Zola-Morgan, 1991; Zola-Morgan and Squire, 1993). This anatomic localization revealed the different physiological sources of the taxonomy of memory, separating higher-order declarative memory from peripherally mediated forms of non-declarative memory like perceptual priming and motor skill learning (Squire, 1992). And finally, the discovery of long-lasting synaptic modification - particularly within the excitatory circuit of the hippocampus – indicates that the structural mechanisms underlying functional processes of memory can be directly addressed (Bliss and Lomo, 1973).

How does the brain reproduce experience, and how is this internal representation structured? The rodent hippocampus is a particularly useful system for neurophysiological analysis, in large part because hippocampal pyramidal neurons possess robust spatial receptive fields, or place fields. The studies reported here examine the representation of mnemonic information within the hippocampus, utilizing the characteristic representation of space as a measure of the information content within neural activity. Spatial locomotor activity provides a characterizable experience with which to study hippocampal representation of that experience, as encoded in the simultaneously recorded activity of multiple hippocampal neurons. These studies address the structure of mnemonic representation during the wake state, the nature of afferent information

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processed by the hippocampus, the reproduction of mnemonic activity during off-line sleep, and the nature of temporally structured reactivation.

1.1 Intrinsic circuitry

The hippocampus represents a substantially large portion of the rodent brain, noted early on by neuroanatomists for its unusual shape and connectivity. This unique morphology was transcribed into the nomenclature, with the distinctive folded laminar structure reflected in both the name 'hippocampus' (derived from the Greek for seahorse) and subregion specification 'CA' (cornus Ammonis, referring the Egyptian god Ammon's ram-like horns). The hippocampus proper can be divided based on cell morphology into two regions, named *regio inferior* and *regio superior* by Ramon y Cajal and corresponding to CA3/CA2 and CA1 under Lorente de No's delineation of fields (Ramon y Cajal, 1911; Lorente de No, 1934). Based on developmental and functional characteristics, regions CA3 and CA1 of the hippocampus proper are grouped with the dentate gyrus and subicular formation (subiculum, parasubiculum, and presubiculum) into the larger hippocampal formation (Paxinos, 1995).

Phylogenetically, the hippocampus is more primitive than six-layer neocortex and consists of two largely acellular layers above and below a single well-defined layer of principal cells, comprised of granule cells in the dentate gyrus and pyramidal cells in the hippocampus. Principal cells are a generally uniform population, possessing elaborate dendritic trees and mediating fast excitatory neurotransmission via glutamate. In contrast, intrinsic neurons, or interneurons, of the hippocampus constitute a much more heterogenous population with varying afferent and efferent connectivity, the vast majority of which mediate local inhibition via gamma-aminobutyric acid (GABA). Recent analyses of the anatomical and functional diversity of hippocampal interneurons suggest that much more heterogeneity exists than previously thought (Parra et al., 1998; McBain and Fisahn, 2001). While the excitatory transmission of information through the hippocampus is often emphasized (Andersen and Bliss, 1971), local inhibition by hippocampal interneurons is equally vital, controlling synaptic plasticity through perisomatic inhibition or dendritic shunting (Miles et al., 1996) and regulating network activity through the generation and synchronization of theta, gamma, and ripple (200 Hz) oscillations (Freund and Buzsaki, 1996).

The distinctive morphology of the hippocampus extends to its intrinsic circuitry, which comprises a

unidirectional excitatory flow of information through the hippocampus (Andersen and Bliss, 1971), unlike the vast majority of reciprocal corticocortical connections. This circuit, or trisynaptic loop, consists of projections from layer II of the entorhinal cortex to the dentate gyrus via the perforant path, dentate gyrus projections to CA3 via the mossy fibers, and CA3 projections to CA1 via the Schaffer collaterals. To complete the loop, CA1 neurons give rise to a return projection to layer V of entorhinal cortex, as well as a projection to subiculum that in turn innervates entorhinal cortex as well. Given the reciprocity of cortical-hippocampal projections and the return of information to neocortical areas for memory storage, it is generally presumed that the transformation of information within the trisynaptic loop is essential to memory formation. Notably, both dentate gyrus and CA3 pyramidal neurons give rise to recurrent projections innervating both ipsilateral and contralateral dentate gyrus and CA3, respectively (associational/commissural projections) (Paxinos, 1995). The existence of extensive recurrent collaterals suggests that the hippocampus may function as an autoassociative network, a model network for memory formation that encodes input and output patterns by strengthening synaptic weights between coactive neurons (Marr, 1971; McNaughton and Morris, 1987; Treves and Rolls, 1992). The importance of the trisynaptic pathway in memory formation was amplified by the discovery of long-lasting synaptic potentiation (long-term potentiation, or LTP) at all excitatory connections within the circuit, providing a possible synaptic mechanism for persistent functional change (Bliss and Lomo, 1973; Bliss and Collingridge, 1993).

While the idea of serial, lamellar processing of information through the trisynaptic loop has provided an initial understanding of hippocampal circuitry, anatomic and functional evidence suggests that this picture of hippocampal processing is incomplete. There is considerable divergent transverse connectivity between hippocampal fields, indicating that the hippocampus is a three dimensional network and does not function in a strictly parallel manner (Amaral and Witter, 1989; Amaral, 1993). Entorhinal cortex perforant path projections innervate not only the dentate gyrus but also CA3, CA1, and the subiculum, with separate projections to dentate gyrus/CA3 (arising from layer II) and CA1/subiculum projection (arising from layer III) (Steward and Scoville, 1976). Therefore CA3 and CA1 can receive convergent afferent information via monosynaptic connections from entorhinal cortex in addition to disynaptic and trisynaptic connections via the dentate gyrus (Yeckel and Berger, 1990), explaining the persistence of spatial receptive fields (place fields) in CA1 pyramidal neurons despite dentate gyrus ablation (McNaughton et al., 1989). Recent work has shown that the perforant path projection to CA1 can modulate synaptic plasticity and the gating of CA1 spiking in the trisynaptic Schaffer collateral (CA3 to CA1) pathway

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(Remondes and Schuman, 2002). These results suggest that both the perforant path and the trisynaptic circuit mediate significant, integrated information processing in the hippocampus (Sybirska et al., 2000).

1.2   Extrinsic afferents
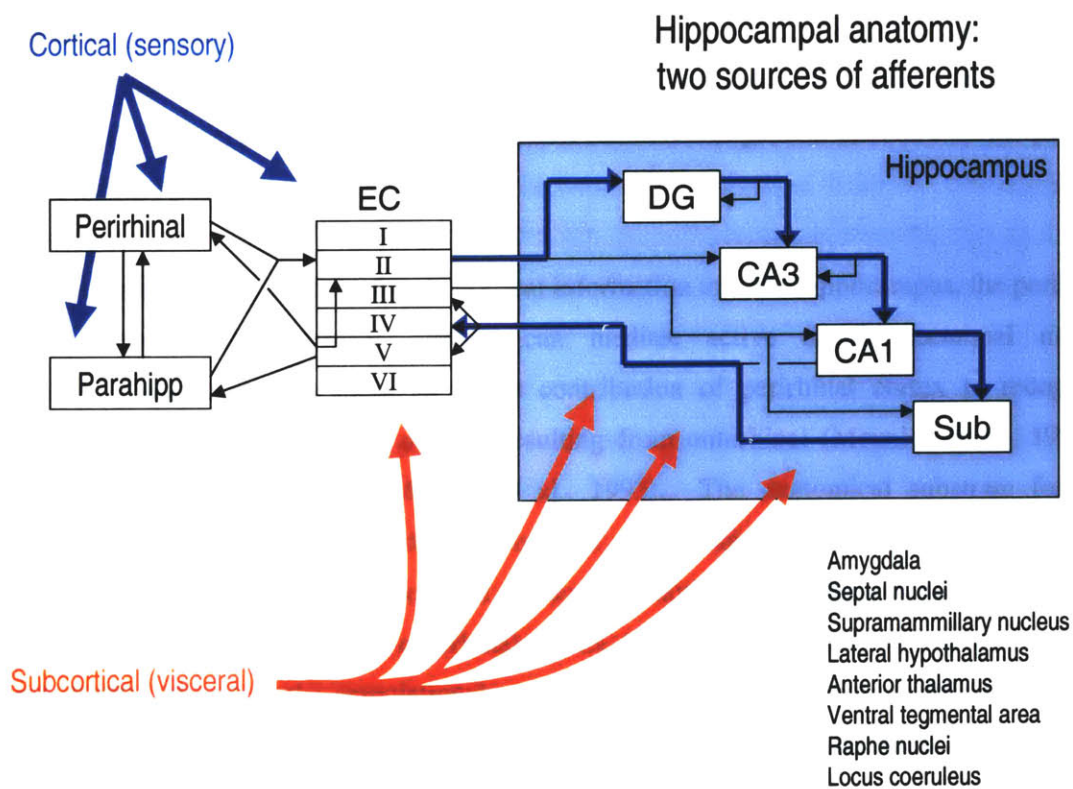
Figure 1.1 Extrinsic afferent inputs to the hippocampus

Excitatory inputs to the hippocampus proper arise from superficial layers of the entorhinal cortex, which receives unimodal and polymodal inputs from sensory associational areas. Significant associational connections exist within the perirhinal and parahippocampal cortices, which provide the majority of inputs to the entorhinal cortex, as well as within the entorhinal cortex itself. A unidirectional cascade of excitatory projections through the subregions of the hippocampus proper returns information to neocortical areas via deep entorhinal cortex. Subcortical afferents to the hippocampus, releasing primarily neuromodulatory neurotransmitters, represent a second major stream of extrinsic input. EC, entorhinal cortex; DG, dentate gyrus; Sub, subiculum.

Cortical afferents

Anatomically, the hippocampus can be viewed as a high-level polymodal sensory association area, receiving convergent afferent input from unimodal and polymodal association cortices. The entryway for neocortical information into the hippocampus is the entorhinal cortex, which receives unimodal olfactory inputs from piriform cortex and polymodal inputs from orbitofrontal, cingulate, insular, and retrosplenial cortices. The entorhinal cortex also receives significant polymodal input from two adjacent limbic regions, the perirhinal and parahippocampal cortices (Suzuki and Amaral, 1994b). These adjacent cortices receive unimodal associational input from somatosensory, auditory, and visual association cortices, as well as polymodal input from orbitofrontal, cingulated, retrosplenial, and posterior parietal cortices and the dorsal bank of the superior temporal sulcus (Suzuki and Amaral, 1994a). Though the majority of this anatomical information has been primarily derived from primate studies, recent work confirms the general structure in rats (Burwell et al., 1995; Burwell and Amaral, 1998).

Rather than being simple conduits for neocortical information into the hippocampus, the perirhinal, parahippocampal, and entorhinal cortices can mediate active extrahippocampal memory processing. Lesions studies show an active contribution of perirhinal cortex to recognition memory, exacerbating the memory deficits resulting from entorhinal (Meunier et al., 1993) or hippocampal damage alone (Zola-Morgan et al., 1993). The anatomical substrate for such processing is likely to be the considerable intrinsic associational connections both within and between the perirhinal and parahippocampal regions, and layer V/VI to layer II and intrinsic layer II associational connections in the entorhinal cortex (Suzuki and Amaral, 1994b; Dolorfo and Amaral, 1998). Note that the associational connections between deep and superficial entorhinal cortex act as a bridge between input and output connections of the hippocampus, though whether this anatomic pathway mediates a functional reentrance of information into the hippocampus has not been examined. The extensive interconnected anatomy of the intermediate MTL cortices are the initial stages in a hierarchy of associative networks culminating at the hippocampus, functioning to integrate and abstract the lower-level information arriving via sensory afferents (Lavenex and Amaral, 2000). *In vivo* electrophysiological studies in primates show that perirhinal, parahippocampal, and entorhinal neurons respond to multimodal stimuli (Desimone and Gross, 1979) and display stimulus-selective activity across delays in delayed-nonmatch-to-sample tasks (Miyashita and Chang, 1988; Suzuki et al., 1997), suggesting a functional integration of information beyond the representation of simple sensory features.

Subcortical afferents

The emphasis on excitatory processing and hippocampal function as high-level association cortex overshadows the fact this region is a major convergence zone for both cortical and subcortical afferents (Figure 1.1). In addition to neocortical projections from the entorhinal cortex, the hippocampus receives significant subcortical innervation from areas including the hypothalamus, brainstem raphe nuclei, basal forebrain septal nuclei, and locus coeruleus (Paxinos, 1995). Rather than providing sensory information, subcortical inputs release neuromodulatory neurotransmitters and control broad states or modes of hippocampal function (Buzsaki, 1989; Haas et al., 1995; Vizi and Kiss, 1998). The major subcortical projection to the hippocampus arises from the septal nuclei, in particular the medial septal nucleus (MS) and the nucleus of the diagonal band of Broca (DBB). Septohippocampal fibers travel via the fimbria, dorsal fornix, and supercallosal stria and reach all hippocampal fields, with particularly prominent innervation of the dentate gyrus. The MS/DBB contains two populations of septohippocampal projection neurons, cholinergic and GABAergic cells, providing GABAergic innervation exclusively to interneurons and cholinergic innervation to both interneurons and principal cells (Amaral and Kurz, 1985; Freund and Antal, 1988; Vertes and Kocsis, 1997).

The most significant function of the septohippocampal projection in rodents is generation of the hippocampal theta rhythm, a large amplitude 5-12 Hz sinusoidal oscillation in the hippocampal local field potential (LFP) (Vinogradova, 1995; Vertes and Kocsis, 1997). Despite ongoing debate about the exact mechanism of theta generation, it is clear that destruction of septohippocampal nuclei abolishes the hippocampal theta rhythm (Stewart and Fox, 1990). Present in the hippocampus during awake exploratory behaviors and REM sleep (Vanderwolf, 1969; Buzsaki et al., 1983), the theta rhythm has been postulated to represent an 'online' or data-input state structured for learning and storage (Buzsaki, 1989; Buzsaki, 2002). Accordingly, the theta rhythm modulates synaptic plasticity, producing optimal LTP induction if stimuli are given at theta frequency (Larson and Lynch, 1986) and at particular phases of the theta cycle (Huerta and Lisman, 1993). Furthermore, increased cholinergic neuromodulation such as that seen during the theta state can shift the balance between afferent cortical input and recurrent feedback excitation, controlling the amount of learning versus recall in CA3 (Hasselmo et al., 1995; Hasselmo, 1999).

Monoaminergic brain stem nuclei, including noradrenergic neurons of the locus coeruleus and serotonergic neurons of the dorsal and median raphe nuclei, are a second source of significant subcortical projections to the hippocampus, with relatively stronger innervation of the dentate

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gyrus than CA3 and CA1 (Loy et al., 1980; Paxinos, 1995). Noradrenaline in particular has been shown to modulate both hippocampal plasticity and spiking activity. *In vitro*, noradrenaline enhances synaptic plasticity at perforant path and mossy fiber synapses and alters the excitability of CA1 pyramidal cells via β-adrenergic receptors; noradrenaline may also function to enhance the signal-to-noise ratio via α-adrenergic receptors by increasing GABA release from inhibitory interneurons. *In vivo*, noradrenergic agonists result in a broad, nonspatial increase in hippocampal place cell firing that is dependent on novelty (Tanila, 2001), consistent with a proposed noradrenergic signal for saliency or behavioral arousal (Aston-Jones et al., 1991; Sara and Segal, 1991; Usher et al., 1999). The role raphe projections in hippocampal plasticity is less clear, but it is known that serotonergic projections to the hippocampus primarily innervate interneurons, and are critical in shifting hippocampal network activity from a theta rhythmic to a desynchronized state.

As reviewed above, the hippocampus receives two major afferent streams, excitatory neocortical projections carrying sensory information and neuromodulatory subcortical projections carrying internal state information. How might these streams interact in memory formation? First, neuromodulation can switch the hippocampal network between different states of information processing (Buzsaki, 1989; Haas et al., 1995; Vertes and Kocsis, 1997). Two-stage models propose distinct modes of hippocampal function: an encoding mode, where afferent neocortical information is temporarily stored in modifiable synaptic weights within the hippocampal circuitry, and a reactivation mode, where previously stored patterns are replayed to drive more permanent changes in the network (Buzsaki, 1989). The encoding mode corresponds with theta rhythmic states, such as active exploration and REM sleep, where large amplitude theta oscillations in the hippocampal LFP are accompanied by sparse, selective high rate discharge of pyramidal neurons. The reactivation mode corresponds to periods of large irregular amplitude (LIA) LFP, during consummatory awake behaviors and slow-wave sleep, accompanied by the synchronous discharge of many neurons in population bursts capable of driving downstream synaptic modification (Chrobak and Buzsaki, 1994). By controlling the timing and strength of these two processing states, subcortical afferents may control the selective encoding of stimuli. A second function of neuromodulation may be the regulation of synaptic plasticity, which can occur on two levels. Post-stimulus enhancement of plasticity, such as proposed for the potentiated consolidation following emotional stimuli (Packard and Cahill, 2001), reflects a broad shift in overall hippocampal state towards enhanced encoding. Such neuromodulation represents a threshold effect and is generally

not stimulus selective. Alternatively, selective neuromodulation during the encoding process may drive the encoding of particular stimuli, equivalent to a targeted filtering mechanism. Such neuromodulation must be much more temporally precise than post-encoding enhancement, functioning through presynaptic heteroreceptor modulation of neurotransmitter release (Vizi and Kiss, 1998) or fast postsynaptic plasticity pathways (Haas et al., 1995; Cahill and McGaugh, 1996).

The goal of this brief and cursory review of hippocampal anatomy is to emphasize that hippocampus processing is likely much more complex than a unidirectional flow of excitatory information that redirects inputs back to reciprocally-connected sensory cortices after simple associative processing. Integration and recoding of information begins in the hierarchy of associative connections between and within the perirhinal, parahippocampal, and entorhinal cortices before activity even reaches the hippocampus proper. Within the hippocampus itself, there exist multiple stages of recurrent connectivity in the dentate gyrus and CA3 and significant transverse divergence of connections in the trisynaptic circuit. Subcortical inputs regulate modes of hippocampal processing and control plasticity during autoassociative processing of neocortical inputs, and represent a pathway for hippocampal integration of internal state variables with external information during memory formation. Lastly, it should be noted that the hippocampus also has extensive connections (though no direct extrinsic afferents) with other limbic structures in the so-called Papez's circuit (Papez, 1937), notably the mammilary bodies and anterior thalamic nuclei, that may subserve mnemonic function (Aggleton and Brown, 1999).

1.3   The hippocampal role in memory

Evidence for a localized memory system in the brain began with a single neurosurgical case, a patient (H.M.) who developed severe retrograde amnesia following bilateral medial temporal lobe resection (Scoville and Milner, 1957). Subsequent neuropsychological studies demonstrated that H.M.'s deficits were specific to a certain kind of memory based on facts and events, called declarative memory (Cohen and Squire, 1980). Medial temporal lobe damage was found to specifically disrupt declarative memory, sparing procedural types of memory such as motor skill learning and priming (Corkin, 1968; Squire, 1992). From additional amnesic cases as well as non-human primate studies, the crucial medial temporal lobe structure for memory formation was narrowed down to the hippocampus (Zola-Morgan et al., 1986).

In the rodent, much of the early work on hippocampal function focused not on declarative or spatial memory but on the much more specific realm of navigation and spatial memory. This can be partly attributed to the difficulty of examining declarative knowledge in rodents, a problem addressed by early behavioral scientists by using ethologically-derived spatial locomotion tasks. However, the major shift towards spatial memory in the hippocampal literature arose from the discovery of place fields in the rodent hippocampus. In 1971, O'Keefe and Dostrovsky showed that the firing of individual pyramidal neurons are tuned to the location of the animal in the environment (O'Keefe and Dostrovsky, 1971). Given the robustness of the spatial activity and the lack of any other strong behavioral correlate, it was proposed that the rodent hippocampus primarily carried a neural representation of the physical environment, or a cognitive map (O'Keefe and Nadel, 1978). Primarily employing the Morris watermaze task (reference memory) or a radial arm maze (working memory), numerous studies have demonstrated the necessity for an intact hippocampus in spatial memory, including lesion studies (Morris et al., 1982; Sutherland et al., 1982; Sutherland et al., 1983; Jarrard, 1993), neuropharmacological blockade studies (Morris et al., 1986), and genetic lesion studies in mice (Rotenberg et al., 1996; Tsien et al., 1996).

The wide gap between spatial memory in rodents and the broader declarative memory observed in primates have led to many researchers to propose more abstract nonspatial (or, in the least, not explicitly spatial) theories of hippocampal function. Central to many of these theories - such as configural association (Rudy and Sutherland, 1989; Rudy and Sutherland, 1995), relational memory (Eichenbaum et al., 1992), and temporal discontiguity theory (Wallenstein et al., 1998) - is the idea that associativity is integral to both memory formation and hippocampal function. Evidence for such theories originally came from behavioral studies showing hippocampal dependence of nonspatial tasks, such as time interval discrimination (Meck et al., 1984) and trace conditioning (Solomon et al., 1986), but more recently investigators have begun examining the *in vivo* electrophysiological correlates of hippocampal activity in both spatial and nonspatial behavioral tasks (see Section 1.4 below). While it is clear the rodent hippocampus plays a prominent and essential role in navigation and spatial memory, the general consensus in the field also supports a hippocampal processing nonspatial information.

1.4 Place fields, supraspatial activity, and the cognitive map

The discovery of place cells in the rodent hippocampus was both revolutionary and contentious for the field of hippocampal neurophysiology (O'Keefe and Dostrovsky, 1971). It was revolutionary because it demonstrated a clear and robust behavioral correlate of hippocampal activity, that of physical space, that to this day continues to shape theories of hippocampal function. It became contentious not because anyone doubted the existence of place fields, but because the strength of the spatial correlation - in the absence of any other robust behavioral correlate - was seen by some as evidence that the hippocampus was uniquely devoted to spatial memory and the representation of space (O'Keefe and Nadel, 1978). O'Keefe and Nadel proposed the existence within the hippocampus of an internal representation of the environment, the so-called cognitive map. Unfortunately, much time and ink has been devoted to the issue of whether the rodent hippocampus mediates a general memory function or spatial memory exclusively; initially addressed with lesion experiments, this approach is increasingly being supplemented with hippocampal electrophysiology.

What is the electrophysiological evidence for spatial processing in the hippocampus? In any physical environment pyramidal neurons in CA3 and CA1 have clear spatial receptive fields. Together place fields form a sparse, distributed, and nontopographic map of physical space (Muller et al., 1987; Wilson and McNaughton, 1993; Redish et al., 2001). Place cell activity is clearly influenced by visual sensory cues, as evident in the strong control exerted by visual cues over place fields (O'Keefe and Conway, 1978; Muller and Kubie, 1987), and place cell firing in closed environments can be simply modeled as functions of distance from boundary walls (O'Keefe and Burgess, 1996). However, the hippocampus does not function like a simple perceptual system and is not tied to a particular modality such as vision (Hill and Best, 1981; Save et al., 1998). Spatial firing persists after salient cue removal or switch to darkness, and the hippocampus can maintain different neural representations of visually identical environments, indicating the contribution of intrahead variables such as proprioceptive and vestibular idiothetic information (Muller and Kubie, 1987; O'Keefe and Speakman, 1987; Quirk et al., 1990; Skaggs and McNaughton, 1998; Tanila, 1999). Sensory stimuli seem to activate the spatial representation, which can then be stably maintained in a self-consistent manner, with sensory information acting as an updating error-correction mechanism (Knierim et al., 1998).

The concept of an exclusively spatial memory system in the rodent hippocampus conflicts with the declarative hippocampal memory seen in primates, leading some to propose broader memory hypotheses. Clearly, hippocampal neurons demonstrate activity to more than just physical location.

In addition to visual influence on the spatial representation, sensory stimuli in auditory (Sakurai, 1994) and olfactory (Wiener, 1996; Wood et al., 1999) modalities can drive hippocampal neurons. Hippocampal activity accompanies conditioned stimuli and learned responses in classical conditioning tasks (Berger et al., 1976; Berger et al., 1983; McEchron and Disterhoft, 1999) and signifies match/nonmatch characteristics in recognition memory (DNMS) tasks (Wible et al., 1986; Wiebe and Staubli, 1999; Wood et al., 1999). The prevalence of nonspatial activity can be comparable to that of spatial activity, but this balance is extremely task-dependent and different for almost every experimental design; one can still view spatial processing as the major mode of hippocampal function.

However, accumulating evidence suggests that even spatial activity in the hippocampus reflects more than a simple representation of space. Despite generally uniform place field activity in open field environments, place fields show a tendency to cluster near walls and salient visual cues (Hetherington and Shapiro, 1997). Likewise, the accumulation of place fields at goal locations in a modified water maze task suggests that the spatial representation is plastic and can be modified to the task at hand (Hollup et al., 2001). In a task that requires successive orientation to a start site, a prominent landmark, and a goal site - each of which were continually shifted relative to one another – different subsets of place cells fire relative to the different behaviorally relevant reference frames (Gothard et al., 1996). It appears the hippocampus is not tied to a single spatial representation even during the course of a single behavior.

The influence of nonspatial information on hippocampal activity should not be surprising in light of the significant subcortical innervation from neuromodulatory transmitter systems. These findings do not suggest that the model of a cognitive map is wrong, only that it is risky to presume too narrow a view of hippocampal function. Spatial activity represents the internal coding of a physical variable, an abstract quantity constructed from sensory information but not directly related to any of the perceptual senses; it cannot be viewed as a simple behavioral correlate. The dichotomy of spatial versus nonspatial activity is misleading because it tempts one to search for physiology that fits preformed concepts, rather than learn the function from the neural activity. Examining hippocampal function as either spatial or nonspatial attempts an ethological or neuropsychological approach to what is ultimately a mechanistic question; understanding how the hippocampus mediates memory processing will explain what kind of memory it subserves.

1.5   Mnemonic activity

The definition of memory at the behavioral level is ultimately a functional and phenomenological one, corresponding to the ability to store and recall information (whether that information is conscious, as in declarative memory, or unconscious, as in motor skill learning). Defining memory at the neural level, however, requires knowledge of the internal representation of information, which is far less transparent. A complete understanding would encompass knowledge of the distributed code by which information is represented as well as the neuroanatomical substrates in which it is encoded. Given limitations on both our understanding of the neural code for information and ability to comprehensively monitor brain activity, neurophysiologists have limited the search for memory-related, or mnemonic, activity in single neurons or small ensembles of neurons.

Viewed broadly, mnemonic activity encompasses any changes in neural activity correlated with changes in experience, with no requirement that such neural activity be necessarily involved in a stand-alone representation of the external experience (Desimone, 1992). Under this rubric, mnemonic activity includes long lasting changes in sensory cortex receptive field organization sensory following modification of afferent inputs (Clark et al., 1988; Allard et al., 1991; Pons et al., 1991; Weinberger et al., 1993; Wang et al., 1995) and changes in motor cortices with motor learning (Mitz et al., 1991; Chen and Wise, 1995). Perceptual responses that reflect a stimulus' novelty/familiarity, as observed in inferior temporal (Miller et al., 1991) cortex, specifically perirhinal cortex (Miller et al., 1991; Miller et al., 1993; Suzuki, 1996), are another class of mnemonic activity and may be involved in recognition memory. With persistent neural activity, such as that seen during delay periods in delayed-match-to-sample and delayed-nonmatch-to-sample tasks, stimulus-specific or associatively linked mnemonic information can be reactivated and sustained (Fuster and Jervey, 1981; Miyashita and Chang, 1988; Miller et al., 1996).

The strongest form of putative mnemonic activity is reactivation, the spontaneous reinstatement of previous patterns of neural activity. Unlike experience-dependent plasticity in sensory cortices, perceptual responses, or persistent neural activity, reactivation does not require inducing stimuli for expression and hence more closely approximates the internal representation of information associated with declarative forms of memory. It is tempting to equate reactivation with evidence for memory processing, but it should be emphasized that without a clear understanding of

downstream effects the presence of re-expressed neural activity only suggests a possible mnemonic function.

Despite the identification of mnemonic changes in primary sensory cortices (e.g., receptive field modifications, experience-dependent plasticity) or higher-order visual cortices such as inferior temporal and prefrontal cortex (e.g., paired-associate responses, delay activity), patterned reactivation is most likely mediated by the hippocampus. First, both the hippocampus and its upstream cortices (perirhinal, parahippocampal, entorhinal) contain substantial recurrent connectivity. Theoretical models of autoassociative memory postulate that such recurrent excitation can mediate a cued recall of stored patterns, even if the cueing pattern is partial or degraded (Marr, 1971; McNaughton and Morris, 1987). Second, the temporally-graded retrograde amnesia seen in temporal-lobe amnesiacs like H.M. suggests that the hippocampus retains a requisite role in memory consolidation long after the initial experience. Because distant memories remain intact in these patients, it is believed that there is a gradual transfer of information to extrahippocampal structures, likely mediated by reactivated patterns of activity (Zola-Morgan and Squire, 1990). Third, the hippocampus demonstrates distinct modes of network activity in the wake and sleep states that correspond to the multiple processing stages theorized for memory encoding. These two-stage models divide memory formation into a learning stage, where afferent information is rapidly encoded in recurrent circuitry, and a reactivation phase, where reinstantiated patterns of activity modify downstream synapses. During slow-wave sleep (SWS), hippocampal activity is characterized by irregularly occurring sharp waves (SPW) in the LFP that represent the synchronous discharge of many CA3 and CA1 pyramidal neurons, with the proper intensity, frequency, and pattern to induce synaptic enhancement (Buzsaki, 1989; Chrobak and Buzsaki, 1994).

1.6   Sleep reactivation

The idea that sleep is involved in leaning far predates neurophysiological models of two-stage learning. A large literature of sleep deprivation studies supports the necessity of sleep for learning and memory consolidation (Fishbein and Gutwein, 1977; Karni et al., 1994; Smith, 1995; Stickgold et al., 2000a), although some argue that sleep serves a general homeostatic rather than a specific memory-processing role (Crick and Mitchison, 1983). Most of these studies focus on rapid-eye movement (REM) sleep, motivated primarily by the association between REM and the structured

cognitive activity of human dreaming (Aserinsky and Kleitman, 1953), but slow-wave sleep (SWS) also subserves memory processes (Plihal and Born, 1999; Stickgold et al., 2000b). Because of its proposed function in memory consolidation and the isolation it provides from external stimuli, sleep offers an ideal condition to investigate mnemonic reactivation.

Given the implied plasticity associated with the temporal dynamics and coactivity conditions, are experience-dependent patterns discernible during SWS? In a seminal study, Pavlides and Winson elicited selective place cell activation by confining an animal to select locations during the wake state; CA1 cells active during behavior, compared to those without place field activation, maintained increased firing rates during subsequent sleep, during both REM and SWS (Pavlides and Winson, 1989). These results suggest that some aspects of experience are in fact reinstated during sleep, but cannot separate general activity-dependent changes in the firing properties of neurons from a more structured, information-bearing mnemonic reactivation.

Successive experiments, however, have identified increasing levels of experience-dependent structure, specifically within SWS patterns, that are more indicative of reactivation. Key to these studies is increasing the dimensionality of the examined patterns – either in the number of contributing neurons or in the temporal length - to better identify neural activity representative of past patterns. Examination of many simultaneously recorded neurons reveals that place cell pairs that are coactive during behavior show increased coactive firing during subsequent SWS (Wilson and McNaughton, 1994; Kudrimoti et al., 1999); this effect is not an activity-dependent confound of increased firing rates, as cell pairs that are active but not coactive during behavior do not show increased sleep activity. Unlike the increased post-behavior firing rates observed in individual neurons, paired reactivation was found only in SWS, specifically during the SPW-concurrent bursts of population activity. Such paired reactivation observed in CA1 presumably reflects the autoassociative binding of simultaneously active neurons via recurrent excitation, most likely in CA3 where SPWs originate. Given the short transient nature of SPW bursts, there is a considerable temporal compression as cell pairs coactive over seconds during behavior are simultaneously reactivated in approximately 100 msec windows.

Paired reactivation indicates the re-expression of a distributed hippocampal representation, but only reflects instantaneous states of activity, retaining no temporal information about the dynamics of the original pattern. Such temporal information is a requisite element of spatiotemporal theories of information coding in cell assemblies (Abeles and Gerstein, 1988; Buzsaki, 1989), but as yet there

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is scant evidence for a temporal component in reactivation patterns. Heteroassociative models suggest that recurrent networks such as CA3 can preserve information about the temporal structure of input patterns, such as the temporal firing order of different neurons (Blum and Abbott, 1996; Levy, 1996; Lisman, 1999). Such a temporal preservation mechanism may underlie the requirement for intact hippocampal function in sequence learning tasks (Honey et al., 1998; Wallenstein et al., 1998). Minimally, such models predict a preservation of relative firing order. The observation of temporal biases in the reactivation of cell pairs during SPWs, matching temporal asymmetries of cell firing during behavior, may reflect such temporal information (Skaggs and McNaughton, 1996), though the reported effect is modest. Some have postulated cortical mechanisms that can reproduce precise firing sequences, preserving not just relative but absolute temporal firing order (Abeles et al., 1993; Prut et al., 1998). Nadasdy et al. first applied such techniques to search for such firing sequences across multiple hippocampal spike trains during sleep and behavior (Nadasdy et al., 1999). They find repeating sequences in excess of chance occurrence across both sleep and behavioral periods, but because template sequences were chosen independent of their relation to behavior (i.e. place field sequence), the detected reactivation does not explicitly carry mnemonic information. It is also risky to assume that the temporal scale of reactivated SWS patterns will match that of behavioral activity in light of the different dynamics of theta modulated and LIA activity.

To move beyond merely cataloging the existence of reactivation, it is necessary to address issues of mechanism, specifically how such patterns are re-expressed and what synaptic and anatomic structures mediate their generation. The occurrence of paired reactivation with SPW bursts, which are initiated within CA3, supports an intrahippocampal storage of the reactivated activity within the recurrent collateral synaptic matrix (Wilson and McNaughton, 1994; Kudrimoti et al., 1999). Pairwise reactivation in the dentate gyrus (Shen et al., 1998) suggests that recurrent circuitry in dentate gyrus as well as entorhinal, perirhinal, and parahippocampal cortices are also potential generation sites; understanding the dynamics of reactivation genesis will likely require simultaneous multi-site information from many of these putative generation sites. Consistent with the proposed role of hippocampal reactivation in memory consolidation, experience-dependent correlated sleep activity also occurs across corticocortical ensembles recorded in parietal cortex (Qin et al., 1997). SPW bursts in hippocampus occur in coordination with spindle oscillations in neocortical structures, providing a possible synchronizing mechanism to integrate hippocampal and neocortical reactivation processes (Siapas and Wilson, 1998). The replay of spike trains during sleep was recently described in the zebra finch RA nucleus, a motor cortex analog that mediates

sensorimotor aspects of song learning (Dave and Margoliash, 2000), suggesting that sleep reactivation is a learning process conserved across species and that reactivation can arise from extrahippocampal structures.

In contrast to the reactivation processes observed in SWS, there is little direct evidence for structured neural activity during REM sleep despite the widely-held assumption of cognitive processing during dreaming. REM does not exhibit correlated reactivation like that seen during SWS (Kudrimoti et al., 1999), which is unsurprising given the absence of SPW bursts and synchronous discharge. However, human neuroimaging studies report an experience-dependent change in brain activation during REM sleep that may signal neural reactivation (Maquet et al., 2000). Furthermore, hippocampal neurons show an experience-dependent theta phase-selectivity in spiking during REM sleep (Poe et al., 2000), suggesting that the hippocampus can gain access to mnemonic information.

1.7   An integrative view of hippocampal function

The widely-accepted fact that the hippocampus is essential to memory formation has led to an effort to parse and delineate the flavors of memory, particularly in the rodent hippocampus where spatial information processing appears disproportionately strong (at least compared to the primate). While useful as a heuristic, this psychobiological approach of classifying cognitive processes has the tendency to overlook the mechanistic constraints, both anatomic and functional.

The task given to the hippocampus is a difficult one: continually arriving convergent multimodal sensory information is somehow transformed, efficiently and selectively, into eventual long term-memory storage. Focusing only on the taxonomy of memory ignores the significant amount of information processing and extraction that must first occur before memory storage, a process that likely has a great deal to do with the type of memory ultimately encoded. The anatomy of both the hippocampus and the polymodal association cortices that act as convergent information gateways to the hippocampus (perirhinal, parahippocampal, and entorhinal cortices) are well suited to associational integration and information extraction, much of which occurs before neural activity reaches the hippocampus proper. Temporal continuity or context models of hippocampal function (Rudy and Sutherland, 1989; Jarrard, 1993; Rudy and Sutherland, 1995; Wallenstein et al., 1998)

come closest to incorporating this associational nature of hippocampal processing, emphasizing the hippocampal role in binding together different sources of information.

Described in the following chapters are *in vivo* electrophysiological experiment conducted in rats, using place cell activity as a means to examine the hippocampal representation of information in the wake state, and what aspects of such structured representations are reactivated in subsequent sleep states. Emphasis is placed on the possible neuroanatomic contributions to structured hippocampal activity, both in the representational selection process during encoding as well as the reactivation process.

In the first series of experiments reported here, we examine the effect of selective spatial reinforcement on the hippocampal representation of space. Previous studies have examined the effect of reward on place cells, but without separating out confounding state-dependent or sensory-driven neural activity. By limiting analysis to periods of uniform behavior, we can filter out instantaneous perceptual effects and isolate encoded effects that are part of the memory representation.

In the second series of experiments, we examine the reactivation of long temporal patterns of hippocampal activity. Off-line periods such as sleep provide an ideal milieu to examine mnemonic activity because, with the isolation of the brain from external sensory inputs, it is possible to investigate the purely internal information content of neural activity. We present a novel way of searching for temporally structured sleep reactivation that addresses the particular, wake-like neurophysiology of REM sleep.

2 Bias in hippocampal activity towards sites of previous reinforcement

2.1 Summary

The human hippocampus is essential for memory formation, specifically mediating episodic or declarative memory. The rodent hippocampus encodes a robust representation of space, but the extent to which nonspatial information is represented and how such processing is integrated with spatial activity is not known. To study the interaction between spatial and nonspatial information, we recorded the simultaneous activity of many pyramidal cells in the hippocampal CA1 field during a reinforced locomotor task. We show that there is a bias in both place field distribution and population spiking activity towards previously reinforced locations, and that this likely reflects a broad network representation given the inverted bias observed in hippocampal interneurons. Restriction of analysis to uniform sampling behavior suggests that this inhomogeneity is a mnemonic effect. These results show that information regarding behavioral salience can reach the hippocampus, and affect the hippocampal representation of experience within memory.

2.2 Introduction

Human memories are episodic, binding together percepts across space, time, and multiple sensory modalities. Lesion studies show that this kind of integrative, fact-based memory requires the human hippocampus (Squire, 1992). Consistent with a role in representational binding, the hippocampus receives convergent ascending input from unimodal and polymodal sensory cortices (Lavenex and Amaral, 2000), and contains recurrent circuitry capable of autoassociative encoding (Marr, 1971; McNaughton and Morris, 1987). Thus the hippocampus has access to both afferent sensory input and the neural architecture to transform it into associative memory. However, the wealth of information arriving at the hippocampus despite limited storage capacity suggests that memory formation must be selective (Gluck and Myers, 1993). What kind of information is selected for representation, and how is this selection accomplished?

Spatial memory formation provides a means to examine such representational selection. The hippocampus is required for tasks involving spatial information processing (Morris et al., 1982; Sutherland et al., 1983; Jarrard, 1993). Furthermore, pyramidal neurons in the rodent hippocampus have robust spatial receptive fields (place fields), exhibiting elevated firing rates when the animal occupies a particular location in space (O'Keefe and Dostrovsky, 1971). These findings led to the proposal of a hippocampal cognitive map, encoding an allocentric representation of space (O'Keefe and Nadel, 1978). Abstracted from visual and idiothetic information, this map appears to be a distributed and nontopographic geometric representation of the physical environment (O'Keefe and Burgess, 1996), with a uniform distribution of place fields over explored environments (O'Keefe and Conway, 1978; Muller et al., 1987; Wilson and McNaughton, 1993).

However, evidence is accumulating that hippocampal activity can also be modulated by nonspatial stimuli. These stimuli are generally of behavioral importance to the animal, such as match/mismatch characteristics in delayed-nonmatch-to-sample tasks (Wible et al., 1986; Wiebe and Staubli, 1999; Wood et al., 1999) and conditioning stimuli in classical conditioning experiments (Berger et al., 1976; McEchron and Disterhoft, 1999). While place fields remain the strongest and most robust firing correlate of hippocampal neurons, these results suggest that the hippocampus can encode particularly relevant nonspatial information. In particular, reports of reward and goal related hippocampal activity (Eichenbaum et al., 1987; Breese et al., 1989; Kobayashi et al., 1997; Hollup et al., 2001) suggests that the hippocampal spatial map may extend

beyond a purely coordinate representation of the environment and signify features of behavioral significance.

To explore the effect of salient nonspatial information on the hippocampal representation of space, we examined the activity of hippocampal CA1 neurons during a spatial locomotion task. Male Long-Evans rats were chronically implanted with tetrode arrays capable of recording multiple single-cell activity (Wilson and McNaughton, 1993; Louie and Wilson, 2001). Animals were trained to run for food reward at select locations on a circular track, in each trial traversing locations that were previously reinforced and ones that were previously nonreinforced (Figure 2.1). Analysis of neural responses at particular locations was restricted to intervals when the animal was actively traversing those sites, excluding intervals when the animal was feeding or still. This approach filters out differences in neural activity that arise between locations simply due to different behaviors at those sites. The circular task is analogous to the modified Morris watermaze task used by Hollup et al., but eliminates the differential behavior at previous goal locations observed in their probe trials (Hollup et al., 2001). By disambiguating state-dependent or sensory-driven hippocampal activity during reinforcement from actual reinforcement-related mnemonic hippocampal activity during locomotion, we can examine the effects of past reinforcement on the current hippocampal representation of space.

2.3   Results

Electrophysiological recordings were conducted while rats ran on a circular track, in each trial traversing three quarters of the track to obtain food reward in a removable well. To eliminate neural activity while rats were feeding, standing still, and accelerating or decelerating, analysis was restricted to the central 180° of the 270° paths and included only trials where the rat maintained a minimum velocity throughout. The different traversals comprised a four-trial sequence that was repeated multiple times (~10) throughout a single recording session, producing four trial-specific spatial firing rate maps for each neuron. Trial-specific rate maps were then combined to produce a composite map of spatial firing activity normalized for behavior (Figure 2.1), such that reinforced and nonreinforced locations differ only in their previous history of reinforcement.

57 pyramidal neurons recorded from four rats in the circular track task exhibited spiking activity in their composite firing rate maps (mean rate threshold, 0.3 Hz). To examine the effect of previous reinforcement on current activity, we investigated differences in neural activity between track segments containing rewarded locations (R) and intervening nonrewarded segments (N). In all four animals, population composite maps derived from simultaneously recorded cells show greater firing in R compared to N segments (Figure 2.2, $p < 0.005$, two-sided t-test). Note that this strong spatial bias in neural activity occurs despite uniform behavior across R and N locations, as evident in examination of composite velocity and occupancy (Figure 2.2A). In comparison, population maps from the same animals performing a free forage task with randomly located reward, recorded prior to training in the circular task, show equivalent R and N segment activity (Figure 2.2C).

To compare this spatial bias across individual neurons, we defined a reinforcement bias measure of a neuron's relative spiking activity in reinforced versus nonreinforced track segments (R_{bias}). In the free forage condition, the distribution of R_{bias} values is symmetric and centered around zero. In the circular track task, there is a significantly larger proportion of positive R_{bias} values (36/57, 63%; $p < 0.05$, binomial test) and a marked asymmetry in the R_{bias} distribution. This distribution under selective reinforcement differs significantly from that under random reinforcement ($p < 0.05$, Wilcoxon rank sum test), suggesting that the bias seen in population spiking activity is mediated by a distributed bias across multiple neurons.

A relative spatial shift in the spiking activity of an individual neuron can occur with or without a change in the location of peak firing (place field center), an important parameter in distributed information coding (Georgopoulos et al., 1986; Wilson and McNaughton, 1993). We examined the distribution of place field centers to determine if the observed spatial bias in firing rates was accompanied by a shift in place field locations. For each neuron, the location of maximum mean firing rate in the composite spatial map was identified. As shown in Figure 4, place field centers are not uniformly distributed, occurring significantly more often in previously reinforced locations than expected by chance (36/57, $p < 0.05$, binomial test).

In the distributed ensemble code for space, place field locations are established within minutes (Bostock et al., 1991; Wilson and McNaughton, 1993) and remain stable for many days (Muller and Kubie, 1987; Thompson and Best, 1990), but specific parameters such as place field size and asymmetry can be rapidly and reversibly modified (Mehta et al., 1997; Mehta et al., 2000). To investigate the temporal nature of hippocampal activity modulation, we examined the time course

of spatial bias progression. Spatial bias in population spiking activity is present early in circular task recording sessions, as evident in the time-varying spatial composite maps and population R_{bias} measures (Figure 2.5).

Putative inhibitory interneurons were distinguished from pyramidal neurons on the basis of their high mean firing rates (>10 Hz) and narrow action potential widths (< 250 μsec). A small number (11) of putative interneurons were identified; all were recorded in the vicinity of pyramidal neurons, in or near stratum pyramidale. Some interneurons display spatially nonspecific spiking, but several show a marked modulation by radial position; this modulation is inverted relative to population pyramidal cell activity, with selective decreases in spiking at previously reinforced locations (Figure 2.6).

2.4   Discussion

The hippocampal code for space represents a high order transformation of sensory information, consistent with the anatomic position of the hippocampus as the highest level of association cortex (O'Keefe and Nadel, 1978; Wilson and McNaughton, 1993; O'Keefe and Burgess, 1996). However, comprehensive mnemonic representation of an event should include information about not only external stimuli but internal state variables as well. Here we demonstrate that hippocampal spatial activity, rather than being homogenous, displays a strong bias towards locations of behavioral significance. This bias occurs during uniform behavior and manifests upon reintroduction to the environment, suggesting that such bias is incorporated into a mnemonic representation of space capable of storage and subsequent reactivation.

Sources of hippocampal bias

While position is the strongest and most robust determinant of hippocampal neuron activity, nonspatial factors also modulate hippocampal firing. First, place field activity can be modulated by direction and velocity (McNaughton et al., 1983). Second, under specific task conditions pyramidal neurons are responsive to sensory stimuli from various modalities, such as auditory (Sakurai, 1994) and olfactory cues (Wiener, 1996; Wood et al., 1999). Third, there is a strong dependence of neural activity upon instantaneous behavioral state. Voluntary motor behaviors such as locomotion and rearing are accompanied by strong theta-band oscillations in the hippocampal

local field potential (LFP) and sparse, spatially selective firing of pyramidal neurons (Vanderwolf, 1969; Buzsaki et al., 1983). In contrast, behavioral states such as drinking, grooming, quiet sleeping, and drowsiness elicit large-amplitude irregular activity (LIA) in the LFP, accompanied by transient high-frequency oscillations and coherent discharge of many CA1 pyramidal neurons, regardless of animal position (Buzsaki, 1986; Buzsaki et al., 1992; Ylinen et al., 1995). Several studies have examined place cell activity at reward locations, but strong nonspatial modulation of hippocampal firing makes it difficult to separate behavioral state-dependent or sensory-driven firing changes at the goal site from modifications to the underlying spatial representation itself (Eichenbaum et al., 1987; Breese et al., 1989; Kobayashi et al., 1997). For example, a shift in pyramidal cell firing from one goal location to another following a shift in the site of selective reward delivery may reflect reward contingency rather than a change in spatial coding.

To isolate place specific firing from modulation by nonspatial factors, we restricted analysis to the middle portion of start to goal paths where animals ran across both previously reinforced and nonreinforced sites, and included only trials with smooth uninterrupted traversals. Given the possible sensory responsiveness of hippocampal neurons, we used a radially symmetric circular track to minimize local visual cues and removable reward wells to eliminate local odor cues. Thus composite spatial maps eliminated hippocampal activity directly elicited by reward and consumption, or driven by different behaviors or different stimuli between reinforced and nonreinforced sites. The existence of biased firing despite equivalent behavior and cues during sampling suggests that the bias is mnemonic and experience-dependent, arising from past differences between the types of locations

Given behavioral and stimulus uniformity, what differences could drive this mnemonic hippocampal bias? One possibility is the amount of experience between locations, since relatively more time is spent in reinforced portions of the track. However, previous work shows that hippocampal place fields develop within minutes of exposure to an environment (Bostock et al., 1991; Wilson and McNaughton, 1993) and remain stable for days (Muller and Kubie, 1987; Thompson and Best, 1990), suggesting that differential exposure plays a minimal role. In addition, because multiple circular task sessions were conducted prior to recording and each session comprised multiple traversals of the entire track, animals received significant exposure to nonreinforced locations as well.

Another difference between reinforced and nonreinforced locations is the relative behavioral significance they carry for the animal. The presence of important stimuli can modify hippocampal firing, as evident in the greater density of place fields near walls with prominent visual cues (Hetherington and Shapiro, 1997) and place cell firing relative to a task-related landmark reference frame rather than the fixed environment (Gothard et al., 1996). Such stimulus-related firing may also underlie reports of hippocampal activity associated with reward sites (Eichenbaum et al., 1987; Breese et al., 1989). Hollup et al. have recently reported the accumulation of place fields at platform locations in a circular Morris watermaze task, suggesting differential encoding of behaviorally significant locations even in the absence of distinguishing stimuli (Hollup et al., 2001). However, rats in the Hollup et al. study searched repeatedly over goal locations, raising the possibility that irregularities in place field distribution arose from either differential behavior or differential motivation (goal search) at platform sites. In our task, behavioral, motivational, and stimulus homogeneity during sampling suggests that the observed hippocampal bias is mnemonic, reflecting a previous experience of spatially selective reinforcement.

Possible neuroanatomical substrates

While it is tempting to attribute the observed bias to the reinforcing nature of the food reward, other processes such as arousal and attention may also contribute in establishing the behavioral saliency of stimuli. Identifying the extrahippocampal structures associated with biased hippocampal activity will help illuminate its genesis and function. In addition to excitatory glutaminergic neocortical projections via the entorhinal cortex, the hippocampus receives significant neuromodulatory subcortical input from areas including the hypothalamus, brainstem raphe nuclei, basal forebrain septal nuclei, and locus coeruleus (Paxinos, 1995). Unlike the high-level sensory input arriving via neocortical inputs, these subcortical afferents mediate broad, state-dependent information and may be the source of the goal-related signal that establishes biased hippocampal activity. For example, there is a rich noradrenergic innervation of the hippocampus from the locus coeruleus (Loy et al., 1980) that modulates place cell firing (Tanila, 2001) and has been postulated to carry a saliency or arousal signal (Aston-Jones et al., 1991; Sara and Segal, 1991; Usher et al., 1999).

Another possible source is the mesencephalic dopaminergic region comprising neurons of the substantia nigra pars compacta and ventral tegmental area, widely cited as a reward signaling system. These neurons show phasic responses to rewards or stimuli that predict rewards (Schultz et al., 1993; Schultz et al., 1997) and project widely and diffusely, providing a global reward-error

signal for reinforcement learning (Schultz, 2000; Waelti et al., 2001). Because midbrain dopaminergic neurons do not strongly innervate the hippocampus, such a reinforcement signal may be indirect, for example operating via modulation of septohippocampal cholinergic projections (Day and Fibiger, 1994; Inglis et al., 1994). Regulation of cholinergic neurotransmission in the hippocampus may be particularly significant since septal cholinergic projections are involved in theta rhythm generation (Stewart and Fox, 1990; Vinogradova, 1995; Vertes and Kocsis, 1997), activate GABAergic hippocampal interneurons via nicotinic acetylcholine receptors (Ji and Dani, 2000; Buhler and Dunwiddie, 2001), and control the balance of intrinsic recurrent versus cortical afferent neurotransmission in CA3 (Hasselmo et al., 1995; Hasselmo, 1999).

The possible participation of interneurons, as suggested by the small number of examples of biased interneuron activity reported here, reinforces the notion that the coding of bias is a systematic, distributed representation across the hippocampus. The temporal gap between peak population pyramidal spiking at reinforcement sites and interneuron spiking at nonreinforced sites indicates that interneurons with a clear inverted bias are most likely not driven by feedforward or feedback excitation from pyramidal neurons. It is possible that these interneurons are inhibited by interneuron-selective (IS) inhibitory cells that are themselves driven by feedforward pyramidal excitation (Freund and Buzsaki, 1996). Or, these interneurons may be modulated by subcortical inputs, and function via perisomatic inhibition (i.e., basket cells) to decrease network pyramidal activity at nonreinforced locations.

The increased density of place fields at previously reinforced locations suggests a strengthening of synaptic connections between neurons encoding those regions, likely through associative mechanisms. Such plasticity mechanisms have been modeled (Blum and Abbott, 1996; Wallenstein and Hasselmo, 1997) and reported (Mehta et al., 1997; Mehta et al., 2000) for the backwards shift and asymmetrization of place fields with experience, and their modulation by neuromodulatory chemicals are a candidate entryway for subcortical influence. Place field clustering also has implications for downstream decoding mechanisms, providing a higher spatial resolution and increased signaling for behaviorally important locations.

2.5   Methods

Behavioral task

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Following implantation male Long-Evans rats (5-7 months old) were acclimated to an elevated circular track (95 cm diameter, 10 cm width). In 3 of 4 animals a recording session was conducted while the animals freely foraged for randomly scattered food reward (chocolate-flavored pellets). Subsequently, animals were trained to traverse the track to receive food reward at specific locations. A trial consisted of travel from the start location to a removable food well placed at the goal location, followed by food consumption; in any given trial the goal was located at a position 270° (clockwise) from the start. After completion of a trial the goal location became the start location for the subsequent trial. After four trials the original start location once again became a start position, and the entire four-trial sequence was repeated. Note that because the animal was always traversing towards a location with a food well, no explicit behavioral criterion for task performance was necessary other than steady, consistent locomotion without interruption between start and goal locations. Recording sessions were 10-15 min duration and consisted of approximately 40 trials. Distal room cues consisted of objects on the periphery of the rectangular recording room, including electrophysiology equipment, computers, and doors.

Electrophysiology

Following surgical implantation with a microdrive array of 12 independently adjustable tetrode wires (AP −3.6, L 2.2) tetrodes were lowered to the CA1 layer over a period of days and individually positioned to obtain maximal unit isolation. Electrical signals were passed through two miniature 25-channel headstage preamplifiers to 8-channel differential amplifiers (Neuralynx), bandpass filtered (300 Hz to 6 kHz), sampled (31.25 kHz/channel), and digitized; suprathreshold events were stored for subsequent analysis. Continuous local field potential (LFP) recordings were obtained from a subset of the tetrodes used for unit recording (filtered at 0.1 Hz to 475 Hz, sampled at 1.5 kHz/channel). Head position and direction during RUN epochs was monitored at 30 Hz with a spatial resolution of 0.5 cm via overhead camera tracking of a headstage infrared diode array. A custom software package (Xclust, M. Wilson) was used to identify clusters of spike waveforms using spike width and peak amplitude on each of the four tetrode channels as primary waveform parameters.

Composite field analysis

Composite place maps were created by first time-delimiting each individual trial by approximate start and end times. Each trial was then grouped into one of the four trial types, and each trial group was position bounded to the middle 180° of the 270° trial arc. Any trial that demonstrated significant decreases in velocity was then removed from analysis. Time and position bounding was

performed four times (corresponding to the four trial types); the four 180° segments were recombined to create a composite map, with double redundancy at each location. This composit procedure was applied to spike train data from each recorded neuron and to the position data.

To measure degree of spiking activity in reinforced versus nonreinforced track segments, we defined the reinforcement bias (R_{bias}) such that:

$$R_{bias} = (R-N)/(R+N)$$

where R and N denote the mean firing rates across reinforced and nonreinforced segments, respectively. R_{bias} is essentially a normalized measure of the relative firing that ranges between -1 and 1. R and N firing rates were calculated for the 22.5° arc subtending reinforced or nonreinforced locations. Note that R_{bias} is a measure that can be equally applied to individual neuron and population spiking activity. Because the underlying distributions of R_{bias} values are not necessarily normal, particularly under selective reinforcement , the nonparametric Wilcoxon rank sum test was used to compare the two distributions.

2.6 Figures

Figure 2.1 Behavioral task and isolation of mnemonic place field activity

(A) Schematic of the four-trial sequence in the circular task.

Rats were trained to run from a start location to a goal location without interruption, traversing 270° clockwise in each individual trial. Food reinforcement was delivered at the goal location in a removable food well. Upon trial completion, the goal location became the start location for the subsequent trial. After four trials, the rat returned to original start location and the four-trial sequence began again. A behavioral session consisted of approximately 40 trials, such that multiple sampling was obtained from each trial type.

(B) Example pyramidal neuron spiking activity in circular track task.

Black lines, animal position as tracked by overhead infrared camera. Red dots, location of action potentials from a single CA1 pyramidal neuron. Note that while this neuron shows place specific activity (place field), spikes also occur at goal locations. Nonspecific activity can arise from broad changes in hippocampal activity that accompany changes in behavioral state (large irregular amplitude, or LIA, activity), such as during feeding, and do not reflect selective positional firing. Composite spatial maps were used to filter out such nonspecific activity (see below).

(C) Composite place fields created from restricted traversals.

Top, spiking activity from each neuron was scaled by position occupancy to create trial-type specific firing rate maps. Red denotes regions of high firing, blue denotes regions of low firing. Middle, electrophysiological and position data was selected from the middle 180° of each 270° traversal. Bottom, position-bounded data from the four trial types were combined to create a composite firing rate map for each individual neuron. In addition, data was restricted to traversals in which the animal maintained a minimum velocity. Thus the composite map reflects the spatial activity of a given neuron sampled during uniform locomotor behavior, despite ongoing nonuniform selective spatial reinforcement.
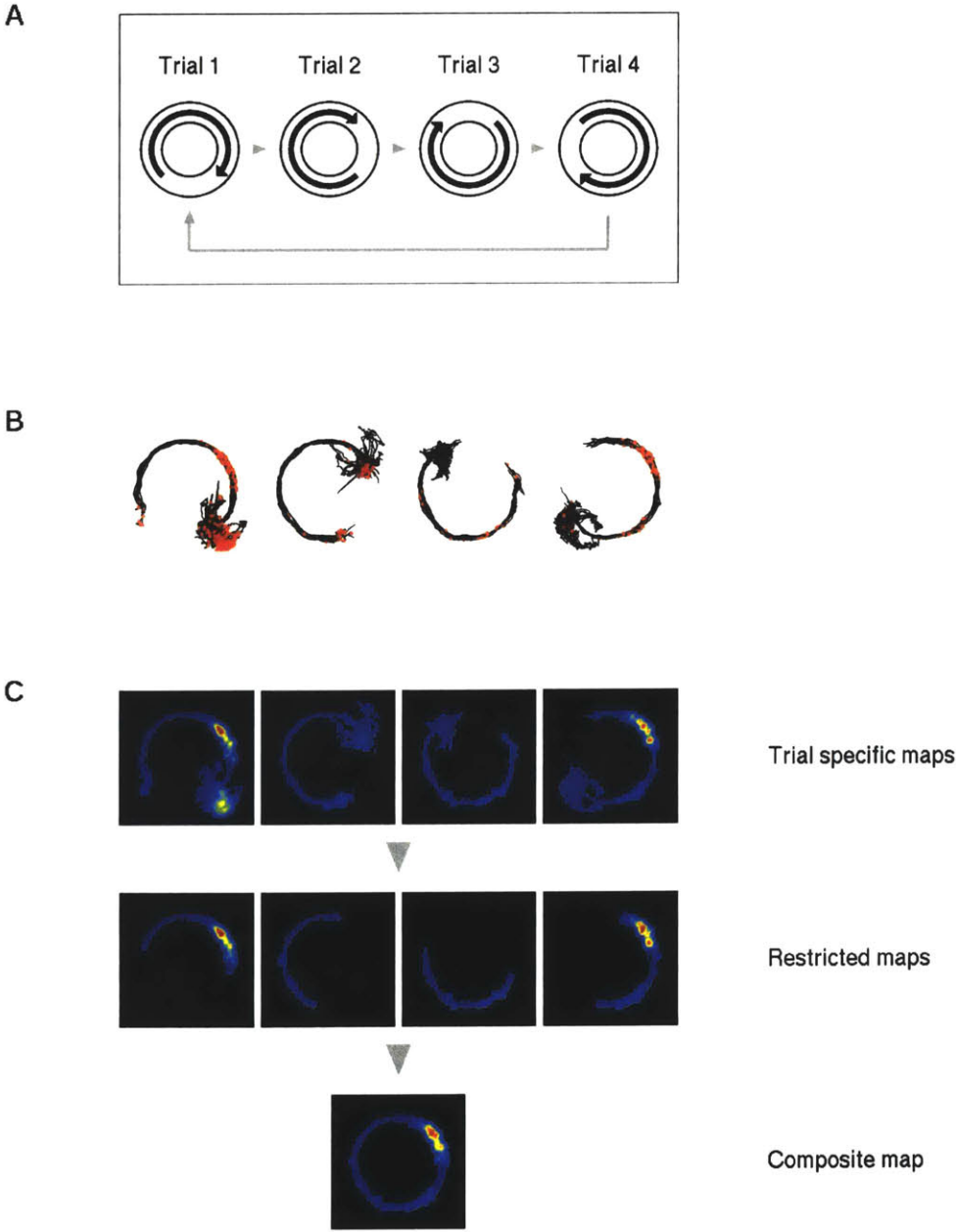
Figure 2.1

A

B

C

Trial specific maps

Restricted maps

Composite map

Figure 2.2 Spatial bias in hippocampal population activity towards sites of previous reinforcement.

(A) Example raster plot of population spiking activity in the circular task.

Top, population spiking activity as a function of radial position. Black dots, action potentials from all 17 recorded pyramidal neurons in a single behavioral session. Spikes were filtered according to the composite spatial map protocol, and are thus selected from the central region of traversals meeting a minimum velocity threshold. Spikes are plotted by radial position (x axis), in increasing order of occurrence (y axis) with a small y-randomization for visualization purposes. Red line, population firing rate as a function of radial position in the circular track. Purple lines, radial position of goal locations. Note the marked nonuniformity of the firing rate function, with selective increases at sites of previous reinforcement. Bottom, behavioral variables as a function of radial position. Small black dots, instantaneous velocity. Blue line, histogram of radial position occupancy.

(B) Bias in composite population spiking activity.

Left, composite spatial map of population activity in a single session. Purple points denote radial position of goal locations. Center, histogram of composite population firing rate in polar coordinates. Red and blue bins represent mean firing rate in 45° track segments corresponding to reinforced (R) and nonreinforced (N) locations, respectively. Right, mean firing rate in all reinforced locations (red) compared to all nonreinforced locations (blue).

(C) Summary of population reinforcement bias across all animals.

Left, mean population firing rates across reinforced and nonreinforced locations, plotted by animal (single session). Red and blue bars, mean firing rate in R and N segments, respectively, during the circular task. Black and white bars, mean firing rates in R and N segments, respectively, during a free forage task performed prior to circular task training. Forage data was obtained from 3 of 4 animals subsequently performing the circular task. Since the location of reinforcement was homogenous in the forage task, track segments were designated as R or N based on subsequent circular task usage. Right, mean population firing rates across reinforced and nonreinforced locations, across all animals. R an N segment firing rates are significantly different (p < 0.005) in the circular task but not the forage task.
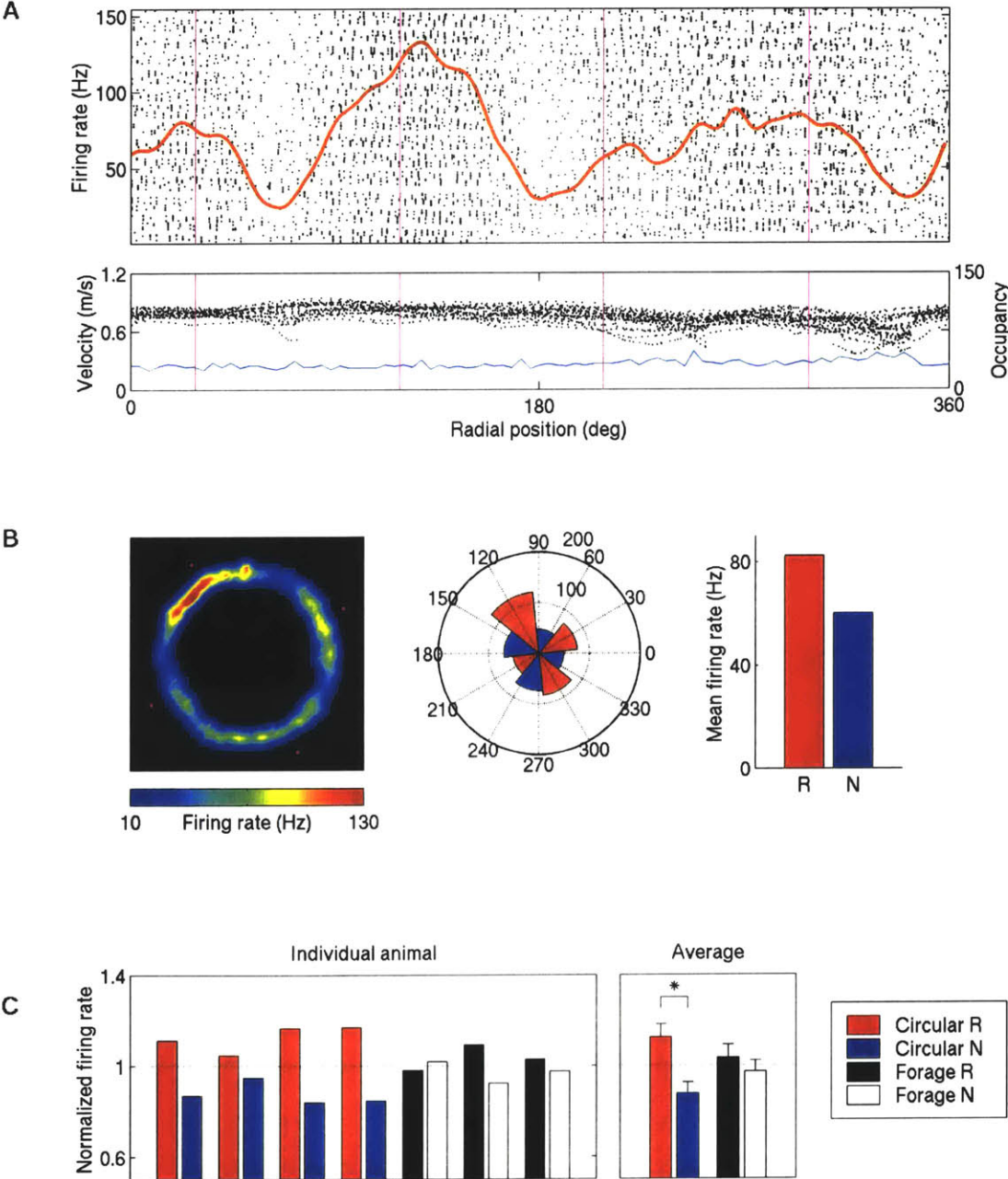
Figure 2.2

Figure 2.3 Shift in distributed reinforcement bias across hippocampal neurons

Histogram of reinforcement bias (R_{bias}) values across all neurons recorded in the forage (gray) and circular (black) tasks. An R_{bias} value represents a normalized difference in firing rate between reinforced and nonreinforced locations, and ranges from [-1,1]. Inset, number of neurons with positive and negative R_{bias} values in the forage and circular tasks.
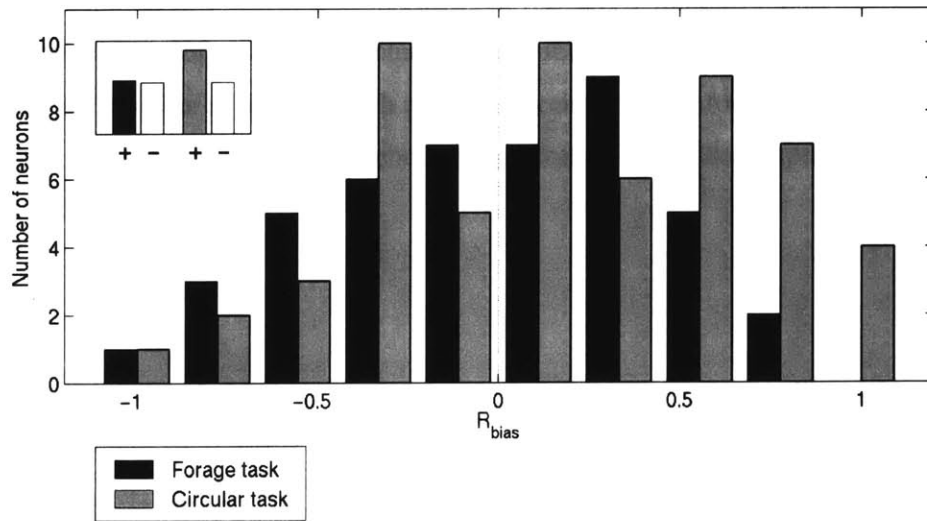
Figure 2.3

Figure 2.4 Place field center distribution skewed towards reinforced track segments.

(A) Distribution of radial position of place field centers.

Black dots, place field centers (radial position of maximum firing rate) for all neurons in the circular task. Schematic of circular track provided for reference, red and blue denotes reinforced and nonreinforced track segments.

(B) Overall distribution of place field centers between reinforced and nonreinforced segments.

Red and blue bars, number of neurons with place field centers in reinforced and nonreinforced 45° track segments, respectively. This difference is significantly more than expected by chance ($p < 0.05$, binomial test).

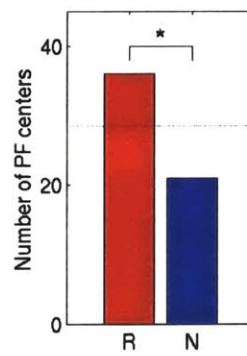**Figure 2.4**

A
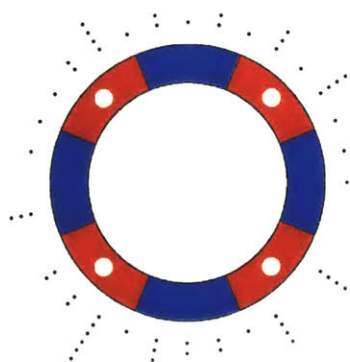
B

Figure 2.5 Time course of reinforcement bias in population activity

Left, population firing rate as a function of radial position and elapsed time in two recording sessions from different animals. Right, time-dependence of population R_{bias}, a measure of overall R vs. N segment spiking activity analogous to the individual neuron R_{bias} measure
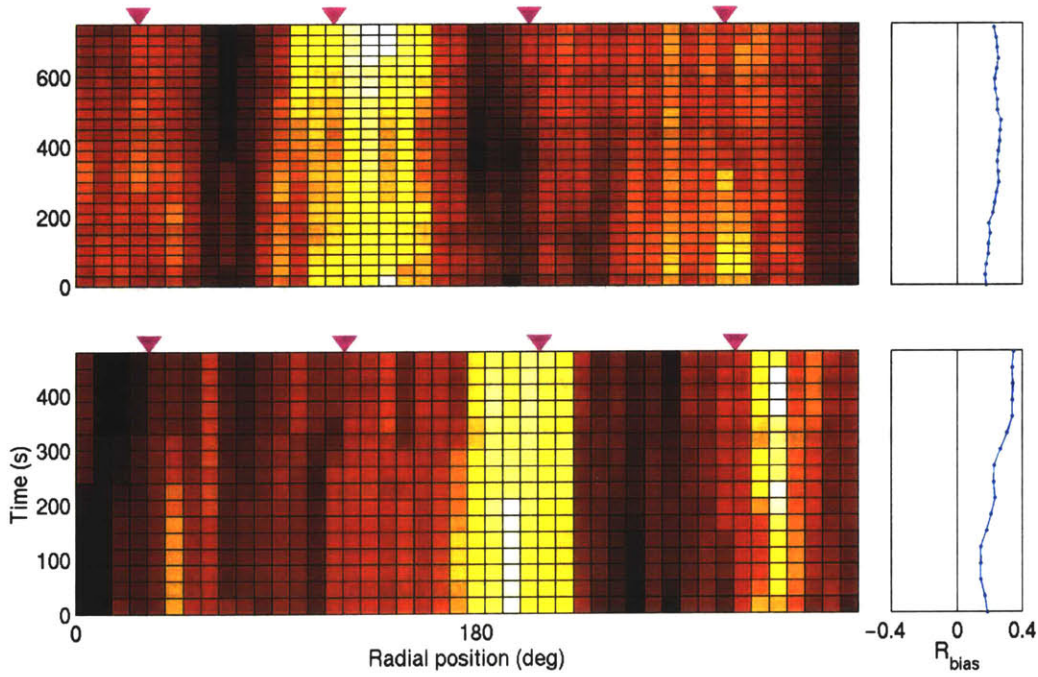
Figure 2.5

Figure 2.6 Evidence for inverted bias in hippocampal interneurons

(A) Identification of interneurons.

Two example of firing characteristics of putative hippocampal interneurons. Lower graphs, autocorrelogram of spike trains. Note the strong modulation of firing at the theta rhythm frequency (~8 Hz). Insets, average spike waveforms on four channels of the recording tetrode. Putative interneurons had higher mean firing rates (>10 Hz) and smaller negative peak widths (<250 μsec) than pyramidal neurons, and displayed little complex spike activity.

(B) Example interneuron composite firing rate maps.

Composite firing rate maps are shown for two example interneurons. Mean composite firing rates are higher than composite rates for pyramidal neurons. Right, some composite maps have little spatial specificity. Left, other interneurons displayed an inverted bias pattern from that seen in pyramidal cells, with decreased spiking activity at previously reinforced locations. Purple dots denote radial position of goal locations.

(C) Interneuron activity biased towards nonreinforced locations.

Spiking activity as a function of radial position in three example interneurons. Note the strong modulation as a function of location, with decreased firing at sites of previous reinforcement. Purple lines, radial position of goal locations.

(D) Distribution of interneuron R_{bias} values.

Histogram of R_{bias} values for 11 putative interneurons recorded in four animals.

Figure 2.6
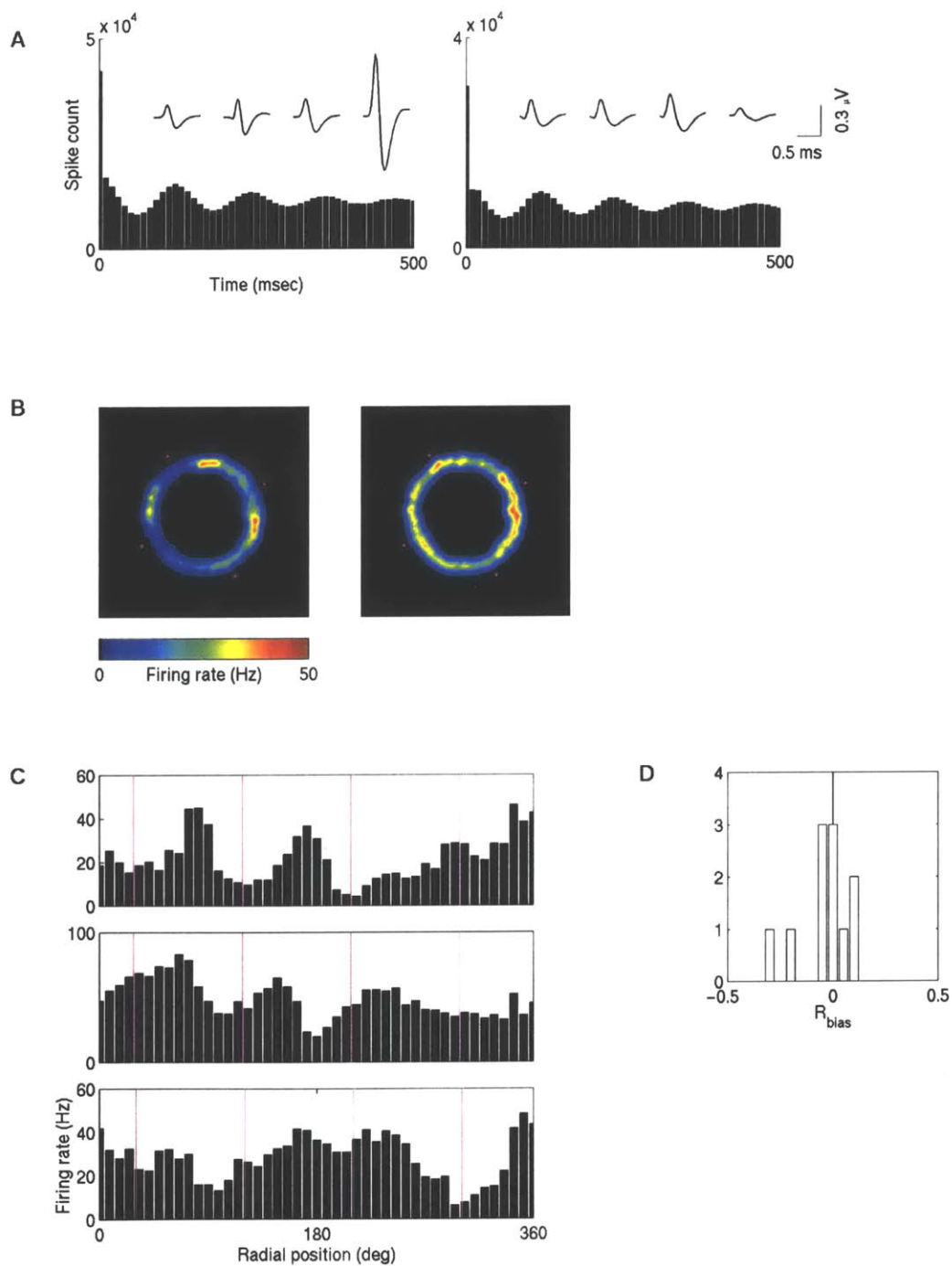
3 Temporally structured replay of awake hippocampal ensemble activity during rapid eye movement sleep

3.1 Summary

Human dreaming occurs during rapid eye movement (REM) sleep. To investigate the structure of neural activity during REM sleep, we simultaneously recorded the activity of multiple neurons in the rat hippocampus during both sleep and awake behavior. We show that temporally sequenced ensemble firing rate patterns reflecting tens of seconds to minutes of behavioral experience are reproduced during REM episodes at an equivalent timescale. Furthermore, within such REM episodes behavior-dependent modulation of the subcortically driven theta rhythm is also reproduced. These results demonstrate that long temporal sequences of patterned multineuronal activity suggestive of episodic memory traces are reactivated during REM sleep. Such reactivation may be important for memory processing and provides a basis for the electrophysiological examination of the content of dream states.

3.2   Introduction

The hippocampus is a region of high-level sensory convergence that is crucial to the formation and encoding of memories (Zola-Morgan and Squire, 1993).   Extensive work in rodents has demonstrated direct behavioral correlates of hippocampal neuronal activity, the most robust of which is the selective activation of CA1 pyramidal cells at particular locations in space (place fields) (O'Keefe and Dostrovsky, 1971).   Consistent with a hippocampal role in memory encoding, these cells exhibit experience-dependent reactivation during sleep that is representative of previous behavior (Pavlides and Winson, 1989; Wilson and McNaughton, 1994; Skaggs and McNaughton, 1996).   Specifically, neurons with overlapping place fields during spatial exploration show increased coactivity during subsequent sleep.   Such short-timescale mnemonic changes are associated with slow wave sleep (SWS), particularly the high-frequency ripple oscillations during which many hippocampal neurons fire in close temporal synchrony.   These oscillations provide ideal physiological conditions for the Hebbian modification of synapses (Bliss and Collingridge, 1993), suggesting that SWS reactivation may be driving downstream synaptic changes to encode memory representations.

In contrast, the possible role of REM sleep during memory consolidation is unclear.   The strong association of REM sleep with human dreaming (Aserinsky and Kleitman, 1953) has generated many theories regarding the information content of dream states as well as the physiological function of REM sleep.   Deprivation studies demonstrate the necessity of REM sleep for the acquisition of certain types of learning (Smith, 1995), but it has been argued that REM sleep may serve a general homeostatic role rather than a specific memory-processing function (Crick and Mitchison, 1983).   Although general experience-dependent changes in neural activity occur during REM sleep, efforts to detect short-timescale mnemonic activity like that observed during SWS have failed to detect such replay (Pavlides and Winson, 1989; Poe et al., 2000), although general experience-dependent changes in activity have been observed (Kudrimoti et al., 1999).   However, unlike SWS, REM sleep is dominated by the robust theta oscillations (6-10 Hz) and EEG desynchrony that characterize the awake exploratory state, raising the possibility that reactivation during REM sleep may be structured more like awake neural activity.

To investigate this, we employed a behavioral task that produces distinct hippocampal firing patterns over extended durations and examined subsequent REM episodes for similar patterns of activity.   Four male Long-Evans rats were chronically implanted with microelectrode arrays to

record multiple single cell activity from the CA1 region of the hippocampus (Wilson and McNaughton, 1993). Animals were trained to run along a circular track for food reinforcement, traversing 3/4 the track circumference in each trial of a four-trial sequence that was continuously repeated for the duration of the task (Figure 3.1A). Following acquisition of the task, electrophysiological activity was monitored during task performance (RUN) and during periods of sleep immediately before and after behavior.

3.3   Results

The ability to simultaneously record the activity of multiple neurons enables the examination of complex patterns of firing structure beyond pairwise firing biases. CA1 pyramidal cells recorded during the behavioral task displayed spiking activity that was strongly dependent upon the animal's position in space (Figure 3.1B). To examine the influence of mnemonic coding on hippocampal activity, analysis was restricted to pyramidal cells that were active and unambiguously isolated throughout all sleep and behavioral epochs. Consistent with previous observations of place cell activity within the hippocampus, in which ~30% of cells are typically active in any given spatial environment (Wilson and McNaughton, 1993), cells with mean RUN firing rates exceeding 0.2 Hz were identified as active, yielding ensembles of between 8 and 13 simultaneously recorded neurons per session (see Methods). While some cells were strongly modulated by location alone, other cells fired in a conjunctive manner combining both location specificity and behavioral specificity (e.g. cell 10, Figure 3.1B), similar to behavioral dependence reported in other tasks (Wiener et al., 1989; Deadwyler et al., 1996). Note that the combination of spatial receptive fields and structured spatial behavior produces a characteristic ordered pattern of ensemble activity (Figure 3.1C). The temporal structure within this pattern is determined by the sequence in which the animal's behavior takes it through the task environment, providing within the ensemble pattern a unique signature of the behavioral experience. Due to the repetitive nature of the task, such patterns of activity were consistently repeated throughout a given session. The repeated activation of these robust patterns during a behaviorally salient task led us to hypothesize that such patterns would be good candidates for subsequent reproduction during sleep.

REM episodes were identified as periods of sleep with sustained (>60 s) increases in the local field potential theta power (quantified in the theta/delta power ratio and confirmed by video monitoring of immobility and sleep posture). The pattern of neuronal ensemble activity over the entire

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duration of each identified REM window (the template) was then examined for correspondence to patterns recorded during RUN (Figure 3.2). In contrast to studies that investigated the recurrence of multineuron spike sequences on the timescale of milliseconds to seconds (Abeles and Gerstein, 1988; Abeles et al., 1993; Nadasdy et al., 1999), we examined neural activity at a lower temporal resolution but over much longer durations on the order of tens of seconds to minutes, with individual neuron spike train data binned at 1 s resolution and Gaussian smoothed ($\sigma = 1.5$ s). This degree of binning and smoothing preserves and emphasizes modulation of neuron activity that occurs at behavioral timescales, such as place field activation, while eliminating millisecond-timescale temporal structure.

To quantify the similarity between a RUN epoch and a given REM episode, we defined a template correlation coefficient C_t between two multiple-neuron spiking patterns. If a spatiotemporal pattern of ensemble activity is represented as a matrix with the dimensions of time and cells, the correlation coefficient C_t between a given REM template and RUN window is analogous to the degree of overlap observed when the two matrices are superimposed. To compare the activity from individual REM episodes to the considerably longer RUN epochs, each REM pattern was used as a sliding template to identify matching patterns within the RUN epoch. As shown in Figure 3.2B, we calculated the template correlation C_t between the REM episode pattern and RUN patterns from windows centered at successive time points across the RUN epoch (step size, 1 s). In addition to obtaining proper temporal alignment, evaluation of correspondence requires consideration of temporal scaling because reactivated activity during REM may be temporally compressed or expanded compared to RUN activity. To account for this, the correlation analysis described above was repeated at multiple temporal scaling factors (SF). SFs > 1 signify a slower corresponding activity during REM, while SFs < 1 signify faster activity. The result is a two-dimensional correlation map of the RUN epoch, with each point $C_t(t,SF)$ signifying how strongly a segment of RUN activity centered at time t corresponds to the REM template at a given scaling factor SF. An example of two correlated ensemble patterns is shown in Figure 3.3 (120 s REM template and corresponding 75 s RUN window, $C_t = 0.32$).

To establish that observed correlations between REM and RUN patterns could not have arisen by chance alone, the significance of C_t was assessed relative to a sample distribution of shuffled-template correlation data generated for each REM episode. Each REM template was randomized to create a sample of possible templates specific to that REM episode (n = 50). The template correlation function C_t was then calculated for every shuffled template to create a distribution of

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possible C_t values for every (t,SF) point. Shuffles were performed upon binned spike count data prior to Gaussian smoothing (see Methods). Because no single shuffle procedure is comprehensive, we used four different shuffled C_t functions, each designed to address different nonspecific population-wide effects that may contribute to measured REM-RUN correlation (Figure 3.4). First, to control for the possibility that REM-RUN correlation was the result of consistent differences in firing rate between cells, spike count data were independently shuffled within each cell, preserving overall firing rate while disrupting longer timescale temporal structure within and between cells (BIN shuffle). Second, binned spike counts were shuffled in time similar to the BIN shuffle but with relative spike count data across cells held fixed (COLUMN shuffle). This would preserve population vectors that are reactivated as discrete states like those observed in SWS reactivation (Wilson and McNaughton, 1994) but would disrupt long timescale temporal ordering between states. Nonspecific correlation could also arise due to broad modulation of overall activity in both RUN and REM. Temporally intact spike count vectors were exchanged between cells in the third shuffle (SWAP shuffle). Finally, to ensure that REM-RUN correspondence depended upon the temporal alignment of activity across cells, spike count data for each cell were randomly displaced in time relative to activity in the other cells while maintaining within-cell spike timing information (SHIFT shuffle). This preserves the temporal structure of individual cell firing patterns while disrupting the relative phase between them. It is important to note that significant REM-RUN correlation requires correspondence in both the firing patterns of individual neurons as well as the temporal alignment of activity across all neurons.

The statistical significance of the template correlation function was calculated relative to these distributions of shuffled-template correlation data. At each timepoint and SF during RUN the observed correlation function C_t was converted into four z scores relative to the individual shuffled-template distributions. Each C_t value was then converted into an overall z score, equivalent to the minimum (least significant) z score relative to the four shuffle distributions. Thus the two-dimensional C_t matrix is converted into a two-dimensional z score matrix describing the significance of correspondence between a given REM template and points across the RUN epoch (Figure 3.5A). While it is possible to characterize the significance of individual peaks in the correlation function, we employed a more stringent test incorporating the repetitive structure of the behavioral task itself. Each RUN period was divided into behavioral epochs corresponding to repetitions of the four-trial task sequence, producing a C_t significance matrix for each behavioral segment (Figures 3.5A and 3.5B). These matrices were averaged across all behavioral epochs, and the correlation significance of each REM template was defined as the maximum value of the

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epoch-averaged matrix.

We examined a total of 45 REM episodes from four animals over seven different recording sessions. REM episode durations ranged from 60 s to 250 s (mean, 114.0 ± 50.2 s). There was a noticeable asymmetry between prebehavior and postbehavior depth of sleep, as quantified by REM episode incidence (prebehavior 3.0 episodes/hr, postbehavior 0.7 episodes/hr) and percentage of time spent in REM (prebehavior 9.3%, postbehavior 2.4 %). This difference may be attributable to the animal's behavioral state immediately following task performance. 20 of 45 (44.4%) REM episodes showed significant correlation to RUN activity (p<0.05, 19/38 prebehavior, 1/7 postbehavior, Figure 3.5C). Peak correlation significance occurred at temporal scaling factors ranging from 0.55 to 2.49 (mean 1.4 ± 0.6), with the majority (65%) of peak significance points corresponding to SF > 1.0, suggesting that REM activity recapitulates RUN activity at approximately the same speed or slower.

The reactivation of hippocampal patterns during SWS is strongest immediately following awake behavior, suggesting the development of an experience-dependent memory trace (Wilson and McNaughton, 1994; Kudrimoti et al., 1999). However, we observed significant RUN correlation from 19 of 38 REM episodes occurring before familiar RUN behavior on any given recording day. Does this reflect persistent mnemonic reactivation? Because animals received repeated daily exposure to the task, the correlation RUN-correlated patterns during postbehavior REM sleep may be attributable to residual activity from previous behavioral sessions.

If structured REM activity represents the experience-dependent reactivation of patterns established during RUN behavior, there should be no significant correlation between REM episodes and novel RUN behaviors to which the animal has never been exposed. We therefore examined three additional experiments where the animal was exposed to both the familiar RUN task as well as a novel spatial task (RUN*, Figure 3.6A). These novel tasks were also spatial locomotor tasks with multiple food reinforcement points and multiple repetitions (see Methods). When we compared prebehavioral REM episodes to these novel RUN epochs, we detected no significant correlation between them (15 REM episodes, Figure 3.6B). Furthermore, the distributions of correlation significance scores were significantly different in novel versus familiar environments (p<0.00005, Kolmogorov-Smirnov test, Figure 3.6C). In addition, three REM episodes identified during sleep following RUN* were tested. While none were found to have significant correlation with RUN* epochs, the small number of samples – consistent with earlier observations of limited

postbehavioral REM episodes - makes evaluation of this result difficult. This may reflect either a difference in quality of postbehavioral sleep as previously indicated or a slower incorporation of mnemonic information into REM. It is important to note that the same REM episodes that failed to match novel RUN* epochs did exhibit a significant distribution of correlation scores to familiar RUN epochs (identical to the distribution of all REM-familiar RUN correlation scores), demonstrating that the lack of novel RUN* correspondence was not due to a bias in the sample of REM epochs. This suggests that the observed correspondence of REM activity to RUN patterns in the 3/4 circular task arises from the replay of previously learned, behavior-specific activity.

To investigate correspondence between REM sleep and broader characteristics of awake behavior, we next examined variations in the theta rhythm, a large amplitude 6-10 Hz oscillation in hippocampal extracellular field potential regulated by medial septum cholinergic and GABAergic inputs (Vanderwolf, 1969; Stewart and Fox, 1990). The theta rhythm strongly modulates single cell firing rates and excitability and may be important for the induction of synaptic plasticity. Theta frequency oscillation is prominent during awake behavior and REM sleep and is highly correlated with specific behaviors in different species, such as exploration and movement in rodents (Buzsaki et al., 1983). Because of this behavioral dependence of theta rhythm strength, different segments within a behavioral task will elicit different amounts of theta activity. Hippocampal local field potential (LFP) traces recorded during RUN epochs exhibited phasic increases and decreases in theta rhythm strength that were tightly coupled to the repetition of single trials within the circular track task (Figure 3.7A).

The strength of theta oscillation (measured as power of the 6-10 Hz bandpass filtered LFP trace, see Methods) was calculated across all REM episodes (n = 20) that exhibited significant RUN correspondence in their ensemble unit activity. To examine similarities between REM and RUN patterns of theta frequency modulation, each REM theta power trace was then aligned with its corresponding RUN theta power trace according to the temporal alignment and scaling values determined by template correlation analysis. For example, Figure 3.7A shows LFP theta power from the correlated REM episode and RUN window depicted in Figure 3.3. Peaks and troughs were identified in the RUN theta trace (red and blue dots, respectively, Figure 3.7B); corresponding REM theta values were measured and divided into two groups depending on their RUN alignment (H, aligns with RUN peak; L, aligns with RUN trough). Mean H and L REM theta power values were calculated for each REM episode and normalized for comparison across all REM episodes. In 75% of REM episodes (16/20), the mean H theta power was greater than the mean L theta value;

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furthermore, mean H and L theta power values averaged across all evaluated REM episodes are significantly different (p < 0.0005, paired t test, Figure 3.7C). This significant difference between REM theta values that were divided according to their alignment to RUN theta values suggests that aspects of theta oscillation modulation generated during the awake behavioral task are also represented during sleep.

3.4   Discussion

The gradual shift in the locus of memory storage from the hippocampus to other, presumably neocortical sites suggests that previously stored memories can be subsequently reactivated (Squire, 1992). Here we demonstrate significant ensemble correlation between periods of awake behavior and REM sleep, despite the absence in REM of the explicit sensorimotor cues that drive distinct neural patterns during RUN. The existence of decipherable mnemonic structure during REM sleep raises further questions regarding the neural mechanisms responsible for such temporally structured activity, as well as the possible role of such reactivation in processes such as memory consolidation and learning.

Specificity of REM-RUN correspondence

Analysis of REM-RUN correspondence demonstrated that the temporal patterns of individual neuronal spiking and the phase or timing of firing between different neurons established during RUN are recapitulated during REM. This analysis employed a template correlation measure (C_t) that quantified the strength of similarity between two patterns of activity. The crucial question is whether REM-RUN correspondence is a specific result of behavioral experience or whether such similarity could arise due to nonspecific patterns of activity. We have addressed this issue through the use of shuffled template variants and the examination of REM correspondence with novel patterns of RUN activity.

Shuffle procedures were selected to control for several potential nonspecific sources of correspondence (Figure 3.4). The BIN shuffle addresses the possibility that correspondence could have arisen from the general equivalence of individual firing rates between REM and RUN. Because both states are marked by increases in theta rhythmicity, cells that systematically changed firing rates during theta-modulated states could contribute to REM-RUN correspondence. This shuffle preserves relative firing rates between cells but disrupts the temporal patterns that are a

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direct consequence of the interaction between behavior and place specificity of firing. This type of nonspecific rate effect was also controlled for by the novel RUN* analyses, since both the familiar and novel behaviors generated prominent theta rhythmic activity, but significant correspondence occurred only in the familiar condition.

A potential source of nonspecific match between RUN and REM is the presence of discrete episodes of characteristic activity that were not related to the specific RUN experience. For example, occasional bursts in synchronized activity across the hippocampus can occur due to normal (large irregular activity) phenomena; the emergence of such phenomena in both RUN and REM could lead to apparent correspondence driven by synchronous population activity. Several of the shuffling procedures directly address this possibility. The SWAP shuffle exchanges the identity of individual cells but maintains the time course of any populationwide modulation that might exist. If populationwide covariance in activity is the source of apparent correspondence, the precise identity of the cell is less significant than the proper temporal alignment of all cells in the ensemble during these discrete events. The COLUMN shuffle preserves the ensemble structure of activity within discrete windows but alters the temporal order of these windows. Correspondence resulting from the appearance of discrete events would be preserved in this shuffle while patterns that are dependent upon the temporal ordering of events across windows, such as patterns of place-related firing, would be disrupted.

Slow rhythmic modulation of population neural activity, such as the cortical slow oscillation, is known to occur during sleep. Another possible nonspecific explanation for the observed match between RUN and REM patterns is that such broad fluctuations in overall neural activity during RUN could match nonspecific slow rhythmicities expressed during REM. However, such slow fluctuations are not likely to account for the observed correspondence between specific ensemble spiking patterns for several reasons. First and foremost, the potential confound introduced by an underlying slow rhythm is periodic activation of entire subpopulations of cells, a result that is specifically controlled for within the shuffle analysis by the COLUMN and SWAP analyses. Second, place specificity of firing of individual cells clearly demonstrates that neurons were not firing in a way that simply reflected behavioral state. Place-specific cells fired at specific points within each trial, and cells with different place fields fired at different times; accordingly, RUN ensemble patterns are composed of neuronal activity with specific temporal offsets rather than phasic activation of the entire ensemble (Figure 3.1). Template correspondence depends then on not just periodic activation within the REM episode but periodic activation with correct temporal

offsets, a result that is not easily explained by an underlying slow rhythm. While existence of slow rhythms that also imposed consistent phase relationships between the firing patterns of different neurons during both RUN and REM cannot be ruled out, such activity has never been demonstrated and would be extremely difficult to reconcile with the observed spatial specificity (place fields) of individual neurons.

The examination of novel RUN* behaviors further demonstrates the dependence of REM-RUN correspondence on the specific patterns of ensemble activity generated during experience in the familiar testing environment. We examined several tasks where similar periodicities in behavior were produced due to the repetitive nature of the task. To explicitly control for the possibility that changes in apparatus might produce subtle alterations in behavior that would impair REM match, we examined a control task in which the animal simply altered the pattern of starting and stopping locations while still remaining on the familiar apparatus (RUN*3). This preserves the overall quality of behavior (circular, periodic running) as well as the general periodicity (~15 s per trial) but significantly alters the precise pattern of neural activity by proceeding through a different sequence of locations within the apparatus. The clear difference in the degree of correspondence between REM patterns and any and all of these novel conditions (Figure 3.6) indicates that ensemble match between familiar RUN and REM was not a simple consequence of nonspecific regularities in neural activity in the familiar RUN due to such factors as periodic fluctuations in behavior.

The robust spatial correlates of hippocampal neuronal activity (place fields) indicate that the activity of these cells is a function of spatial location, but the apparent periodicity of firing across the RUN task raises the possibility that these cells have simply been entrained into firing with a slow periodicity that leads to the appearance of spatially specific firing. If the consequence of RUN activity was to reinforce slow periodic firing patterns of individual cells and the tendency to fire with similar slow periodicity was reflected during REM, it could be argued that C_t correspondence was not due to a match with the behaviorally specific ensemble pattern of RUN-related firing but simply the periodicity of firing of individual cells. This possibility was addressed through the use of the SHIFT shuffle. The signature ensemble activity that characterizes a specific RUN episode is dependent not only upon the temporal pattern of firing of individual cells, but also upon the relative timing or phase of firing between cells. The SHIFT shuffle preserves the temporal firing patterns of individual cells but disrupts the phase information between cells. The demonstration that significant REM-RUN correspondence is only achieved when both the firing patterns of individual

cells as well as their phase relationship with other cells is preserved indicates that this correspondence is due to the explicit match with the behaviorally specific patterns of ensemble activity produced during RUN.

In contrast to experience-dependent changes observed during SWS, significant RUN-correlated activity occurred during REM episodes prior to awake behavior. Does this correspondence reflect previously encoded memories? Examination of neural activity during REM episodes preceding three different novel behaviors revealed no RUN correspondence, despite the fact that approximately half of the same REM episodes were significantly correlated to patterns from the familiar task. Thus, RUN-correlated REM patterns recorded before task performance on any given day represent persistent experience-dependent activity from previous task sessions. This time course differs from that of mnemonic changes in SWS, which are strongest immediately following awake behavior. However, some residual SWS reactivation can be observed in prebehavioral sleep (Kudrimoti et al., 1999), and behavioral studies of experience-dependent changes involving REM sleep mirror this longer time course. Increases in REM following learning occur as much as 24 hr after the end of training, and acquisition of certain memory paradigms requires REM sleep hours to days after learning (Smith, 1995). The apparent difference in the robustness of reactivation between pre- and postbehavioral REM episodes may either reflect a simple difference in the quality of REM between these periods that consequently limits the expression of reactivation or may reflect differences in the processing of mnemonic information within the sleep cycle. While present data suggests a difference in REM reactivation during these periods, further study will be required to evaluate the significance of this effect.

Neural mechanisms

These results demonstrate that the relative temporal firing order within an assembly of neurons can be preserved and reproduced. It is critical to note that the timescale of these temporal patterns extended over tens of seconds to minutes. In contrast to previous studies of temporal sequence activity that examined sequences on the timescale of milliseconds to seconds (Abeles and Gerstein, 1988; Abeles et al., 1993; Nadasdy et al., 1999), reactivation of temporal sequences at this timescale has never been previously observed. Previous studies that failed to identify behaviorally related ensemble activity during REM looked at short latency correlation but did not examine long timescale temporal structure (Kudrimoti et al., 1999). The surprising length of reactivated

sequences raises the question of how temporal information at such a scale is encoded. Hippocampal CA1 place fields develop a strong asymmetry with experience, which provides a synaptic mechanism capable of encoding a sequence of locations (Mehta et al., 2000). Sequence information is crucial for generating neural activity dependent upon temporal order, such as the trajectory-dependent cell firing observed in the hippocampus and entorhinal cortex (Frank et al., 2000; Wood et al., 2000). Trajectories represent task-specific temporally ordered spatial locations, and linkage of such trajectory representations either within the hippocampus or entorhinal cortex could provide a mechanism for reconstructing extended sequences of behavior.

The establishment of temporal order may involve extrahippocampal brain areas such as neocortex. In particular, the prefrontal cortex can play a role in maintaining information relating temporally adjacent states such as the beginning and end of a trial. Neurons in the prefrontal cortex have broad behavioral correlates during spatial behavioral tasks (Jung et al., 1998) and exhibit prospective activity that can encode temporal relationships across delay periods (Quintana and Fuster, 1992; Watanabe, 1996; Asaad et al., 1998; Rainer et al., 1999). Coordinated interactions between the hippocampus and prefrontal areas during REM sleep could provide a mechanism for organizing temporal order of hippocampal or entorhinal states representing behavioral sequences.

Given the subcortical generation of the theta rhythm, the recapitulation of patterns of theta modulation in addition to ensemble patterns of pyramidal cell activity suggests a broad recapitulation of behavioral state. Interestingly, in accordance with the mild temporal expansion of REM reactivation suggested by the occurrence of optimal scaling factors between 1 and 2, the frequency of theta during REM sleep is ~1.2 times slower than during RUN (data not shown). This approximate temporal concurrence between theta rhythm and REM-RUN correspondence may reflect globally slower neural processing during sleep. For example, the frequency of the theta rhythm is sensitive to brain temperature (Whishaw and Vanderwolf, 1971) and brain temperature is typically lower during sleep (Andersen and Moser, 1995), suggesting that the neural processes underlying REM reactivation may be similarly slowed. Alternatively, there may exist a specific link between the theta rhythm and sequence reactivation, with the theta rhythm serving as a pacing mechanism during temporal information storage and reactivation, perhaps to coordinate interactions across multiple brain regions. Further experiments will be required to explore the role of the theta rhythm in temporal scaling.

Functional implications

What could be the function of REM sleep replay of awake activity? One possible interpretation is that REM activity reflects neocortical activation of hippocampal circuits in a later stage of the memory consolidation process (Hennevin et al., 1995). The reactivation of hippocampal patterns is enhanced during periods of SWS immediately following behavior (Wilson and McNaughton, 1994; Kudrimoti et al., 1999). In particular, the synchronization of subsets of hippocampal neurons during oscillatory ripple events has been suggested as a strong mechanism for synaptic modification (Buzsaki, 1989). Recently acquired information within the hippocampus would activate neocortical circuits as the initial stage of consolidation, as suggested by the correlation between neocortical spindle activity and high-frequency hippocampal discharges during SWS (Siapas and Wilson, 1998). Neocortical circuits established via SWS hippocampal-neocortical interactions could subsequently engage the hippocampus during REM sleep. While it is possible that such reactivation may only reflect encoded information and not serve an explicit function, the reactivation of previous behavioral episode representations may be important for the learning and performance of procedural tasks, which is dependent upon REM sleep (Karni et al., 1994). Mnemonic information that may have shared characteristics along a particular behavioral axis such as emotion could be juxtaposed and evaluated for common causal links, allowing adaptive behavioral change based on prior experience (Hobson et al., 1998). The ability to identify specific mnemonic content within REM sleep will allow explicit evaluation of such hypotheses and further the examination of the role of sleep and dreaming in memory formation and consolidation.

3.5   Methods

Behavioral paradigm

Following implantation, Long-Evans rats (5–7 months old) were trained to run from a start location to a goal location for a food reward on an elevated circular track (95 cm diameter, 10 cm width). This task (RUN) was considered familiar because all animals were trained daily on the task for at least 5 days prior to the first recording session. A trial consisted of travel from the start location to a removable food well placed at the goal location, followed by food consumption; in any given trial the goal was located at a position 270° (clockwise) from the start. After completion of a trial the goal location became the start location for the subsequent trial. After four trials the original start location once again became a start position, and the entire four-trial sequence was repeated. Note that because the animal was always traversing toward a location with a food well, no explicit

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behavioral criterion for task performance was necessary other than steady, consistent locomotion without interruption between start and goal locations. A recording session consisted of a 1–2 hr sleep epoch, a 10–15 min behavioral epoch (RUN) of ~40 trials, and a subsequent sleep epoch. All sleep sessions were conducted in a separate sleep enclosure within the recording room. Recording sessions were conducted daily, always at the same time of day such that ~18–20 hr separated the end of one session with the beginning of the subsequent session.

Three additional novel spatial locomotor tasks (RUN*) were conducted in the fourth experimental animal to directly address the issue of experience-dependence. Novel RUN* behaviors were chosen to be similar to the familiar RUN task, and each consisted of a spatial locomotion task between food reward sites with trials repeated throughout the RUN epoch. RUN*1 was performed on an elevated T-shaped track (115 cm central arm, 60 cm choice arms), and a behavioral trial consisted of food travel from the goal location on the central arm to a goal location on one of the arms, food consumption, and then return to the central goal location. The animal tended to perform this task in a delayed alternation pattern, alternating between left and right arms in subsequent trials, and analysis was restricted to behavioral segments where the animal reliably visited one arm followed by the other arm. RUN*2 was performed on an elevated U-shaped track (160 cm arms, 15 cm connector), and a behavioral trial consisted of travel from one goal location down one arm, across, and up the other arm to the opposite goal location, food consumption, and return to the original goal location. RUN*3 was performed on the same circular track as the familiar RUN task, and the behavioral task was identical to RUN except the animal traveled 90° during each behavioral trial.

Electrophysiology

Following surgical implantation with a microdrive array of 12 independently adjustable tetrode wires (AP –3.6, L 2.2), tetrodes were lowered to the CA1 layer over a period of days and individually positioned to obtain maximal unit isolation. Electrical signals were passed through two miniature 25-channel head-stage preamplifiers to 8-channel differential amplifiers (Neuralynx), bandpass filtered (300 Hz to 6 kHz), sampled (31.25 kHz/channel), and digitized; suprathreshold events were stored for subsequent analysis. Continuous LFP recordings were obtained from a subset of the tetrodes used for unit recording (filtered at 0.1 Hz to 475 Hz, sampled at 1.5 kHz/channel). Head position and direction during RUN epochs were monitored at 30 Hz with a spatial resolution of 0.5 cm via overhead camera tracking of a head-stage infrared diode array. A custom software package (Xclust, M.A.W.) was used to identify clusters of spike waveforms using

spike width and peak amplitude on each of the four tetrode channels as primary waveform parameters.

Putative pyramidal cells and interneurons were differentiated based on bursting (complex spiking) and waveform characteristics. To restrict our analysis to patterns of RUN activity composed of actively firing units, only pyramidal cells with mean RUN epoch firing rates above 0.2 Hz were included for subsequent analysis. Cells that were not cleanly isolated or with unstable waveforms over the 4–6 hr recording period were excluded. The number of cells that met these criteria in the eight recording sessions ranged between 8 and 13 per session. While the number of cells in the ensembles used in the present analysis is somewhat lower than those found in earlier studies using this technique (Wilson and McNaughton, 1993), it should be noted that the present study placed additional constraint on unambiguous isolation over the extended recording session and restricted sampling to cells active during RUN periods, which typically make up ~30% of available cells. No other selection bias that might have influenced temporal sequence expression or detection was used to identify cells.

Template correlation analysis

For a given REM template, the spike count data recorded simultaneously from C cells can be transformed into a $C \times N$ matrix, where N is the length of the window in time bins (1 s). Ensemble pattern correspondence can then be evaluated by examining the overlap between the $C \times N$ REM matrix and a corresponding $C \times N$ matrix of RUN activity.

To create the REM template array, the REM episode spike train of each unit was binned into 1 s intervals and the resultant vector was smoothed (convolution with Gaussian, $\sigma = 1.5$ s). The multicell collection of binned and smoothed spike train vectors defined the REM firing rate array. For a given timepoint t in RUN, the analogous RUN array was constructed over a temporal window centered on that timepoint; each unit spike train was binned into the same number of intervals as the REM array and the vectors were Gaussian smoothed. The template correlation coefficient (C_t) is defined as:

$$C_t = \frac{\frac{1}{N \cdot C} \sum_{c=1}^{C} \sum_{n=1}^{N} \left(\frac{x_{nc}}{X_c} - \bar{x} \right) \left(\frac{y_{nc}}{Y_c} - \bar{y} \right)}{\sigma_x \sigma_y}$$

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where: $x_{1c}, x_{2c}, ..., x_{Nc}$ and $y_{1c}, y_{2c}, ..., y_{Nc}$ are the binned smoothed spike counts for cell c in the REM and RUN windows, respectively, N is the number of bins, and C is the number of cells. Each cell vector is normalized by its respective dimensionless root mean square amplitude X_c or Y_c, $\bar{x}$ and $\bar{y}$ are the mean bin values in the REM and RUN arrays, and σ_x and σ_y are the REM and RUN standard deviations of the normalized binned spike counts across all cells, where:

$$X_c = \sqrt{\frac{1}{N}\sum_{n=1}^{N} x_{nc}^2} \cdot spikes^{-1}$$

$$\bar{x} = \frac{1}{N \cdot C}\sum_{c=1}^{C}\sum_{n=1}^{N} x_{nc}$$

$$\sigma_x = \sqrt{\frac{1}{N \cdot C}\sum_{c=1}^{C}\sum_{n=1}^{N}(\frac{x_{nc}}{X_c} - \bar{x})^2}$$

Note that C_t defined in this manner ranges between -1 and 1.

A high C_t value at time t indicates that the REM template strongly matches an equivalent RUN window centered at time t. C_t was then evaluated between the REM template and multiple RUN windows across the behavioral session in a sliding window fashion (step size 1 s). To account for temporal expansion or compression of replayed activity in REM, the fixed length REM template was compared to varying length RUN windows at each timepoint. The size of the RUN window was determined as the length of the REM episode divided by a temporal scaling factor (SF), which ranged from 0.3 to 3.0. Defined in this manner, SF values > 1 denote temporal expansion of activity and slower replay during REM as compared to RUN; SF values < 1 denote temporal compression and faster replay. Thus, template correlation analysis produces a two-dimensional map of RUN activity representing the strength of a given REM template's correlation to RUN at any given timepoint and scaling factor.

Theta rhythm modulation analysis

To quantify the time-varying strength of theta frequency oscillation, REM and RUN theta power traces were computed at a 1 s resolution as described above from the LFP signal that exhibited the strongest theta oscillation. REM episode theta power traces were individually aligned with corresponding RUN traces, as dictated by the temporal alignment and scaling values indicated by

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the template-correlation identified peak epoch-averaged z score. Peaks and troughs were identified in the RUN theta power trace, and REM theta power values that aligned with either peaks and troughs with a ±1.5 s accuracy were identified and separated into two groups designated "H" and "L" based on RUN alignment. H REM theta values aligned with peaks in the RUN theta power trace, while L REM theta aligned with troughs. Thus, a mean H and mean L theta power value was calculated for each REM episode. To allow comparison across REM episodes, REM theta power values were normalized by the mean theta power in each individual REM episode.

3.6   Figures

Figure 3.1   Behavioral task and hippocampal unit activity

(A)   Schematic of the four-trial sequence in the circular track task.

A single trial consisted of travel from the start location to a removable food well placed at the goal location, followed by food consumption; in any given trial the goal was located at a position 270° clockwise from the start. After completion of a trial the goal location became the start location for the subsequent trial. After four trials the animal is at its original starting location and the sequence begins again.   A recording session consisted of a sleep epoch (conducted in a separate sleep enclosure), a behavioral epoch (RUN) of 40 trials and a subsequent sleep epoch.

(B)   Spatial firing characteristics of three example CA1 cells.

Spiking activity was normalized by positional occupancy at each location to produce a firing rate map. Each column represents activity grouped by behavioral trial type.

(C)   Periodic repetition of characteristic ensemble spiking pattern.

Top, ensemble activity over a representative 5 min window of RUN. Each vertical tick mark represents a single action potential. Note the regular repetition of the spatiotemporal pattern that corresponds to single pass through the four-trial sequence. Bottom, expanded segment of RUN epoch ensemble activity. Horizontal bars represent the timecourse of the four different trial types; black bars denotes portion of trial animal is traversing from start to goal location.
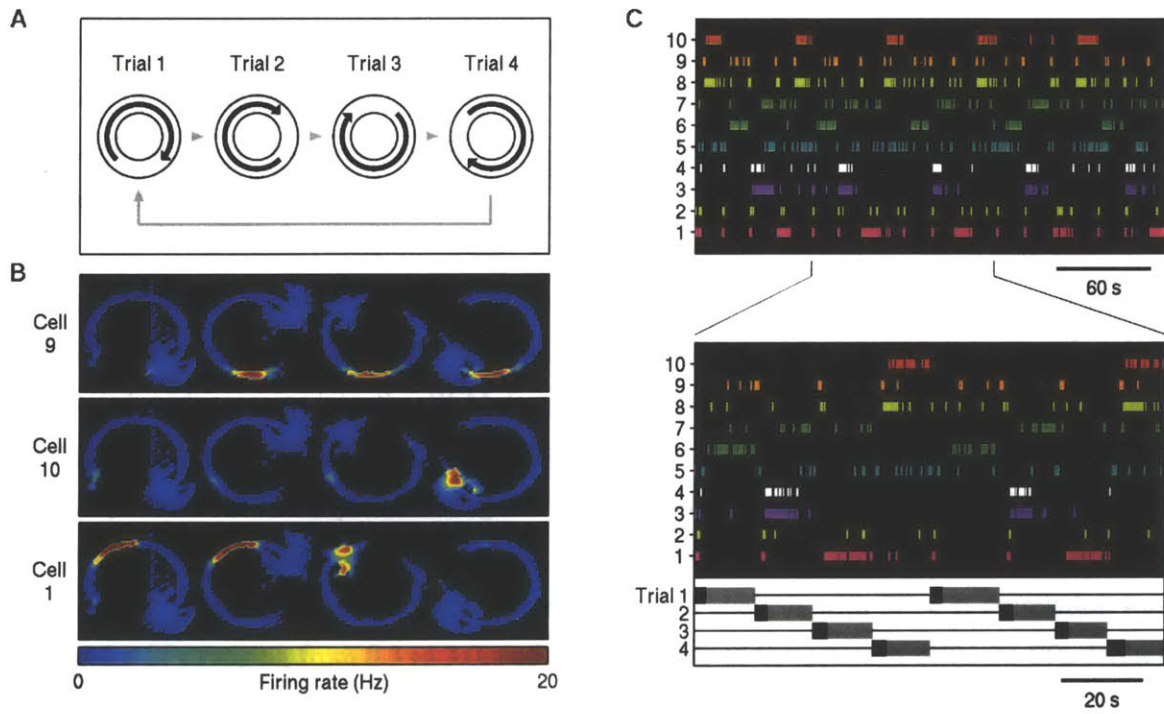
Figure 3.1

Figure 3.2 Identification of REM sleep templates for correlation analysis

(A) Experimental design.

REM episodes identified by increases in LFP theta power are used as templates in independent searches across the RUN epoch. The template correlation coefficient (C_t) is calculated between the template and multiple RUN windows in a sliding window fashion. The width of the RUN window is defined by the scaling factor (SF); SF = 1 corresponds to equivalent REM and RUN window lengths, while SF > 1 corresponds to relatively smaller RUN windows (i.e., slower REM activity). Scale bar, 4 min.

(B) Schematic of sliding window correlation analysis.

For each timepoint t_i in the RUN epoch, a window of RUN ensemble activity centered at that time is extracted and compared to the REM template activity. The result is a correlation vector encompassing the entire RUN epoch and signifying the strength of correspondence between the REM template and different points during RUN. Note that temporal scaling is introduced into the correlation by varying the width of the RUN window taken around each timepoint ($width_{RUN}$ = $width_{REM}$/SF). The correlation depicted here represents C_t analysis with SF = 1; correlation was repeated for SFs ranging from 0.3 to 3.0, and the collection of C_t vectors at different SFs defines the C_t matrix.

Figure 3.2

A

Sleep 1   Run   Sleep 2

θ power

rasters

REM episode

$C_t(t,SF)$

⊗

RUN window @ time = t

width = REM duration

width = (REM duration)/SF

B

RUN

t_i

$[\,\cdots\, C_t(t_i,1)\, \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\,]$
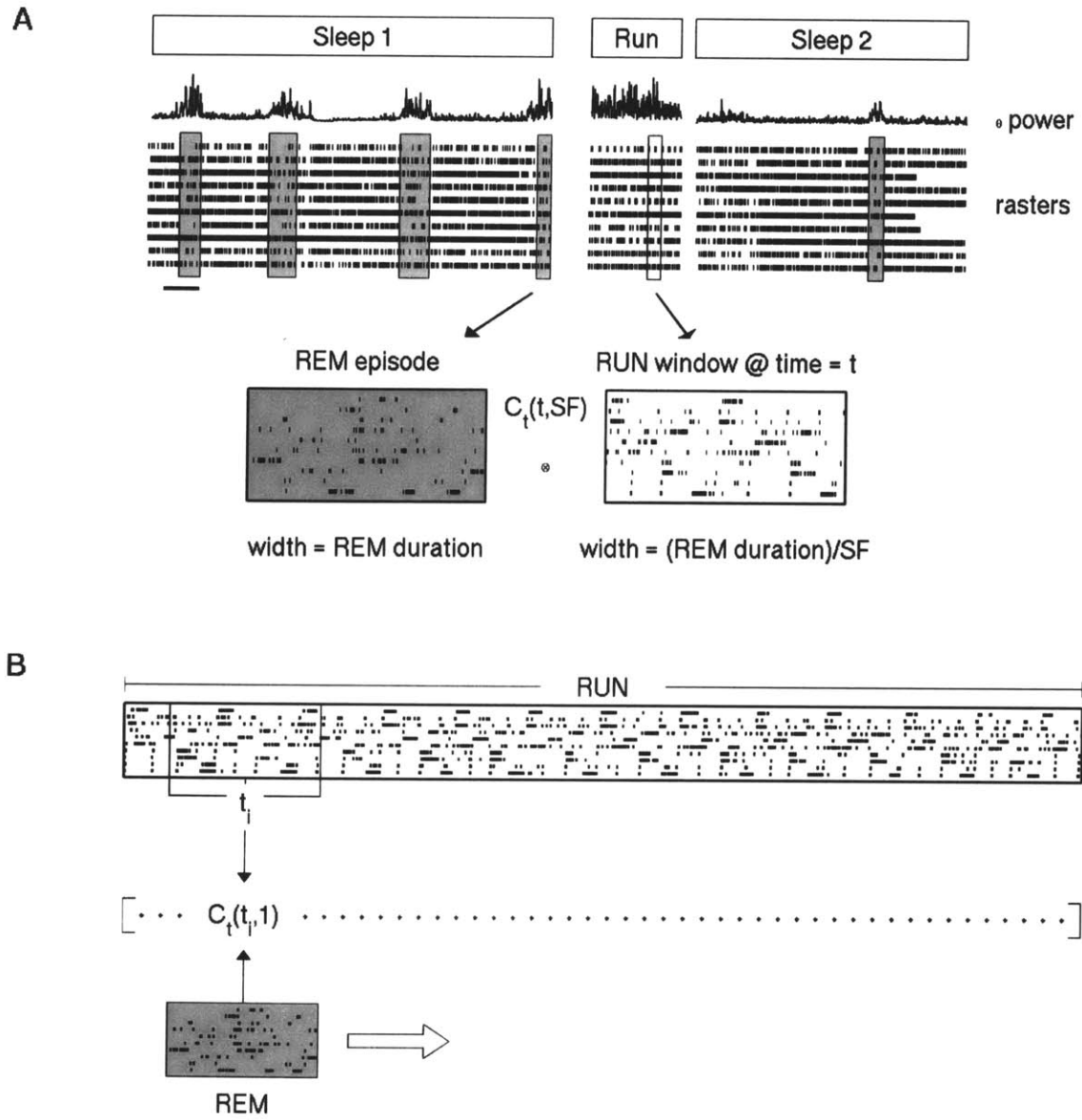
REM

Figure 3.3 Example correspondence between a REM template and RUN activity

Top, rasters of 10 pyramidal cells during a 75 s window from RUN. The RUN time axis is scaled to maximize raster alignment with REM (SF = 1.6). Bottom, rasters of the same cells over the duration of a 120 s REM template.
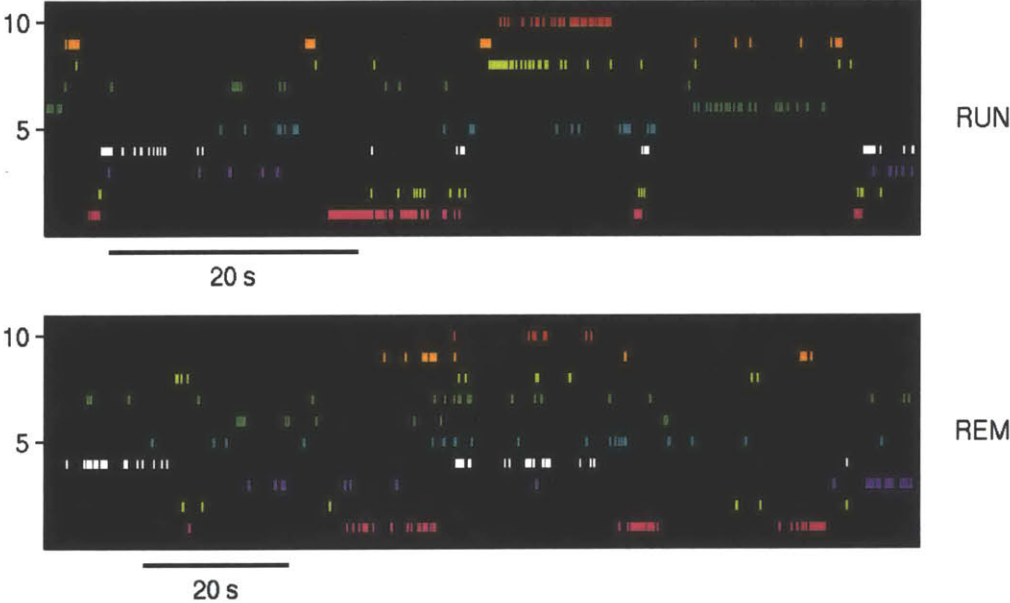
Figure 3.3

Figure 3.4 Ensemble pattern shuffle analyses

(A) BIN shuffle.

All shuffles performed on binned ensemble spike train data, represented here as a two-dimensional matrix. In the BIN shuffle, bins are pseudorandomly exchanged within each cell spike train vector, with shuffling performed independently on each spike train.

(B) COLUMN shuffle.

Similar to BIN shuffle, except temporal alignment of spike train data is preserved across cells.

(C) SWAP shuffle.

Entire spike train vectors are pseudorandomly reassigned between cells. Note that the temporal order of spike activity within each spike train is preserved.

(D) SHIFT shuffle.

Entire spike train vectors are temporally shifted relative to original alignment, with relative temporal order preserved within each spike train. The shift distance is pseudorandomly chosen and ranges between half the window length backward and half the window length forward. The shift is circular, such that data removed from the pattern at one end is reinserted at the opposite end.
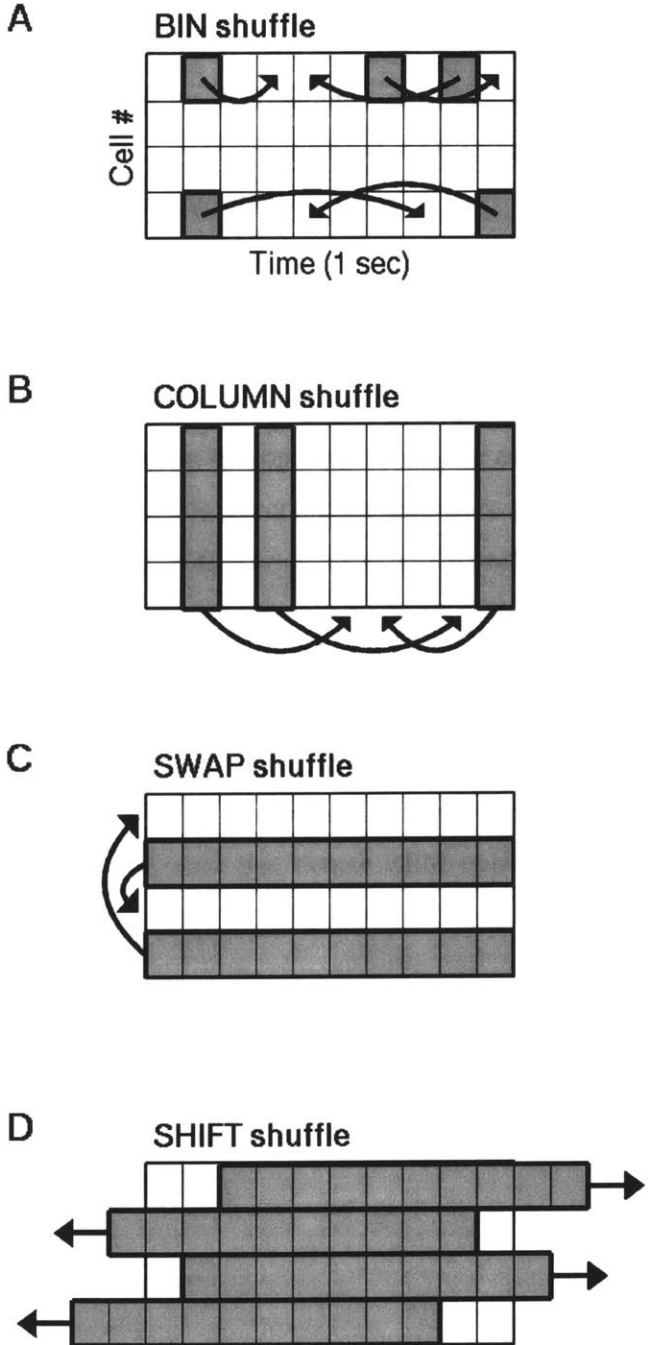
Figure 3.4

Figure 3.5 Template correlation analysis of RUN-REM correspondence.

(A) Example correlation z score analysis.

False-color image represents correlation z score data between one REM episode (Animal 3 REM 4) and the entire RUN epoch. The C_t value at each (t,SF) point during RUN is converted to four z scores relative to the shuffled-template distributions; significance of the template correlation coefficient at each point is designated by the minimum z score. The repetition of behavioral trials during RUN are represented in the timeline at top.

(B) Behavioral epoch analysis of two example REM episodes.

Each left hand panel plots the minimum z score across a single repeat of the 4-trial behavioral sequence, calculated at temporal scaling factors from 0.5 to 2.5; plots have been normalized along the time axis within each epoch. Data in the top row is from the analysis shown in 4A. Only the first six behavioral epochs are shown. The general correspondence of a REM episode to RUN was evaluated by averaging minimum significance values across repeated behavioral segments. The result is an epoch-averaged z score function for each REM episode, as displayed in the right most panels.

(C) Distribution of peak epoch-averaged z scores for all REM templates.

Black portion of bars, prebehavior REM episodes; white portion of bars, postbehavior REM episodes. Bars to the right of the dashed line denote REM episodes with significant template correlation (p<0.05).

Figure 3.5

A

RUN

Trial 1
2
3
4

SF

Time(min)

z score

B

Animal 3 REM 4

SF

1 2 3 4 5 6

AVG

epoch avg z

Animal 2 REM 3

SF

1 2 3 4 5 6

AVG

epoch avg z

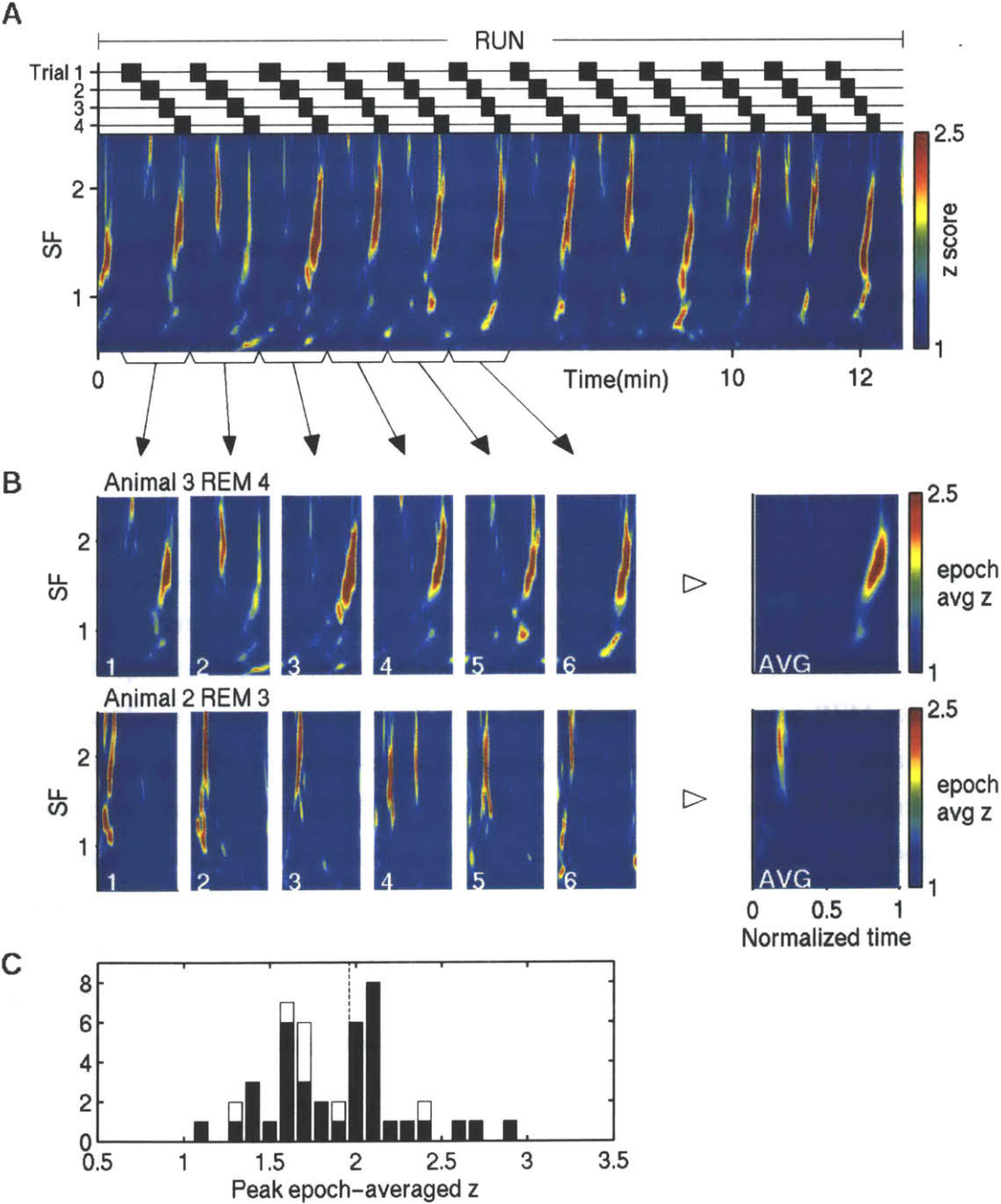Normalized time

C

Peak epoch–averaged z

Figure 3.6 REM correspondence to novel versus familiar RUN epochs

(A) Schematic of recording session timecourse.

Black bars, familiar RUN epochs. White bar, novel RUN* epochs. Gray bars, REM episodes. Recording sessions are separated by approximately 24 hrs, as indicated by the diagonal lines. Note that REM episodes occurring before a familiar RUN epoch on any given day actually follow the previous day's RUN epoch. To investigate the experience-dependence of prebehavior REM correspondence to familiar RUN behaviors, ensemble activity from REM episodes were also compared to neural patterns recorded during novel behaviors (RUN*). The extent of this novel RUN*-REM correspondence (red arrow) can be compared to familiar RUN-REM correspondence, both for the same REM episodes used in the novel analysis (blue arrow) and for all other prebehavior REM episodes (black arrow).

(B) Distributions of epoch-averaged correlation z scores from REM episodes recorded before novel RUN* versus familiar RUN behaviors.

Scores greater than the dashed vertical line indicate a significant correlation ($p<0.05$). Top, correlation scores between REM episodes and familiar RUN epochs. White portion of bars, all prebehavior REM episodes during novel condition; blue portion of bars, prebehavior REM episodes during familiar and novel condition. Bottom, correlation scores between REM episodes and novel RUN* epochs. (C) Cumulative distributions of correlation significance scores. Red line, significance of correlation to novel RUN* behaviors. Blue line, significance of correlation to familiar RUN behaviors, same REM episodes as novel data. Black line, significance of correlation to familiar RUN behaviors, all prebehavior REM episodes. The distribution of correspondence to novel RUN* behaviors differs significantly from the distributions of correspondence to familiar RUN behaviors, both for the subset of REM episodes tested against novel behaviors as well as for all REM episodes ($p<0.00005$, Kolmogorov-Smirnov).
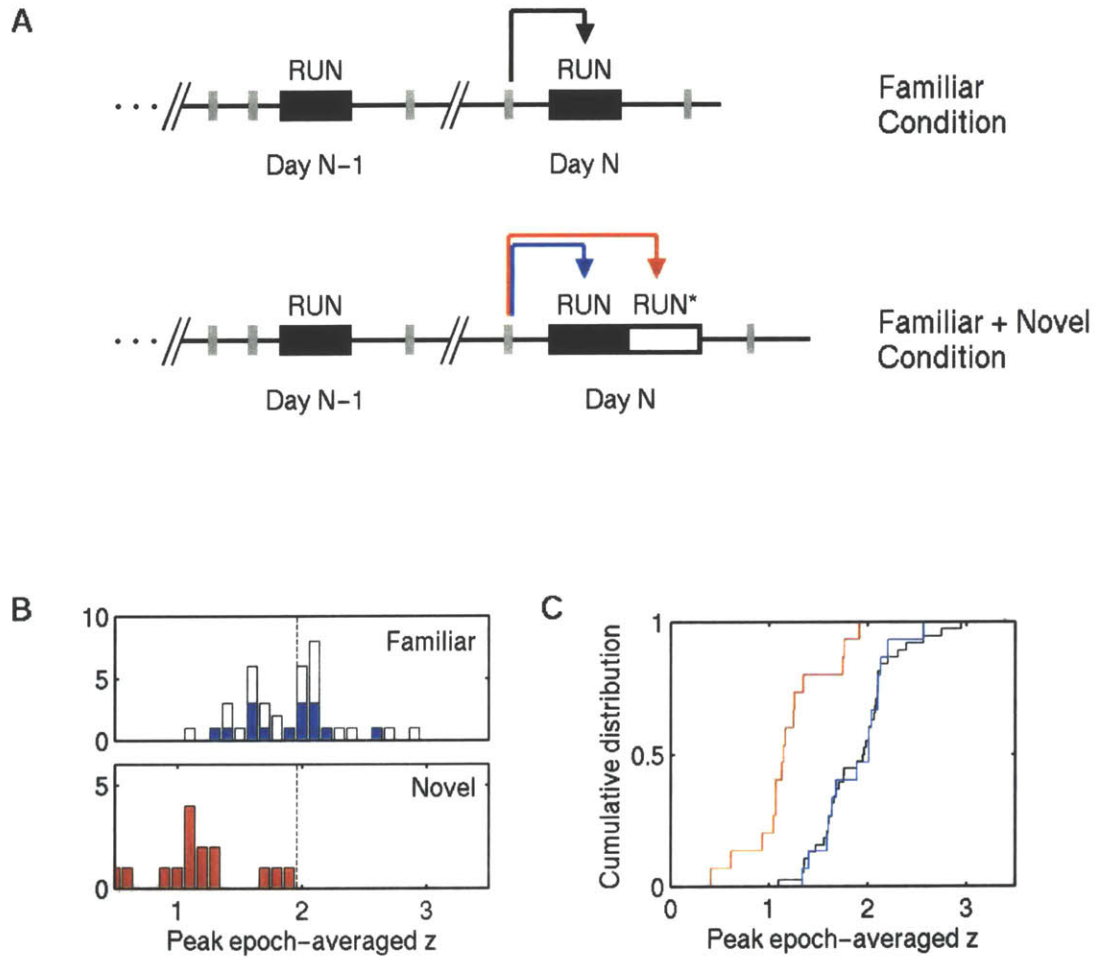
Figure 3.6

Figure 3.7 REM-RUN correspondence in theta rhythm modulation

(A) Broad patterns of modulation in the theta rhythm.

Top trace, LFP theta frequency power during the 80 s RUN window displayed in Figure 3.3, with theta power evaluated as the squared amplitude if the 6-10 Hz bandpass filtered LFP signal. Line above the RUN trace denotes the starting and ending points if individual behavioral trials; note the regular phasic modulation RUN theta power by behavior. Bottom trace, LFP theta frequency power during the 120 s REM episode displayed in Figure 3.3. Theta power traces are aligned and scaled based on template correlation analysis, i.e., at the time and scaling factor corresponding to maximal ensemble pattern correlation derived from the unit rasters (maximum C_t).
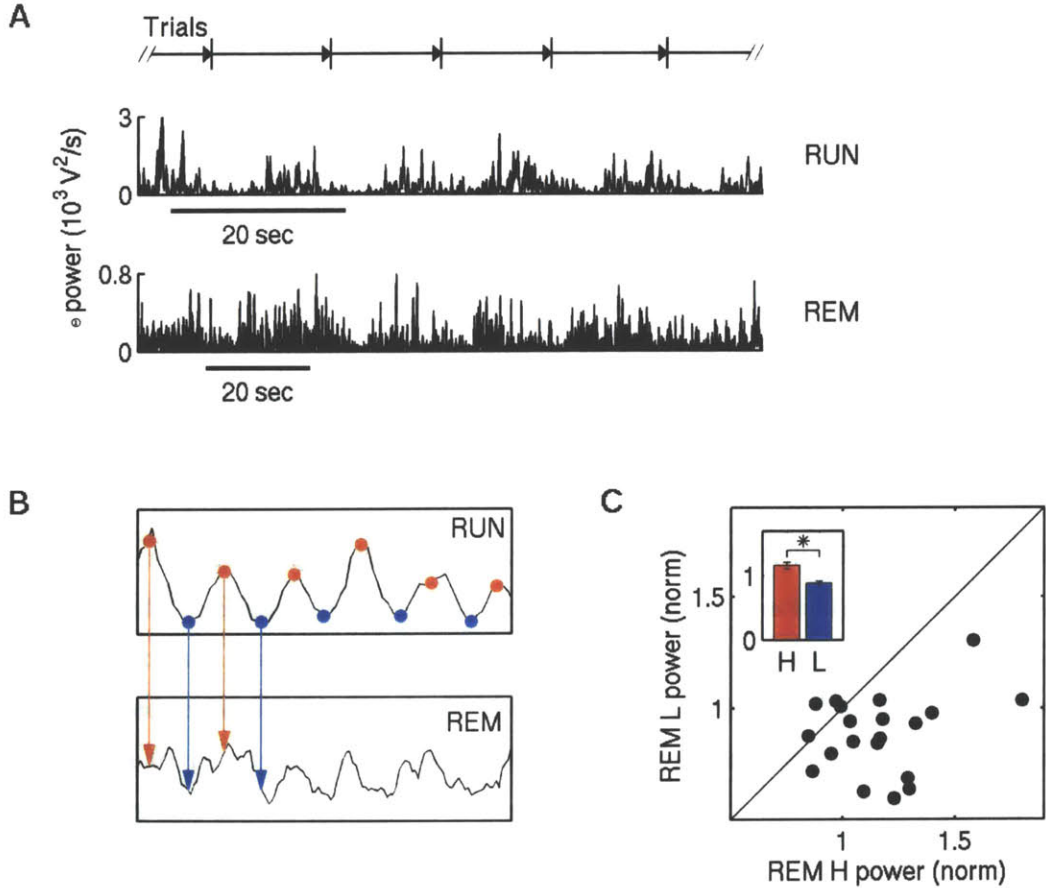
(B) Evaluation of REM-RUN theta power correspondence.

REM and RUN theta power data are binned at 1 s intervals and aligned and scaled to the optimal values derived from template correlation analysis (Figure 3.4). REM theta power values that aligned to either peaks or troughs in the RUN theta trace (red and blue dots, respectively) were identified and grouped according to their alignment with RUN (H, REM theta values that aligned with RUN theta peaks; L, REM theta values that aligned with RUN theta troughs). Example H and L values depicted by red and blue arrows, respectively.

(C) REM-RUN theta power correspondence in all REM episodes with significant ensemble correspondence.

Each point plots the H theta power versus the L theta power for a single REM episode; values are normalized within REM episodes by the mean theta power amplitude for comparison across episodes. Under the null hypothesis of independence between REM and RUN theta power modulation, there should be no difference between H and L REM theta values calculated from template correlation-derived alignment to RUN data. Inset, mean H and L theta power ($\pm$ SEM) across all REM episodes. There is a significant difference between REM H and L values ($p <$ 0.0005, paired t test), suggesting that aspects of theta oscillation modulation generated during the awake behavioral task are represented during REM sleep.

Figure 3.7

A

Trials

⊖ power (10^3 V^2/s)

3

0

20 sec

RUN

0.8

0

20 sec

REM

B

RUN

REM

C

REM L power (norm)

1.5

1

*

H L

1

1.5

REM H power (norm)

4 Conclusion

Summary of results

Three primary results were found in the first experiment, examining the integration of spatial and nonspatial information in the hippocampal representation of space: 1) Despite primarily spatial activity in individual place cells, the distributed representation of space differentially encodes previously reinforced and nonreinforced locations. 2) This bias can be measured as a modulation in population spiking activity, with increased firing rate at previously reinforced sites. However, it is mediated by a shift in individual place fields centers towards these locations, leading to a clustering of place fields at these locations. 3) There is evidence that inhibitory interneurons are also modulated by behavioral saliency, but demonstrate an inverted bias compared to pyramidal neurons; inhomogenity in interneuron activity both addresses possible mechanisms for biased representation and implicates a broad involvement of the hippocampal network.

The second experiment, examining sleep reactivation of structured, behaviorally-correlated wake activity, demonstrated: 1) Spatiotemporal neural patterns, reflecting the activity of many neurons recorded in parallel across a given window in time, are reactivated during select REM episodes. 2) Unlike the compressed replay seen during the SPWs of slow-wave sleep, REM reactivation occurs at a similar timescale to the original pattern, or slightly slower. 3) This reactivation is experience-dependent and occurs only after establishment of the pattern during behavior. 4) Broad patterns of theta-rhythmic modulation are also re-expressed during REM episodes, in alignment and scale with pyramidal cell activity, suggesting a widespread mnemonic reactivation of cortical state.

Goal-related information

The clustering of place fields at behaviorally significant locations, shown here for food reinforcement and previously reported for platform locations in a watermaze (Hollup et al., 2001), indicates that the representation of space is both dynamic and subjective. The robustness of the spatial code in rodents suggests a possible role in spatial behaviors such as navigation, but the place code by itself merely specifies instantaneous location. One possible solution is the learning of sequences of locations, driven by the temporally-asymmetric nature of long-term potentiation (Blum and Abbott, 1996; Levy, 1996; Gerstner and Abbott, 1997; Mehta et al., 2000). Most of

these models view sequence learning as a consequence of the temporal dynamics of synaptic plasticity, but the regulation of these plasticity processes may be influenced by the behavioral demands of the animal. For example the presence of distinct goals, such as locations of food reward or escape platforms in watermazes, has been postulated to drive navigational behavior via reinforcement-learning algorithms (Sutton and Barto, 1998; Foster et al., 2000).

Though we could not directly assess the dynamics of place field redistribution, the perceived shift in spatial fields towards reinforcement locations is reminiscent of the gradual shift in dopaminergic activity from US to CS in classical conditioning experiments (Schultz et al., 1997). Furthermore, these mesocorticolimbic dopaminergic projections mediate appetitive reward-related activity, possibly via a reward-error signal. There is only a weak dopaminergic projection to the hippocampus, but the effects of dopaminergic neurotransmission may be amplified through interaction with cholinergic septohippocampal projections (Day and Fibiger, 1994; Inglis et al., 1994) or other neuromodulatory systems.

Behavioral salience

The valence of goal designation may depend on multiple processes, among them reward, emotion, arousal, and attention. The biased distribution of place fields in both a food reinforcement paradigm and an escape task (Moser and Paulsen, 2001) raises the possibility that these different rewards tap into the same reward system, or that there is a more general saliency system affecting hippocampal activity. In light of the sensitivity of place fields to the behavioral task being performed (Markus et al., 1995) and their relation to multiple significant reference frames in a landmark paradigm (Gothard et al., 1996), place field activity appears responsive to some general aspect of behavioral significance. Nonspatial hippocampal activity also associates with behaviorally-relevant events, shifting from unconditioned stimulus to conditioned stimulus over the course of learning in trace eyeblink conditioning studies. The bias in hippocampal activity towards reinforcement locations reported here not only demonstrates the influence of behavioral salience on hippocampal activity but also shows that this information is part of the encoded memory, stored for subsequent reactivation and reference. In fact, the strong structured reactivation observed during REM sleep may be related to such saliency processing. Consistent with the characteristic prevalence of emotional thoughts in human dreaming, REM sleep is linked to the enhancement of emotional memory (Wagner et al., 2001) and may exhibit selective processing of behaviorally important experiences.

Concluding remarks

From the influence of previous reinforcement on the current representation of the environment, seen as a shift in place field locations towards reinforced locations in the first experiment, it is clear that mnemonic activity is affected by the nature of past experience and does not represent a simple objective translation of the physical environment. The hippocampus is able to integrate nonspatial and spatial information, even within a spatial representation, suggesting a broader memory function in rodents than one exclusively devoted to spatial processing. The encoding of spatial and nonspatial information is distributed, emerging in the place field distribution and mean firing rate of the population of place cells; the participation of putative inhibitory interneurons, displaying an inverted reinforcement bias, indicates a broad coding of experience, spatial and otherwise, at the network level. The representation of information at this broad level is reinforced by the reactivation of these temporally structured patterns of behaviorally-established activity during sleep. The existence of structured neural reactivation is consistent with evidence that sleep contains crucial mnemonic processes, and provides an avenue to explore the mechanisms behind putative memory consolidation. Finally, the detection of structured REM sleep reactivation demonstrates that distributed network representations of both spatial and temporal details of experience can be re-expressed within the brain, a process which is the hallmark of memory.

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