HIPPOCAMPAL PLACE FIELDS REQUIRE DIRECT EXPERIENCE

by

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In humans and other mammals the hippocampus is critical for episodic memories, or memories of events that happen in a particular place and at a particular time. When one records from hippocampal pyramidal neurons in awake, behaving rodents, however, the most obvious firing correlate of these neurons is the animal's position within the environment, earning them the name "place cells". Their aggregate activity is thought to provide the animal with a “cognitive map”: a map-like neural representation of the external world used to solve spatial problems. Since rats’ ability to take shortcuts through novel space was the major evidence leading Edward Tolman to propose the concept of a cognitive map, it follows that place cells should exist for parts of the environment that the animal has not directly-experienced. We therefore compared the relative stability of place cells recorded from rats in observed versus directly explored parts of an environment in response to a pharmacological manipulation that preferentially destabilizes newly-generated place fields. In contrast to the classical cognitive map hypothesis, the formation of stable place fields clearly requires direct experience with a space, suggesting place cells are part of an autobiographical record of events and their spatial context rather than a map-like representation of space automatically calculated from observed environmental geometry.

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To Ken and Susan Rowland, My Parents

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INTRODUCTION

Patients like patient H.M with damage to the hippocampal formation exhibit an anterograde amensia for events along with when and where those events occur. This form of memory has alternatively been called "declarative," "explicit," and "episodic." The deficit, first described by Scoville and Milner in 1957, is perhaps the most striking ever reported in the neuropsychological literature; the patients remain able to learn new skills and facts, have no obvious psychoses, do not exhibit any clear perceptual deficits, and have little retrograde amnesia (Scoville and Milner, 1957). Perhaps most surprising of all, such a profoundly dehumanizing deficit was revealed by removing a single structure in a relatively old part of the brain. The hippocampus is a primitive form of three-layered cortex called the allocortex, and the rhinal cortices, the major inputs to the hippocampus, are periallocortex. Indeed, prior to Scoville and Milner's pioneering work, the hippocampus was largely thought to participate in "fight or flight" decisions. This makes rodents, who have a comparatively greater proportion of these allocortical structures than humans, a highly attractive model organism for studying episodic memory.

The 1970's produced two additional groundbreaking discoveries in the hippocampus. In 1973, Bliss and Lomo discovered long-term potentiation (LTP) at dentate gyrus (DG) synapses (Bliss and Lomo, 1973). In their experimental design, the inputs to DG neurons were stimulated 100 times per second for 3-4 seconds. The

efficacy of a single stimulus in driving DG cells was then compared before and after the stimulus train. They found that a stimulus produced a greater response in DG after delivery of the stimulus train than controls, suggesting that the synapses had strengthened after repeated stimulation. Such synaptic modification resembled the plastic changes that Donald Hebb (1949) believed formed the cellular basis of learning: " Let us assume that the persistence or repetition of a reverberatory activity (or "trace") tends to induce lasting cellular changes that add to its stability.…When an axon of cell *A* is near enough to excite a cell *B* and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that *A*'s efficiency, as one of the cells firing *B*, is increased." In 1971, when John O'Keefe recorded the firing patterns of hippocampal neurons in awake, behaving rats, he found that by far the most obvious firing correlate was the animal's position in space, he therefore called the cells "place cells" and the area circumscribed by their firing the cell's "place field" (O'Keefe and Dostrovsky, 1971; O'Keefe, 1976). He and Lynn Nadel later hypothesized that hippocampal place cells provided a "cognitive map" of the animal's current environment (O'Keefe and Nadel, 1978). They borrowed the term "cognitive map" from Edward Tolman. Tolman used the term to describe a hypothetical comprehensive mental map of the environment used to solve spatial problems (Tolman, 1948). The key experimental evidence favoring his hypothesis was the ability of rats to take shortcuts through novel space. O'Keefe and Nadel (1978) likened the map of space to Kant's theory that the sense of space develops prior to experience. The map is *a priori*. Bliss and Lomo's (1973) discovery identified a potential mechanism for storing memories in hippocampal neurons, while O'Keefe's revealed some of their content.

These three discoveries, occupying distinct levels of analysis, frame the current debate over hippocampal memories and the mechanisms underlying them. However, a straight-forward correspondence between the different levels has been elusive, in part due to incompatibilities between some of the major theories generated at each level. The work that I did for my dissertation resolves one such incompatibility, and clarifies the role of plasticity in constructing hippocampal representations. To illustrate the central issue, consider attending a concert in an unfamiliar auditorium. You take your seat before the lights go down, so with a few glances you have created an internal representation of the entire auditorium, including your rough location within it relative to salient landmarks (e.g., the stage, balconies, exits). You could easily generate a number of distinct novel routes to these other locations, with your eyes closed if need be, and you could draw or otherwise describe a conception of that space. In other words, you have a reasonably comprehensive cognitive map of your environment without visiting more than a small portion of it. Your episodic memories of the concert would be quite different, however. They could involve particular pieces of music, perhaps, or conversations with people at the concert, or whatever else happened that was memorable, entirely from a *first-person* perspective, rooted in the physical space you actually occupy at the time. The experiments I describe are rooted in this fundamental difference, and demonstrate that the formation of place fields is strictly a “first-person” process.

Such a first-person record of events resembles what Richard Morris and colleagues have called the "automatic recording of attended experience" (Morris and Frey, 1997). His definition of "attended experience," like William James's (1890) initial definition of "attention," was subjective; the hippocampus stores behaviorally relevant

information in real time as the subject experiences them. Following Morris's idea, we have suggested that neuromodulators, such as dopamine, enable the preferential storage of behaviorally salient information by affecting synaptic weights in the hippocampus in a review and a book chapter. However, one of the major gaps in this argument was the lack of data regarding whether the formation of hippocampal place fields is in fact subjective. The sections below review data showing that hippocampal place cells are far more plastic than initially appreciated. These studies suggest that plasticity and attention are critical components of forming, maintaining and manipulating hippocampal representations, while my study shows that place fields are developed only with direct experience. Thus, rather than being a map of space that is geocentric and comprehensive, as O'Keefe and Nadel (1978) predicted, hippocampal representations are at least initially egocentric, dynamic and preferentially weighted by attention. These properties resemble Tulving's 1972 definition of an episodic memory system: "[episodic memory] receives and stores information about temporally-dated episodes or events, and temporal-spatial relations among those events." The final section of this Introduction deals with the models of place cells, and their predictions for how or if place cells represent unexplored space. Thus, another important consequence of my work is that it places boundary conditions on previous and future models of the formation of place fields.

Plasticity of Hippocampal Representations

When John O'Keefe and colleagues began recording from hippocampal pyramidal neurons in awake behaving rats (O'Keefe and Dostrovsky, 1971), he found cells fired in response to the animal's position in space (though in these early days both he and Jim

Ranck (1973) found a number of other correlates as well.) This led to O'Keefe to call these cells "place cells." The area circumscribed by their firing he called the "place field" of that cell, by analogy to receptive fields in other structures (O'Keefe, 1976). The initial discovery of place cells was followed by O'Keefe and Nadel's publication of the book "The Hippocampus as a Cognitive Map," (1978) which proposed that the hippocampus provided a map-like representation of space akin to Tolman's cognitive map hypothesis of the late 1940's. The hypothesis is not based only on the place cell phenomenon, but is also supported by the pronounced deficits exhibited by animals with hippocampal damage in spatial tasks, most notably the Morris water maze (Morris et al., 1982).

Since the discovery of place cells in the hippocampus, three additional cell types have been discovered in the parahippocampal region (entorhinal cortex and subicular complex) that complement place cells: grid cells, head direction cells and border cells. Grid cells fire when the animal occupies the vertex of a hexagonal grid (Hafting et al., 2005), head direction cells fire when the animal's head is pointed in a particular direction (Taube et al., 1990), border cells fire when the animal is near an environmental boundary (Solstad et al., 2008). The discovery of these additional cell types in the structures that surround and project to the hippocampus bolster the claim that the place correlate of hippocampal firing is not simply an epiphenomenon.

The presence of place cells suggests a spatial memory system, suitable for the "where" component of episodic memory (Kentros, 2006), but is equally consistent with a static representation of the animal's current geometric environment. Indeed, O'Keefe and Nadel's book equated the hippocampal representation of space with a Kantian, *a priori*, map that exists without direct experience. Such an automatic, hard-wired calculation

would be inconsistent with a role for attention and plasticity in forming and maintaining a spatial representation, thus we first review evidence that the map of space is in fact plastic and "soft-wired."

Remapping in the Hippocampus

The anatomical arrangement of the hippocampus is unique. Neurons of the sensory cortex are destined to respond to a limited aspect of the sensory environment because features (e.g., frequency, position) are parsed initially at the receptor level, kept separate until the information reaches the sensory cortex, and once in the sensory cortex the segregation is maintained by the largely local connectivity of neocortical neurons. In contrast, hippocampal neurons exhibit long-range, global connectivity with little or no bias for local neighbors (Li et al., 1994). Moreover, neurons in the hippocampus (particularly in the dentate gyrus) outnumber their inputs from the entorhinal cortex by several orders of magnitude, suggesting a redistribution over a vast network of neurons (Marr, 1971). These two facts together suggest that the hippocampus creates a large memory space similar to the random access memory (RAM) found in computers.

The functional consequence of this anatomical arrangement is easily seen in the firing of place cells. In any given environment, only about 40% of CA1 neurons will be "on" with the remainder firing very few spikes (Guzowski et al., 2004; Lee et al., 2004; Leutgeb et al., 2004). When the animal is placed in a second environment, 40% of the neurons will again be active, but the subsets of active cells are statistically independent of one another. If a cell happens to be active in both environments, then the place fields bear no resemblance to one another (i.e., a cell that has a field against a wall in one

environment might have a cell in the center of the second(Redish et al., 2001). This process is called "complete remapping" (Leutgeb et al., 2005). However, the rule is not absolute; if the environments share some common features (e.g., identical cues but unique environmental geometry) then some cells may remap to the second environment while others may exhibit some plasticity but do not remap (Bostock et al., 1991). This process is called "partial remapping."

These data suggest that the firing of place fields is not hard-coded in the way that receptive fields of neocortical neurons are. However, these data also do not preclude the possibility that a new "chart" is randomly selected every time the animal is placed into a novel environment (Samsonovich and McNaughton, 1997). In this conception, a unique map of space is simply selected, not constructed with experience.

Place Fields Are Learned

The hippocampal representation of space in rats is not immediately present in novel environments, but develops over 4-6 minutes of experience in the space (Wilson and McNaughton, 1993; Frank et al., 2004). This plasticity takes a variety of forms; some cells that were completely silent over the first few passes through the field suddenly begin firing, other cells begin firing robustly only to stop firing, while others fire from the outset but gradually become more tuned to a location. This process is especially pronounced in mice, in which place fields can require multiple familiarization sessions before reaching peak specificity (Cacucci et al., 2007). Thus, the general capacity for forming spatial representations is innate, a claim bolstered by recent evidence that place cells, head direction cells and grid cells are all present from the onset of spatial

experience in developing rat pups (Langston et al., 2010; Wills et al., 2010), but a particular map is constructed with experience.

This phenomenon suggests that the representation is plastic, but what is the relationship between this plasticity and the plasticity observed at the subcellular level in studies of long-term potentiation (LTP)? Two of the most central and robust phenomena relating plasticity and long-term behavioral memory are the apparent requirement of both *N*-methyl-D-aspartate (NMDA) receptor activation and new protein synthesis. At a behavioral level, injecting antagonists against NMDA receptors or protein synthesis inhibitors create anterograde amnesia similar to hippocampal lesions, creating a correlation between LTP and memory (McDonald et al., 2005). What is the effect of these manipulations on place cells? Kentros et al., (1998) showed that injecting animals with antagonists of NMDA receptors does not prevent the expression of a new map, but does prevent the *stability* of the newly-formed map. Similarly, injecting protein synthesis inhibitors prevents the long-term but not short-term stability of place fields (Agnihotri et al., 2004). In other words, the cells remap a second time when re-introduced to the novel environment after a long delay, as if the animal had never seen the environment, mirroring their effects on memory.

These data suggest that forming a spatial map is a learning process, however memory space is a finite resource. Do hippocampal place cells preferentially represent behaviorally relevant stimuli? Is this capacity limited to the spatial domain or can attention to non-spatial stimuli also be selected and preferentially stored? The data described in the next section address these issues.

Attention Works to Stabilize Hippocampal Representations of Behaviorally Relevant Stimuli

A place cell's firing field will almost always faithfully reinstantiate itself again and again on repeated visits to the same environment. The field will often be stable for at least as long as the experimenter is able to track the cell. One study reported the same stable field for 153 days of recording (Thompson and Best, 1990). Somewhat surprisingly, this long-term stability occurs in environments that have little or no significance to the animals; in a typical experiment rats are simply foraging for food that is randomly scattered on the floor (Muller et al., 1987). When Kentros and colleagues first began recording from mice, they expected that wild-type mice would also show stable place fields, and LTP mutants would show impairments. However, even place cells recorded from wild-type mice showed high levels of baseline instability (Kentros et al., 2004). Each new introduction to the environment elicited a partial remapping in mice that were presumably perfectly capable learners. Kentros and colleagues were initially perplexed by this discovery, but decided to train the animals in a spatial task to see if the animals could accurately navigate despite unstable place fields. The task was a land-based version of the Morris water maze in which entry into an unmarked goal zone, placed in a constant position relative to the cues painted on the environment, turned off bright lights and car alarms. Most animals did indeed learn the task, and, surprisingly, their place fields stabilized with training. On the other hand, animals that never learned the task had unstable and impoverished place fields. Mouse place fields, therefore, do not stabilize until the animal has some reason to care about its visual environment. Although this finding was contrary to the place cell literature it nevertheless matches our

expectations of (and personal experiences with) a constrained and finite memory system. This intuition was again elegantly captured by William James (1890): "Millions of items of the outward order are present to my senses which never properly enter into my experience. Why? because they have no interest for me. My experience is what I agree to attend to. Only those items which I notice shape my mind -- without selective interest, experience is an utter chaos."

The data from the Kentros et al., (2004) study suggested that mouse place fields stabilized after the animals selectively attended to the visual cues, bu the results could just as readily, and perhaps more simply, be explained by general arousal. To examine the effects of selective attention to sets of cues, Muzzio and colleagues (2009) performed a follow-up study. They exploited the fact that hippocampal "place" cells can also respond to olfactory cues (Wood et al., 1999), and hypothesized that selective attention to either space or olfactory cues would strengthen the representation of that modality at the expense of the other, in accordance with models of selective attention. To test the hypothesis, mice were trained to dig for a reward that was either specified by the visual cues or by a particular odor. Thus, in one group only the visual information mattered to the animal while the olfactory information was irrelevant, and in a second group the reverse was true. Consistent with the hypothesis, attention preferentially stabilized the representation of the attended cues, at the expense of the unattended cues, whose representation was degraded. These data provide strong evidence for selective attention in stabilizing hippocampal representations.

Dynamic Selection of Information

The above experiments suggest that hippocampal place fields are plastic during new learning and that attention can enhance and stabilize the representation to the relevant cues. This may leave the mistaken impression that the hippocampal representation becomes ossified after learning. In fact, the hippocampus retains the capacity for learning new information. Place cells can even make new maps for the same physical space, and later reactivate that information separately.

Markus et al.,(1995) were the first to recognize that place cells can remap based on an animal's ongoing behavior. In their study, rats were trained to either freely forage for randomly scattered pellets or, when cued, perform a sequential search for food, all within the same physical space. A percentage of cells adopted distinct firing fields as the animal switched between the two tasks. Therefore, place cells do not simply provide a single immutable representation of the animal's physical environment, but rather the firing of place cells depend on the animal's ongoing behavior. In support of this idea, several groups have now shown that place cells can respond to which direction the animal is about to turn in a maze (Frank et al., 2000; Wood et al., 2000). Perhaps most convincingly, when the animal is placed on a slowly rotating arena and required to perform a place preference task, some cells will be bound to the animal's position with respect to the room (the "room frame") and others to the animal's position with respect to the arena (the "arena frame") (Zinyuk et al., 2000). This finding strongly suggests that multiple reference frames can be expressed simultaneously within the hippocampus.

These results suggest that the hippocampus can generate multiple representations for the same physical space, but do not show that the animal can dynamically switch

between the different representations. The key evidence supporting this hypothesis comes from a strange source: the "noise" in place cell firing. Using ensembles of active place cells, researchers have been able to reconstruct the position of the animal to accuracy levels that approach the limit of the tracking system, suggesting that these cells tightly encode the position of the animal. However, if we reverse the question and ask how predictive the animal's position in space is of the firing of the neuron, the answer is far more ambiguous. This observation was first made by Fenton and Muller (1998). They recorded from single place cells and compared the firing of an individual cell on runs through the place field that were similar in direction and speed, then quantified the variability in firing using an overdispersion statistic. Rather than responding reliably, the spike trains were in fact more variable than a Poisson process; they were "excessively variant." Harris and colleagues (2003) found that the prediction improves if one considers the activity of other simultaneously-recorded cells, echoing Hebb's cell assembly hypothesis. Harris and colleagues called the unobserved binding force an internal cognitive process (ICP), but recognized the similarity between an ICP and attention (Harris, 2005). In a series of follow-up studies, two labs have independently observed that the overdisperssion of place cell firing can be reduced if the animal is engaged in a behavioral task, or if the spike trains of simultaneously recorded cells are clustered into two distinct ensembles (Jackson and Redish, 2007; Fenton et al., 2010). The choice of two ensembles is largely practical. Increasing the number of ensembles typically leaves too little data to analyze. However, it is reasonable to believe that a place cell may be responding to both the locally generated self-motion cues as well as the animal's visual environment, and so two ensembles might be fitting.

In an extension of this thinking, Kelemen and Fenton (2010) trained animals in the rotating arena task while recording place cells, as described above. They found that, at a milisecond to second time scale, cells tuned to one frame tended to be active while cells of the other frame were silent and vice versa. It therefore seems that the network vacillates between the two frames, approximately once per second, as if the animal is shifting its attention between the local arena frame and distal room frame. These data are the best to date that the hippocampus can recall some information while specifically suppressing others on a moment-to-moment basis.

Finally, place cells can fire in variety of non-local yet spatially structured ways, often suggesting that the animal is recalling the past or imaging the future. During rest periods in a recording session, for example as the animal grooms or feeds, the predominant theta oscillation in the EEG disappears and is replaced by irregularly spaced ripple oscillations (Buzsaki et al., 1992). Ripple oscillations bring with them a burst of activity in both pyramidal cells and interneurons. Unlike the place cell phenomenon, this burst of activity has no obvious relationship with the animal's behavior in the past, present, or future, and seems at first like unstructured noise. However, the burst of activity is far from random. Ripple events can: replay sequences of place cells in a forward or reverse order (Foster and Wilson, 2007), reactivate place cells that represent previously experienced environments (Karlsson and Frank, 2009), or even preplay paths through space that the animal hasn't taken (Gupta et al., 2010). In a related and equally fascinating study, Johnson and Redish (2007) showed that when an animal pauses near choice points in a maze, but does not leave the theta state, the place cells representing the path from the animal to upcoming destinations in the maze become active in a sequential

sweep. A sweep proceeds separately for each of the upcoming arms of the maze and the animal makes its choice only after all sweeps are complete, as if the animal is evaluating potential outcomes before committing.

Anatomy

The electrophysiological evidence described above suggests that the hippocampal representations are plastic, respond to be geometric and non-geometric information, and preferentially represent attended stimuli. In this section I consider how such information is routed to the hippocampus from an anatomical perspective, with a particular focus on the neuromodulatory influences from subcortical structures, and connectivity with the medial prefontal cortex (mPFC) and the anterior cingulate cortex (ACC). Several connections and potentially important details are left out for clarity. The majority of the cited literature concerns rats, though the connections are very similar in other mammals. I have adopted the nomenclature of Amaral and Lavenex (2007) for this section. They define the hippocampus as CA3, CA2, and CA1; the hippocampal formation includes the hippocampus plus the dentate gyrus (DG), subiculum, entorhinal cortex, pre- and para-subiculum. See Figure 1.1 for a schematic illustration of the connectivity.

Sensory-motor to Mnemonic Transformations Occur in the Entorhinal-Hippocampus Loop

The entorhinal cortex (EC) provides the majority of excitatory input to the hippocampus via the perforant pathway. The perforant pathway is the first step in the

classical "tri-synaptic circuit"(Andersen et al., 1969). The EC projects to the DG (synapse 1), DG then projects to CA3 (synapse 2), and finally CA3 projects to CA1 (synapse 3). The actual circuit is more complex. The perforant path has two components. One component heads from Layer II of the entorhinal cortex, perforates through the subiculum and innervates the dentate gyrus and CA3. The other heads from layer III, then either through the subiculum with the fibers from Layer II or through the alveus above CA1, and innervates the CA1 region (Amaral and Lavenex, 2007). The dorsal parts of the EC preferentially innervate septal levels (septal meaning the very front, closest to the medial septum) of the dentate gyrus and hippocampus. The spatial topography of inputs has a functional consequence as well; the dorsal parts of the medial entorhinal cortex show tightly spaced, small grid fields while the ventral parts show broadly spaced, large grid fields (Hafting et al., 2005). Hippocampal place fields show a commensurate topography; place cells at septal levels show small place fields and get progressively larger as the electrodes are moved to the temporal pole of the hippocampus (Kjelstrup et al., 2008). Both the lateral and medial subdivisions of the entorhinal cortex project to the hippocampus. Whereas the medial entorhinal cortex has sharply peaked grid cells, the lateral entorhinal cortex lacks well defined spatial responses, raising the intriguing possibility that the lateral subdivision supplies the event or item component of episodic memory while the medial entorhinal provides spatial information (Hargreaves et al., 2005). Correspondingly, the proximal part of the hippocampus shows more robust place fields than the distal part of CA1. The functional heterogeneity suggests that the hippocampus receives both spatial and non-spatial information and the spatial information is represented at different spatial scales. The extensive recurrent

connectivity in the CA3 region likely means that CA3 can mix the spatial and non-spatial information together. The hippocampus is therefore well positioned to create associations between events and their spatial and temporal context. The loop is then completed by return projections from CA1 and the subiculum to the deep layers of the entorhinal cortex. The extensive connectivity between the deep layers of the entorhinal cortex and other cortical structures (Swanson and Kohler, 1986) theoretically allows information to be transferred to the neocortex for long-term storage (Jones and Witter, 2007).

The Hippocampus Receives Pronounced Neuromodulatory Inputs from Subcortical Structures

Monoaminergic inputs arrive in the form of dopamine from the ventral tegmental area (VTA) , seretonin from the raphe nucleus, and norepinephrine from the locus coeruleus (Swanson et al., 1987). The dopaminergic input is of particular interest (Lisman and Grace, 2005). Dopamine has been extensively implicated in attention deficit disorders and intact dopaminergic systems are required for normal performance on attention tasks. Adding dopamine antagonists or agonists to the hippocampal slice preparation will impair or enhance LTP. Finally Kentros and colleagues (2004) showed that dopamine agonists improve, while antagonists impair place field stability. Surprisingly, fibers from the VTA preferentially terminate in the CA1 region, suggesting that the CA1 region may be particularly well suited for selectively tagging information for long-term storage.

The hippocampus also receives cholinergic inputs from the medial septum and the vertical limb of the diagonal band of Broca (MSDBB). When reviewing the lesion or stimulation literature on these structures, it is important to consider that the majority of the inputs from these structures are GABA-ergic (inhibitory), and the return projection from the hippocampus is also GABA-ergic. The combined cholinergic and inhibitory influences from the MSDBB make up the core of the classic theta pacemaker hypothesis (Buzsaki, 2002 provides an excellent review). Finally, the hippocampus, particularly the temporal portions of the dentate gyrus(Pitkanen et al., 2000), receives excitatory inputs from the amygdala. The role of the amygdala in the consolidation of emotionally-arousing experiences is well-described (McGaugh, 2004).

Hippocampal-Prefrontal Dialog: Direct and Indirect Pathways

The hippocampus, prefrontal, and anterior cingulate cortex are functionally overlapping structures (subserving long-term memory, working memory, and attention), suggesting that there should be anatomical connections should link them. Indeed, the hippocampus is directly connected to both the mPFC via CA1 and the subiculum (Amaral and Lavenex, 2007), but the return projection is more circuitous, with stops either in the entorhinal cortex, or nucleus reunions of the thalamus, before being fed forward to the hippocampus (Vertes, 2002). Likewise the ACC projections to the hippocampus are mediated by nucleus reuniens, the claustrum, or the entorhinal cortex (Jones and Witter, 2007; Rowland and Kentros, 2008). These anatomical data suggest that the prefrontal areas cannot *directly* influence hippocampal firing patterns, but rather act on upstream structures.

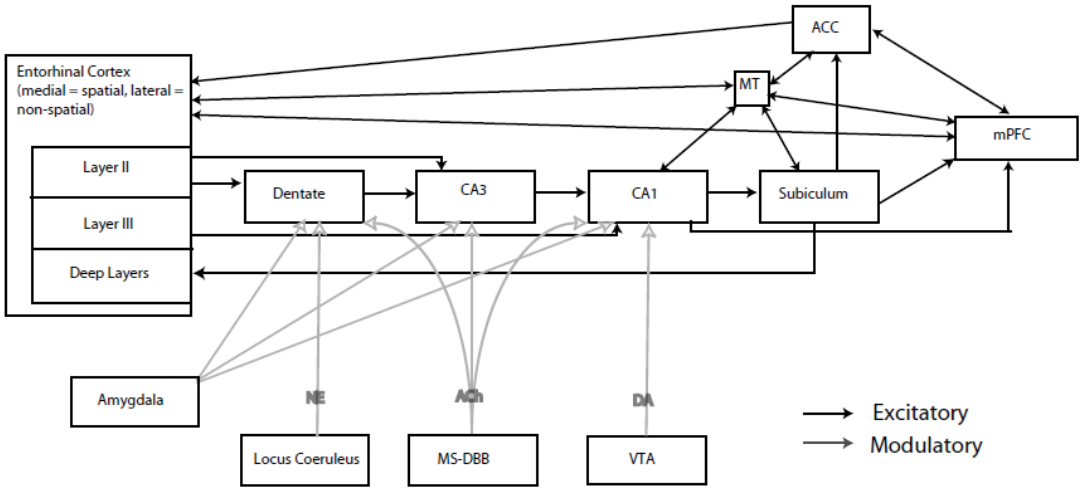

Figure 1.1 Schematic illustration of hippocampal connectivity. Some connections are omitted for clarity (e.g., the connections with the pre- and para-subiculum). ACC, the anterior cingulate cortex; MT, the midline thalamus, which includes nucleus reuniens; mPFC, the medial prefrontal cortex; MS-DBB, the medial septum-diagonal band of Broca; VTA, the ventral tegmental area; NE, norepinephren; Ach, acetylcholine; DA, dopamine. (From Rowland and Kentros, 2008)

Do Hippocampal Place Cells Provide a Cognitive Map of the Environment?

The studies discussed above suggest that the firing patterns of place cells are learned, and propose a role for plasticity and attention. These data tell us that the map of space is not hardwired, and at least not entirely precongifigured as the animal enters a novel space. However, none of the above experiments deals directly with the question of whether hippocampal place fields can represent places that the animal has visually observed but not explored. Here I discuss Edward Tolman's initial proposal (1948), and the skepticism that followed it. I then consider some prominent models for the development and maintenance of place fields. Some of these models predict that place fields should develop without direct experience with a space, as in Tolman's cognitive

map, while others predict the opposite. Most of these theories were developed more than 15 years ago yet remain equally valid.

Do Animals Have Cognitive Maps?

In the 1940's Edward Tolman designed a series of experiments to test whether rats were bound by the strict rules of stimulus-response learning that was prevalent at the time (Tolman, 1948). In the most famous of these studies, he trained rats to find a goal location in a "sunburst" maze. Initially, the rats were confined to a single path that ultimately led to the goal. After overtraining in this paradigm, he then removed the barriers from the remaining arms and allowed the animal to freely choose which arm to take. He reasoned that if the animals followed a stimulus-response learning rule they would choose the familiar path, but if they could make a mental "field map" of the environment then they would take the shortest available routes. He found that the majority of the animals rapidly choose the novel shortcut. He believed that the rats had "acquired not merely a strip-map to the effect that the original specifically trained-on path led to food but, rather, a wider comprehensive map to the effect that food was located in such and such a direction in the room." Similarly, O'Keefe and Nadel (1978) saw a cognitive map as a viewer-independent map of allocentric space. Nevertheless, some confusion exists over the precise meaning of the term, which sometimes stifles arguments over whether animals possess cognitive maps (Bennett, 1996). To this end, Trullier et al. (2007) provide a helpful set of four terms to describe potential navigation strategies: "guidance," "place-recognition triggered response," "topological navigation," and "metric navigation." Guidance behaviors include using a specific cue or set of cues to navigate to

a goal; for example, if an animal learns that a cue gives the position of a reward, then he can easily get the reward by simply navigating toward the cue. A place-recognition triggered response occurs when an animal performs the same action whenever he finds himself in a given place. Topological navigation is when an animal uses concatenations of previously experienced routes to form a new path through familiar space. Finally, metric navigation occurs when an animal navigates through space regardless of previously travelled routes. These four strategies, Trullier et al. argue, exist as a hierarchy with simpler strategies overriding more complicated ones. For example, an animal may use a metric map of the environment until he sees the goal, and can then switch to a guidance strategy. Of these strategies, only the final two require a mental representation of space at all and only the last requires a true "cognitive map."

Even when the terms are clearly defined, however, it is extremely difficult to prove that animals are in fact using a true cognitive map. Such a proof requires exquisitely designed behavioral experiments and careful analysis. For example, in Tolman's sunburst maze, there was a light over the goal location, providing a cue for guidance navigation. In fact, attempts to reproduce his behavioral result have been unsuccessful (D. Redish, personal communication). In one of the more famous controversies surrounding whether animals have cognitive maps, James Gould (1990) tracked honey bees in a large open field. The bees were then moved to a previously unoccupied part of the field and released. He found that the bees were able to rapidly find a goal (food source), which he interpreted as a cognitive map-like representation of the environment. However, critics of this study note that the bees actually used a variety of behaviors inconsistent with a map-based navigation strategy; for example, after the

bees were displaced they circlee high in the air prior to choosing a route (Wehner and Menzel, 1990). These behaviors suggest that the strategy actually employed by the bees was to move around until they could find a better match to a stored visual representation, then follow a set of pre-programmed routes towards the goal.

The idea of a cognitive map, as examined from a behavioral level, therefore remains controversial. The initial back-and-forth between Tolman at the University of California at Berkeley and Hull at Yale University was cleverly summarized by Tulving and Madigan (1970), and could just as well describe the present debate: "place-learning organisms, guided by cognitive maps in their head, successfully negotiated obstacle courses to food at Berkeley, while their response-learning counterparts, propelled by habits and drives, performed similar feats at Yale."

Place Cell Models and Spatial Representations in the Hippocampus

The previous section discussed the controversy surrounding the idea that animals have a cognitive map of their environment. However, even if we assume that they do, there are still no guarantees that hippocampal place cells are the neural implementation of the cognitive map. In fact, many models of place cells suggest quite different spatial representations, with some models even returning to a strict stimulus-response learning rule. Here I discuss some of the major models of place cells and what, if any, predictions they make for representations of observed but unexplored space.

Geometric Models of Place Cell Firing

O'Keefe and Burgess put forward a series of models that use a hypothetical set of boundary vector cells each with a Gaussian shaped tuning curve oriented towards a particular boundary of an environment (O'Keefe and Burgess, 1996; Barry et al., 2006). A cell's place field is then specified when the animal occupies a position where the summed activity of the input boundary vector cells is greatest. The boundary vector cells are similar to the barrier cells discovered by the Moser (Solstad et al., 2008) and Knierim (Savelli et al., 2008) groups in the medial entorhinal cortex, but those cells fire when the animal is close or even touching the boundary, not as a function of distance from the boundary. The O'Keefe and Burgess groups have recently reported a set of cells in the subiculum that fire as a function of distance from a given boundary, in a manner formally consistent with the BVC model (Lever et al., 2009). However, the cells discovered so far represent only a minor percentage of cells in their respective structures, and it remains unclear whether the cells are actually anatomically-connected to place cells. The geometric models account for the clear and predictable effects that removing barriers or manipulating the geometry of the testing enclosure have on place fields, but struggle to account for why cells can respond differently in the same geometric space depending on the animal's ongoing behavior.

Hippocampus as a Cognitive Graph

In 1990's Robert Muller and the graph theorist Janos Pach (Muller et al., 1991; Muller et al., 1996), developed a theory that the hippocampus can be conceptualized as a weighted graph. The nodes of the graph are the individual place cells, with each node

connected randomly to other nodes due to the random wiring of cells in the recurrent CA3 network described above. The weights of the connections, however, are assigned based on distance between place fields in a given environment. They arrived at this conclusion by first assuming a Hebbian learning rule; cells that fire closely in time would have their synaptic connections strengthened while cells that fire farther apart in time would have their synaptic connections weakened. They reasoned that, because place fields fire as the animal moves through the environment, the cells that fire together closely in time will also fire together closely in space. Therefore, the connection matrix will naturally be weighted by the Euclidian distance between the fields. Using simulations, they showed that such a weighted graph could efficiently produce novel paths through an environment by searching the graph for the path of least synaptic resistance between the animal's current position on the graph and its desired end-point. The model is simple and elegant, but struggles to account for the some of the complexities seen in modern place cell studies, particularly instances where multiple maps seem to exist for the same space or non-spatial firing. Nevertheless, elements of the model still persist (Dragoi and Buzsaki, 2006), particularly in a more general class of sequence learning models. Because the connection matrix depends on the animal moving through space, the model makes the strong prediction that a stable representation will only exist for those areas that the animal has actually moved through.

Path Integration Based Maps

The fact that place cells can maintain their spatial relationship even when the room lights are switched off (Quirk et al., 1990) suggests that place cells are not

exclusively driven by visual input but are also responding to the animal's sense of its own position, known as path integration (McNaughton et al., 2006). Mittelstaedt and Mittelstaedt (1980) were the first to show that mammals were capable of homing by path integration. They exploited the tendency of mother gerbils to carry their pups back to the home nest if the pups are displaced. In total darkness mothers retrieved their pups and returned, in a bee-line fashion, to their home base. Moreover, if the arena was smoothly rotated beneath the vestibular threshold of the animal, then the return vector was off by the amount of rotation. These data show that the animal is able to track its own movements through an environment and calculate a return trajectory back to an initial staring point. This process of continuous updating based on self-generated cues requires the sort of self-sustained activity that is mathematically realized by continuous attractor network models. In attractor map models of path integration, a bump of activity is moved between nodes on a chart (similar to the cognitive graph) by a displacement vector (comprised of linear speed and orientation information from the head direction system). In Samsonovich and McNaughton's version, the path-integration based map of activity forms the basis layer for other information such as local view, but such local view information is not required for either the formation or maintenance of the map. Whether the map of the environment extends beyond the places the animal has explored depends on whether the chart is pre-wired or not. Samsonovich and McNaughton suggested that the charts are pre-wired and extend over the entire surface of the environment, and indeed over a near infinite scale. However, Redish and Touretzsky did not assume that the chart was pre-wired. Instead, they suggested that both the path integration information and the

local view information need to be coincidentally experienced in a space in order to form its stable hippocampal representation.

Egocentric "Snapshot" Models

The last class of models that I will discuss rely heavily on Marr's view that the hippocampus is an auto-associative network capable of forming, and briefly storing, assocations between neocoritcal activity (Recce and Harris, 1996) . As an animal explores the environment it is constantly taking in the external cue information and the internal, path-integration information. Incoming stimuli are treated as provisional and are compared to stored representations and if a match is not found, then a new snapshot is stored. The recurrent collaterals of CA3 allow for a pattern completion process whereby a partial or degraded snapshot is treated as familiar. A map is therefore constantly updated as the animal explores a new environment or even a previously unvisited part of a familiar environment. These models make the explicit prediction that place cells do not represent observed but unexplored locations in the environment.

Summary

The electrophysiological data reviewed in this chapter suggest that hippocampal representations are more plastic than expected under O'Keefe and Nadel's (1978) hypothesis. The anatomical data suggest that hippocampal neurons should respond to a variety of spatial and non-spatial information and are potentially subject to attentional modulation. Finally, models of place cells are conceptually diverse, but little experimental evidence is available to truly distinguish them. The experiments described

in Chapter II show that place fields require direct experience. In Chapter III, I discuss the relevance of the work to memory research beyond place cells.

CHAPTER II

HIPPOCAMPAL REPRESENATATION OF SPACE REQUIRES DIRECT EXPERIENCE

This chapter was a paper recently submitted to *PLoS Biology*. Yelizaveta Yanovich contributed to the data collection. Clifford Kentros, my advisor, helped design the experiments, and helped with the writing. I helped design the experiments, collected the majority of the data, analyzed all of the data, and did the majority of the writing.

Introduction

Edward C. Tolman's proposal that the rodent brain contains a "cognitive map" (Tolman, 1948), a map-like representation of the animal's environment used to solve spatial problems, surely counts as one of the most influential ideas of behavioral neuroscience in the 20th century. The key finding supporting it was rats' use of shortcuts through novel space towards a familiar goal. This suggested they had a map-like internal representation of the entire environment, providing compelling evidence against the strict stimulus-response theory prevalent at the time. It therefore made perfect sense for O'Keefe and Nadel to use Tolman's term to explain the equally seminal finding that hippocampal neurons act as "place cells" in awake, behaving rodents (O'Keefe and Dostrovsky, 1971), equating them with a Kantian spatial representation of the entirety of the animal's environment (Wills et al., 2010). Such a comprehensive map of the animal's entire environment could be achieved through a variety of mechanisms: first a preconfigured map or chart can be selected as the animal enters a novel environment (Samsonovich and McNaughton, 1997), second, a set of specialized cells outside of the hippocampus that respond to the geometry of the environment could sum together within the hippocampus to form tuned place fields (O'Keefe, 1991) or finally the animal may be able to mentally project itself to unvisited locations in the environment through mental imagery (Emery and Clayton, 2004).

However, structural tension exists between the hippocampus as a cognitive map and the central role that has been ascribed to the hippocampus in the formation of episodic memories (Morris and Frey, 1997; Eichenbaum et al., 1999) (i.e. memories of *what* happened, *where* and *when*)(Clayton et al., 2001). A cognitive map is an

allocentric, third-person, representation of the external environment encompassing both directly experienced space and distal, observed, space; in contrast to the egocentric, first-person nature of episodic memory in humans (Conway, 2001) and other animals (Buzsaki, 2005). To illustrate, imagine going to dinner at a new restaurant. While you are led to your seat you generate what is in effect a cognitive map of the entire dining room, as opposed to just the particular path you happened to traverse. However, your memory of the behavioral episode of going to your table is strictly autobiographical, a first-person synthesis of the egocentric sensory information available in the space that you physically occupied. Accordingly, place cells take several minutes to form stable place fields in novel environments and will continue to modify over days under special circumstances (Lever et al., 2002), suggesting that the hippocampal representation of a space is not innate but learned (Wilson and McNaughton, 1993; Frank et al., 2004). From f this experimental fact, several models of place field development propose that place fields might develop only as the animal occupies the space. Redish and Touretzky used the fact that hippocampal place cells respond to both the local sensory information and the animal's own internal "path integration system"as the basis for a model whereby the place cells associate the local sensory environment with the animal's internal estimate of position(Redish and Touretzky, 1997). Muller and others have alternatively suggested that the distance between place fields must be calibrated through active movement and stored as a set of synaptic weights (Muller et al., 1996; Buzsaki, 2005). The requirement for the animal to explore an environment to reveal where its hippocampal neurons have place fields has precluded the direct demonstration of whether they can form by observation alone. The following experiments address which of the above kinds of

representations hippocampal place cells resemble by determining whether they represent the entirety of the animal's environment or only directly experienced portions. We find that, in contrast to the classical cognitive map hypothesis, the animal must physically occupy a space in order to form a stable hippocampal representation of it.

Results

Recordings were performed in a customized behavioral apparatus (Figure 2.1) consisting of two concentric boxes: an optically clear Plexiglas inner box inside an opaque outer box with geometric shapes painted on it as spatial cues. Throughout familiarization and screening the animal was restricted to the inner box such that the perimeter of the outer box was extensively observed, but not explored, while the inner box was directly experienced. Upon finding place cells, the outer box was rotated 90 degrees. The place fields followed the rotation, demonstrating that the outer box cues were used to orient its place cells (see R1, Figure A.1, in the Appendix: Supporting Information). Following an injection of the NMDA receptor antagonist CPP ((±)-3-(2-Carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP), 10mg/kg I.P) or saline and another recording session in the inner box (I2), one wall was removed from the inner box (O1), allowing the animal to enter the previously unexplored outer box. After a 6-12 hour delay the animal was recorded again in this modified environment (O2), after which a final session (I3) was run in the initial configuration.

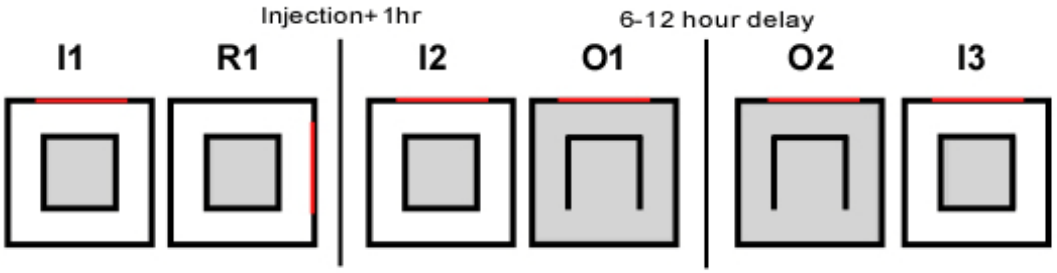

Figure 2.1. Experimental design. The recording environment consisted of two concentric boxes: a clear inner box with no asymmetric cues, and an opaque outer box with geometric shapes on its sides to provide spatial cues. The rats were extensively familiarized to the inner box of the environment (I1), after which the outer box was rotated (R1). Following injection and another inner box session (I2), an inner wall was removed, allowing exploration of the entire environment (O1). After a delay the animal was reintroduced to the open configuration (O2) and then back into the closed inner box (I3). Grey portions indicate regions explored by the rat, the red bar indicates cue orientation.

The experiment is predicated on the requirement of NMDA receptor dependent plasticity for stable hippocampal "remapping", the drastic changes in place fields seen in response to environmental novelty (Bostock et al., 1991; Leutgeb et al., 2005). Since NMDA receptor blockade specifically destabilizes newly formed place fields while sparing previously formed place fields (Kentros et al., 1998), CPP injection prior to session O1 reveals *when* the outer box place fields were formed. If place fields form by observation they should all be stable, but if they form only after direct experience, then CPP should specifically destabilize the outer box cells alone.

Figure 2.2 shows rate maps of place cells recorded from a saline-injected animal throughout the experiments described above. Comparing I2 to O1, the majority of place fields in the inner box did not remap following wall removal. However, consistent with prior work involving manipulations of barriers and walls (Barry et al., 2006), some neurons (particularly those with place fields near the removed wall) did remap following wall removal (e.g cell 4, Figure 2.2; Figure A.2). Place fields were evident in the outer

box as well, with approximately 20% of the cells with fields in the inner box also having fields in the outer box (see cell 3 in Figure 2.2). Removal of the barrier also revealed a previously inactive population of pyramidal cells (e.g. cells 5-7 in Figure 2.2) that had place fields just in the outer box. To compare the stability of place fields of directly experienced versus observed portions of the environment, we computed separate Pearson's correlation coefficients (stability scores) for the inner and outer box areas (see Methods). Place fields in both compartments were nearly always stable in the saline animals (Figures 2.2 and 2.4), which would be expected whether the outer box fields were formed during observation or exploration (Frank et al., 2004). Thus, the saline animals give us three main results: 1) wall removal did not cause a global remapping of place fields in the inner box; and 2) "new" place cells appear only in the outer box of the environment and 3) place cells often have fields in both boxes.

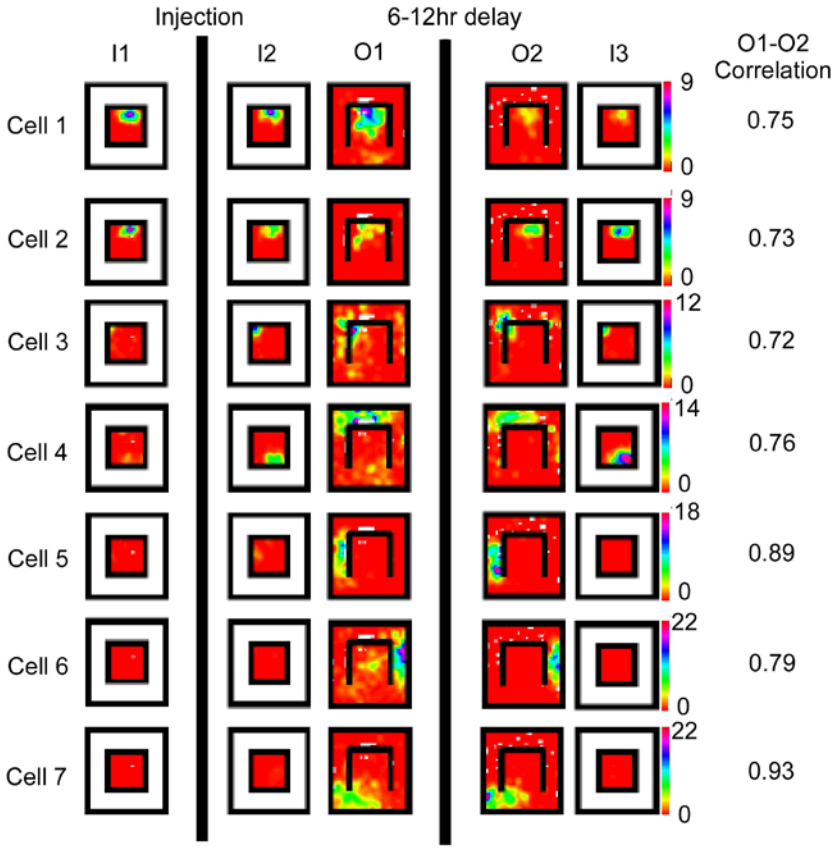

Figure 2.2. Place fields of 7 simultaneously-recorded hippocampal pyramidal neurons across the entire behavioral sequence from a saline-injected rat (with the rotation session omitted for clarity). Rows are cells and columns are sessions. Color bar shows the cell's firing rate values for the entire set of sessions, unvisited pixels are white. Note that all place fields are stable throughout the experiment, with the exception of those inner box cells that remapped in response to wall removal (e.g. Cell 4). Whole-environment correlation scores for the O1-O2 comparison is shown to the right of the ratemaps.

The CPP-injected animals' place fields responded similarly to wall removal, at least in the first session (O1). Most of the inner box fields remained stable, while others, particularly those near the removed wall, remapped (cell 4, Figure 2.3; Figure A.2), and new fields appeared in the outer box. When one compares the O1 and O2 sessions, like the saline animals the vast majority of inner box place fields remained stable (cells 1-3, Figure 2.3), except for those cells that remapped in response to wall removal. Unlike the saline animals, these cells remapped again in the CPP animals (cell 4, Figure 2.3) as did

all of the place cells with fields in the outer box (cells 4-7, Figure 2.3). This is in sharp contrast to the simultaneously recorded inner box place fields which remained stable if they had not remapped in response to the wall removal. We quantified this difference by dividing place fields into inner and outer groups for analysis of variance (ANOVA). Post-hoc comparisons showed no differences in the stability of the inner and outer box place cells in saline injected animals, nor any differences between these two groups and the inner box place cells in the CPP injected animals. In fact, the only significant differences in place field stability were between the outer box place cells in CPP-injected animals and all other groups (Figure 2.4; $F = 36.4373$, $p < 0.001$).

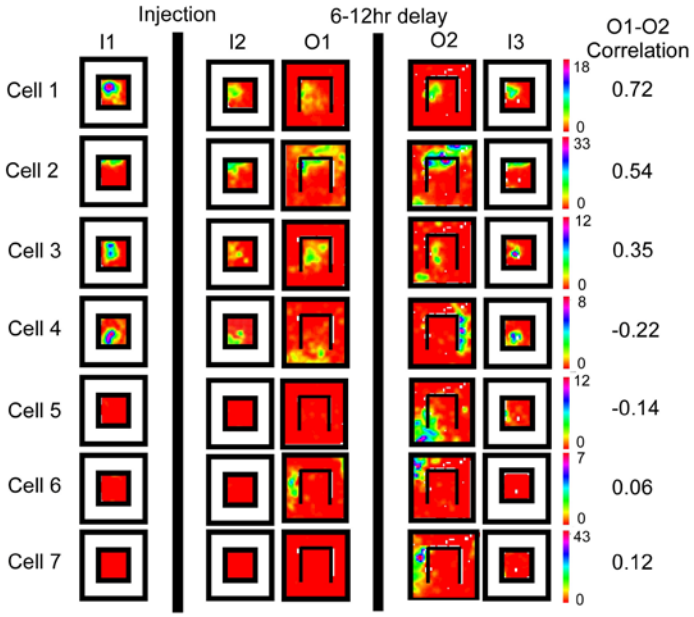

Figure 2.3. Place fields from 7 identified pyramidal neurons across the entire behavioral sequence from a CPP-injected rat. Format is the same as in figure 2.2. Note that most neurons with fields in the inner box (e.g. cells 1-3) maintained firing position throughout the experiment, while cell 4, which remapped in response to barrier removal in O1, remapped again in O2, consistent with the known effects of CPP on remapping. All outer box firing fields remapped between sessions O1 and O2, even those of cells (2&3) with stable inner box fields, which resulted in midrange stability scores for the entire environment.

The data in Figure 2.4 strongly suggest that the place fields representing the newly explored outer box were made more recently than those of the inner box, presumably as the animal first directly experienced it in Session O1. Extensive observation of a space is therefore clearly not sufficient for making a stable place cell representation of that space. Indeed, the initial exploration of the outer area has all the hallmarks of the "complete" remapping one gets when one puts an animal into a distinct and truly novel environment (Leutgeb et al., 2005): "new" place cells started firing in the outer box, and a fraction (~1 in 5) of cells had place fields in both parts of the environment, which is rare in a single environment. Moreover, fast-spiking putative interneurons (i.e. "theta" cells) significantly decreased their firing rate (Figure 2.5) as the animal explored the outer box for the first time, consistent with previous studies in novel environments(Wilson and McNaughton, 1993; Frank et al., 2004).

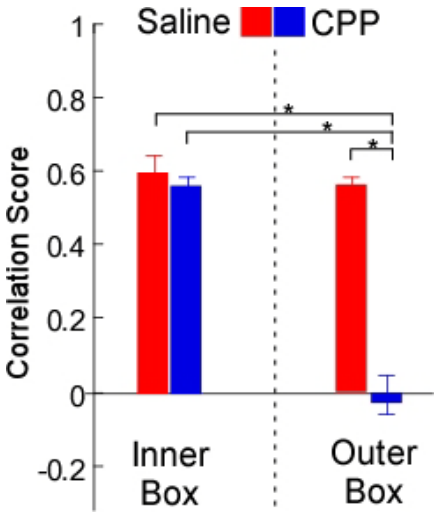

Figure 2.4. CPP preferentially destabilizes outer box place fields. Mean stability scores for the O1-O2 comparison when broken out into inner and outer box areas (see Methods), error bars are SEM. An ANOVA revealed a significant difference between the four groups ($F = 36.4373$, $p < 0.001$). Posthoc comparisons showed that this difference came entirely from the CPP-outer box group, which significantly differed from all three other groups (CPP-inner, and saline inner and outer; * = $P<0.01$).

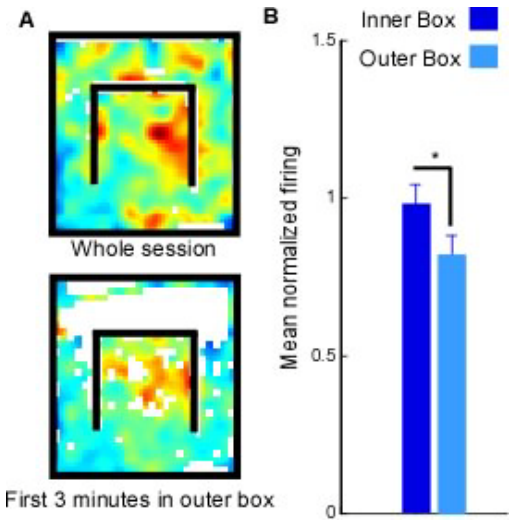

Figure 2.5. Suppressed activity of putative interneurons in the outerbox area compared to the inner box area for saline injected animals. **A** Rate maps showing activity of a putative interneuron over the whole session (top) and the first three minutes of experience in the outer box area (bottom). Note the suppression of activity in the outer box. **B** Group data. Bars show the mean normalized activity of cells (n = 12) in the first 3 minutes of experience in the familiar inner box compared to the first three minutes of experience in the outer box area (Error bars are SEM; Paired t-test, p = 0.0262).

Discussion

Cognitive Maps in Rodents, Monkeys and Humans

We found that NMDA receptor blockade preferentially destabilized place fields in areas that were previously observed but not explored. These data strongly suggest that place fields require direct experience of a space, rather than observation, to form, and thus do not comprise a comprehensive cognitive map of the entire environment as envisaged by Tolman. This result constrains the ability of place cells in rodents to guide spatial navigation, particularly through unvisited areas, but also further emphasizes the

inherent plasticity of hippocampal neurons. Place cells may be predisposed to respond to the animal's position of space (Wills et al., 2010), but the resulting "map" is quite plastic, forming only as the animal directly experiences it, and changing to reflect the animals' changing experience in an NMDA receptor-dependent manner. Although our study was designed to test spatial representations in the hippocampus, hippocampal cells can respond to a variety of non-spatial cues, such as odors(Wood et al., 1999), and consolidation of non-spatial memories also requires NMDA-receptor mediated plasticity (Fortin et al., 2002; Day et al., 2003; Rajji et al., 2006). Direct experience may therefore be a general requirement of hippocampal memories, with plasticity linking together the disparate elements that comprise a memory as they are experienced. The structural homology between the rodent hippocampus and the human hippocampus (Manns and Eichenbaum, 2006) suggests that this result should generalize between species. However we cannot rule out the possibility that place cells in monkeys or humans might extend beyond immediately experienced spaces, particularly in light a of a growing body of evidence suggesting that people lacking a hippocampus have difficulty imagining future events or places, a finding corroborating by neuroimaging studies in normal humans (Byrne et al., 2007; Hassabis et al., 2007; Hassabis and Maguire, 2007; Eichenbaum and Fortin, 2009).

Grid Cells, Place Cells and Complex Environments

Our results raise the question of how observed space would be represented in other brain regions, most notably by the "grid cells"(Hafting et al., 2005) of the medial entorhinal cortex, the major input structure to hippocampus. In many ways, the grid cells

appear to be more “hard-wired” and therefore may be better suited to provide a holistic representation of an environment like that envisioned by Tolman. Their repeating, regular organization is largely maintained in novel environments, suggesting each cell represents distance in space rather than part of a particular environment. Moreover, a grid cell has a similar orientation as its neighbors, unlike place cells that show little or no neighbor relationships, and their firing patterns are immediately present in novel environments (Hafting et al., 2005; McNaughton et al., 2006). The grid cell representation of space therefore seems regular and preconfigured, properties that were once predicted for hippocampal place cells (Samsonovich and McNaughton, 1997) but never fully supported (Touretzky and Redish, 1996; McNaughton et al., 2006).

These data obtained with this quite artificial environment also inform our thinking about how hippocampal place fields and entorhinal grid cells chunk larger and more complex natural environments. Unless there is something special about clear barriers, animals would therefore not have a place cell representation of purely observed space regardless of whether the space is separated by a physical barrier or not, which would encompass most of their natural environment. A recent study showed that grid cells "fragment" into distinct submaps in environments with multiple barriers (Derdikman et al., 2009). It is therefore possible that the grid cells create a separate submap for the observed area.

Constructing a Map Through Plasticity

Even though the rotation experiments (Figure A.1) show that the animal is aware of the outer box area prior to exploring it and uses its cues to orient its place cells, its

place cells appear to treat the outer box as if it were a completely novel environment, requiring new NMDA receptor-dependent plasticity to stably form. This was also true for the inner box cells that remapped in response to wall removal, they remapped yet again in the CPP group. Clearly, both extending the representation by adding new fields and modifying a representation after removal of a barrier are active processes requiring NMDA receptor mediated plasticity to stabilize. In this respect, our results parallel a recent study showing that CPP prevents the stability of place fields that remap in response to a novel behavioral task, but did not destabilize the previously learned representation of the space where the task was conducted(Dupret et al., 2010) (Legault and Wise, 2001)

Our data also brings to mind two studies (Wilson and McNaughton, 1993; Frank et al., 2004)that used high density recordings and reconstruction techniques to show that place fields in novel parts of an environment (revealed by removing opaque barriers) take several minutes to form with little interference to the representation of the familiar space. Here we extend this finding in two ways: first, extensive visual observation is not the same as occupying the space and second, extending a place field map to include novel (in our case observed areas) requires NMDA receptor activation. Furthermore, previous work has shown that dopamine is released as rats explore newly accessible parts of their environments, and this release is blocked by inactivating the subiculum(Legault and Wise, 2001). Taken together these results suggest that not only is plasticity required for the stability of totally new hippocampal map of space, but it is also required for augmenting and updating a previously formed map.

Reorganizing and Using the Map

A recent study by Gupta and colleagues showing that sequences of behaviorally novel paths through familiar spaces can be “preplayed” during sharp waves(Gupta et al.) does not conflict with this interpretation since in these experiments the path was novel, but not the space. It is therefore possible that once the animal experiences the space, then the hippocampus can plan routes based on concatenations of experienced paths or subpaths, sometimes called "topological navigation", however it may not be able to plan routes over novel spaces, sometimes called "metric navigation" (Trullier et al., 1997). However, once the map is formed, a variety of mechanisms can be exploited to evaluate choices (Johnson and Redish, 2007)distinguish between distinct behavioral epochs within the same space (Wood et al., 2000; Ferbinteanu and Shapiro, 2003) and reform based on behavioral contingencies (Markus et al., 1995; Dupret et al., 2010). Moreover, our data measure the “consolidation” of place fields rather than their formation per se, so are also consistent with the equally interesting possibility of a “proto-map” that projects out to observed, unoccupied space. This could be realized either as partly preconfigured map of the environment that is refined with experience(Redish and Touretzky, 1997) or perhaps as a set of cells that resemble the "view cells" found in the monkey hippocampus that respond to the current view of the animal(Rolls, 1999). Nevertheless, the complete remapping of the outer box area in our study suggests that such a proto-map, if it exists, is not only independent of the map of the inner environment, it is formed *de novo* each time the animal experiences the environment, only becoming a stable representation of a space upon consolidation by NMDA-receptor dependent plasticity-driven by the direct experience of that space.

Summary

In conclusion, our evidence strongly suggests that hippocampal place fields do not form a holistic representation of the animal's environment akin to Tolman's "cognitive map". Although our results clash with this classical formulation of a cognitive map and some(Hartley et al., 2000) models of place field formation, they are consistent with a variety of other such models(Redish and Touretzky, 1997; Eichenbaum et al., 1999; Buzsaki, 2005; McNaughton et al., 2006) that require coincident input spanning multiple internal and external cues. In other words, while place fields are indeed an allocentric (third-person) representation of the external world, their formation is inherently egocentric, or "first-person". This property would make them more useful in planning how to get from one familiar place to another by a familiar path than the kind of complex navigation that led Tolman to formulate the cognitive map hypothesis. These properties, however, are entirely consistent with the critical role of the hippocampus, and hippocampal place cells, in episodic memory (Scoville and Milner, 1957; Squire and Zola, 1998). In this view, place fields provide the "where" information that is combined with nonspatial (what) (Wood et al., 1999) and temporal (when) (Pastalkova et al., 2008) information via synaptic plasticity to create an autobiographical record of what is experienced in both animals (Buzsaki, 2005) and humans(Eichenbaum et al., 1999).

Materials and Methods

Animals

We included data from 18 (9 saline-injected and 9 CPP-injected) implanted male Long Evans rats (Charles River; 3-9 months old; 300-450g at time of testing). After surgery, the rats were given a recovery period lasting one week, following which the rats were food restricted to 85-90% of their free-feeding weight. All procedures were carried out in accordance with the Oregon Institutional Animal Care and Use Committee (IACUC) and National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23).

Surgery

Rats were anesthetized with 1-4% Isoflourane mixed with oxygen and implanted with a microdrive containing 6 tetrodes moveable as a bundle. The tetrode bundle was aimed over the CA1 region of the left hemisphere (3.5mm posterior from Bregma; 2.5mm lateral from midline) and lowered to approximately 250μM above the CA1 pyramidal layer. Stainless steel anchoring screws (2-4) were set into the skull. The microdrive was bonded with the skull using Grip Cement (Dentsply, Milford, DE).

Behavioral training

Rats were screened and familiarized in the testing apparatus. The rats were trained to freely forage for scattered food pellets dropped from an overhead feeder. Pellets landed in both the inner and outer box. During this period, the rats were restricted to a clear inner box (50x50cm) within an outer box area (130x130cm) as shown in Figure 2.1 (I1). The position of a red and a green LED attached to the headstage was recorded by an overhead camera. Rats were familiarized to the environment for at least 6 sessions

lasting at least 10 minutes. At the end of each familiarization session the bundle of tetrodes was advanced 25-75µM and the rat was returned to its home cage for at least 8 hours. The floor paper was changed and the floor and walls of the chamber were wiped down with 90% ethanol after every session. This procedure was continued until large-amplitude, well-isolated place cells were present (number of exposures ranged from 7 to 29, median was 15, with no differences between groups). The 6 session experiment illustrated in fig. 1 and described in the main text was then initiated.

Electrophysiology

Tetrodes were made from 17µM platinum 10% iridium wire (California Fine Wire, Grover Beach, CA) twisted together. Wires were plated with platinum (Technic Inc., Anaheim, CA) to a final impedance of 250 to 750kΩ. Spiking activity was filtered from 600-6000Hz and sampled at 32kHz online using the Cheetah-32 system (Neuralynx, Bozeman, MT). Clusters were cut in MClust (A. D. Redish, University of Minnesota) and SpikeSort 3D (Neuralynx, Bozeman, MT). Single units were judged to be the same if similar cluster boundaries could be applied across sessions. We allowed the cluster boundaries to be stretched or contracted between sessions, as necessary, to account for changes in size and shape of the cluster resulting from dramatic increases or decreases in firing rate during remapping. Finally, the waveforms of each cell were compared across sessions.

In total, 934 well-isolated clusters were analyzed (547 CPP; 387 saline) with a median number of 93cells/session in the CPP group and 65 cells/session in the saline group. Cells that were not held across the entire testing sequence were used for single-

session statistics and for comparisons where similar cluster boundaries could be applied (see Data Analysis).

Data analysis

The spiking activity of single units was associated with the rat's position in space at the time of the spike. All data were filtered for epochs of walking by removing any data points were the rat's instantaneous running speed was less than 3cm/sec. The position of rat and the spikes were then binned into 4cmX4cm bins. The binned spikes were then divided by the binned occupancy to create an unsmoothed rate map. A smoothed rate map was created by smoothing the rate map with a 3x3 Gaussian kernel.

Correlation scores based on smoothed rate maps were generated for session pairs by correlating the two maps. A Pearson's correlation score was calculated between equivalent bins, with unvisited and common-zero bins ignored. A cell was eligible for the measure only if it was judged to be the same between the two sessions and showed a place field in either of the two sessions being compared. In addition, the rat must have occupied >85% of the bins in the rate maps and the majority of fields needed to follow the rotation of the cues. These three requirements (recording stability, rotation, and coverage) reduced the number of rats to 6 saline rats (48 cells) and 6 CPP (51 cells) for the critical O1-O2 comparison. Data from the other rats were included in single-session statistics and other comparisons, when appropriate. For the O1-O2 comparison, we divided the environment into an inner and outer box area and computed a separate stability score for the two regions. To do this, we first found the place fields (defined as a contiguous 80cm^2 region where the cell fired above 20% of its peak firing rate for the whole environment) of a cell. If a place cell had a field centered in the inner box in either

session, then a stability score was taken for that cell in the inner box. This procedure was then repeated for the outer box, thus creating an inner and outer group. Some cells contributed to both the inner and the outer box groups, as approximately 1/5 of the cells showed fields in both compartments (e.g cell 3, Figure 2.2). In total, 21 saline and 26 CPP cells contributed to the inner box correlation, and 30 saline and 36 CPP cells contributed to the outer box comparison.

Mean firing rate was taken as the number of spikes divided by the total length of the session. Coherence was the z-transformed Pearson's correlation score between a pixel and its eight nearest neighbors in the unsmoothed rate map. Peak firing rate was the highest firing rate bin in the smoothed rate map. A field was identified as above. Single session statistics were compared between sessions and across groups (Table A.1). All analyses were performed using custom-written MATLAB (the MathWorks, Natick, Massachusetts) code.

Histology

Following completion of the experiment, a brief pulse of current (~25µA) was passed through the wire that yielded the best recordings. The rat was then euthanized with Euthasol (100mg/kg, i.p) and perfused transcardially with 10% formaldehyde. The brain was sliced into 50µM thick coronal sections and stained with cresyl violet. Only data from recording locations confirmed to be in the CA1 region of the dorsal hippocampus were included in the present study (Figure A.4).

CHAPTER III

CONCLUSION

The experiments described in Chapter II clearly show that hippocampal place fields, at least in rats, do not form prior to direct experience with a space. This result suggests that the hippocampus does not contain a cognitive map of the environment. It does not mean, however, that the animal does not have a cognitive map of the environment. Indeed the grid cells of the entorhinal cortex in many ways better resemble a cognitive map: their firing is instantly present in novel environments; the spacing, and field size is largely preserved as the animal is moved into arenas of different size; and the sheer mathematical precision of the grid suggests that a grid could extend on indefinitely (McNaughton et al, 2006). The discovery of grid cells in the entorhinal cortex has proven to both liberating and confining for hippocampal memory research. On the one hand, if space is so accurately represented in the entorhinal cortex, then the hippocampus is free to perform other functions. On the other hand, the main excitatory inputs to the hippocampus are fundamentally and clearly spatial in nature, and so the spatial firing properties can no longer be ignored or discounted as it was in the declarative, configuration-association theory and the relational theories of the hippocampus. If we assume that grid cells would complete a representation of observed space, which has yet to be shown, then why would place cells not follow suit? In this concluding chapter, I put forward the speculative idea that place cells combine the spatial location acquired from the grid cells of the medial entorhinal cortex with the event, item, or scene

information from the lateral entorhinal cortex. The hippocampus has anatomical access to all of the relevant information necessary to perform this function, as discussed in more detail in Chapter I. Because of the time-dependent nature of synaptic plasticity, the encoding of this information must occur in a synthetic, real-time fashion and therefore also gives a temporal context to hippocampal memories. These properties make it formally similar to the episodic memory. I therefore start with a discussion of episodic memory in both humans and animals.

Episodic Memory in Humans and Other Animals

Tulving coined the phrase "episodic memory" in 1972. He considered the concept itself obvious, and introduced the term merely to make clear that the focus of his contemporaries on "semantic memory", or memory of facts, was qualitatively and philosophically different from what we usually think about when we think about memory. He defined episodic memory as:

> "Episodic memory receives and stores information about temporally dated episodes or events, and temporal-spatial relations around these events. A perceptual event can be stored in the episodic system solely in terms of its perceptible properties or attributes, and it is always stored in terms of its autobiographical reference to the already existing contents of the episodic memory store. The act of retrieval of information form episodic memory, in addition to making the retrieved contents accessible to inspection, also serves as a special type of input into episodic memory and thus changes the contents of the episodic memory store." (Tulving, 1972)

Later, in the same paper, he provides a more common-sense definition:

“Most, if not all, episodic memory claims a person makes can be translated into the form: ‘I did such and such, in such and such a place, at such and such a time’”(Tulving, 1972).

This construction emphasized that the subject was the center (the reference) of the memory.

In subsequent papers, it became clear that Tulving viewed episodic memory as a uniquely human ability, requiring what he called "autonoetic", or self-knowing, consciousness and mental time-travel (Tulving, 2002). Proving that animals have these capacities requires a clear demonstration that animals are consciously recollecting their past, which is difficult or impossible to do without a way to communicate with the subject. It is worth noting, however, that two recent reports in rats suggest that animals have some elements of conscious recollection. First, Fortin and Eichenbaum (2004) used receiver operator characteristics (ROCs) to show that rats, like humans, can retrieve memory through recognition or conscious recollection, and damage to the hippocampus selectively impairs the conscious recollection component. Second, the recently discovered "replay" phenomenon observed in hippocampal place cells closely resembles mental time travel (Foster and Wilson, 2006).

Not wanting to lose the utility of the term, animal research frequently uses "episodic-like", defined as what happens as well as when and where it happened (Clayton et al., 2001). Note that this definition is essentially a restatement of Tolman's 1972 version. The reluctance to just abandon the term all-together is because it succinctly describes the deficit seen in patients with hippocampal damage, even the dissociation between episodic and semantic memory in patients with hippocampal damage. In the most striking demonstration of this dissociation, Vargha-Khadem (1997) and colleagues followed the progression of several children born with hippocampal damage. They summarized the main deficits as follows:

> "(i) Spatial: None of the three patients can reliably find their way in familiar surroundings, remember where objects and belongings are usually located, or remember where they have placed them. (ii) Temporal: None is well oriented in date and time, and they must frequently be reminded of regularly scheduled appointments and events, such as particular classes or extracurricular activities. (iii) Episodic: None can provide a reliable account of the day's activities or reliably remember telephone conversations or messages, stories, television programs, visitors, holidays, and so on. According to all three sets of parents, these everyday memory losses are so disabling that none of the patients can be left alone, much less lead lives commensurate with their age, circumstances, and aspirations."

Despite these debilitating impairments, the children test normally for verbal memory and fare well in school, suggesting normal semantic memory. These data clearly implicate the hippocampus in Tulving's episodic memory system.

Episodic-like Memory in Animals

The "what-where-when" formulation makes episodic memory testable in animals, but also requires well-designed behavioral paradigms capable of creating "single-trial" learning. Most currently available learning paradigms require several trials before the animal has demonstrably learned the task. Clayton and Dickinson (Clayton and Dickinson, 1998) provided arguably the most convincing example of animals' ability to form "what-where-when" associations. They exploited the natural caching behavior of scrub jays. One group of birds cached worms in one of two wells (the other well was covered). After 120 hours, the first food well was covered and the second exposed, and the birds cached peanuts. The birds will normally prefer to eat worms, unless the worms stay out for too long and become unpalatable. Clayton and Dickinson found that the birds in this behavioral group chose the well where they stored the peanuts, knowing that the worms had gone bad after 124 hours of storage. In the control condition (where the

birds cached first peanuts then worms) the birds chose the worms, because the 4hr old worms were still palatable. The birds therefore remembered "what", the type of food, "when", the time when the food was cached, and "where", the spatial location of the well.

No behavioral paradigm suitable for rodents captures all three components of episodic memory, but Day and colleagues (Day et al., 2003) recently introduced an "event arena" that forces the animals to make what-where associations. In the event arena, rats learn to associate a flavored food reward with single well within 48 possible choice wells. In the recall phase the animal is cued with the same flavor and asked to choose the food well where that flavor was experienced. Thus the food location ("where") is associated with the type of food ("what"). Acquisition of the task is sensitive to local infusion of NMDA receptor antagonists into the hippocampus, while recall is sensitive to AMPA receptor antagonists. Similarly, monkeys with hippocampal lesions are unable to learn that seemingly simple scene-place associations such as learning that object A is always rewarded on one side of the room and object B on the opposite side (Gaffan and Harrison, 1989).

It is also clear that rodents are sensitive to temporal context and that the hippocampus is critically involved in encoding that temporal context into memory. Trace conditioning is perhaps the most famous example. In delay fear conditioning, the conditioned stimulus (e.g. the tone) co-terminates with the unconditioned stimulus (e.g. mild foot shock), while in trace conditioning there is a gap between the CS and US. Animals can learn delay conditioning without a hippocampus, but are impaired in the trace version, suggesting that the hippocampus is required for associating the two events when they are separated in time (Thompson, 2005).

The hippocampus is also required for learning the sequence of events in animals. In one such experiment, Fortin et al. (2002) trained rats to discriminate between a sequence of odors A,B,C,D,E. They were required to learn that A came before B, B before C and so on, for a food reward. Rats with hippocampal lesions did not learn the sequence of odors, but importantly could still distinguish the odors from odors not presented in the sequence, suggesting that memory for the sequence memory but not recognition memory is dependent on the hippocampus.

Neural Responses to What, Where, and When Information in the Hippocampus

As mentioned in the introduction, rodent hippocampal neurons respond robustly to the animal's position in the environment. Place cells have been seen in every other mammal tested, including bats (Ulanovsky and Moss, 2007), and monkeys (Ono et al., 1993), and humans (Ekstrom et al., 2003). Rodent hippocampal also respond to a variety of non-spatial variables including odors (Wood et al., 1999), objects (especially boundaries) (Rivard et al., 2004), emotional valence (Moita et al., 2003), and behavioral context (Markus et al., 1995; Wood et al., 2000; Ferbinteanu and Shapiro, 2003; Dupret et al., 2010). These responses tend to be weaker than, or conjoined with, spatial responses. In monkeys, the non-spatial properties of hippocampal neurons are by far the most widely reported. It is worth noting, however, that most of these studies have been performed in restrained animals, so any spatial preference can't be expressed. Nevertheless, the neurophysiology is clear. In one typical study where the monkeys were performing an object Rolls and colleagues found that 10% of hippocampal neurons

responded differently to objects independent of location, 13% to the spatial view and 12% responded to a combination of space and position.

The above data show that both "what" and "where" information is amply represented. What about "when"? Time has been the elusive property of hippocampal neurons, but recent studies are beginning to change that. A pair of studies (Wood et al., 2000; Ferbinteanu and Shapiro, 2003) recorded place cells as rats moved through either a plus maze or a t maze. They found that many cells responded to either the turn that either the animal is about to make or just made suggesting that the neurons are responding to the where the animal is within the timing structure of the task. Pastalkova et al., (2008) recorded neurons from rats as they performed a delayed alternation task. In their task, the rat was delayed by running inside a wheel for 15 seconds before making a choice at a choice point. They found that the cells that were active inside the wheel were not continuously active but only active for short bursts. Surprisingly, the short bursts occured at the same time whenever the animal was in the wheel. These "episode" fields were only present when the animal had to hold in mind what turn to make after leaving the wheel but not when either arm was rewarded.

Summary

The hippocampus has anatomical access to and shows firing correlates of all the relevant information for encoding episodic memories. The results I presented in Chapter II, showing that hippocampal place fields require direct experience by the animal, add a critical missing piece to puzzle: the combination of the events, space are referenced from the animal's perspective, fulfilling the autobiographical reference criteria of Tulving's

original definition. Taken together, these elements make the hippocampus unlike a cognitive map and better resemble an episodic memory system. This formulation does not deny the existence of place cells or the over abundant representation of space within the broader hippocampal formation, but instead considers space to be an essential, but not exclusive, part hippocampal memories.

APPENDIX A

SUPPORTING INFORMATION

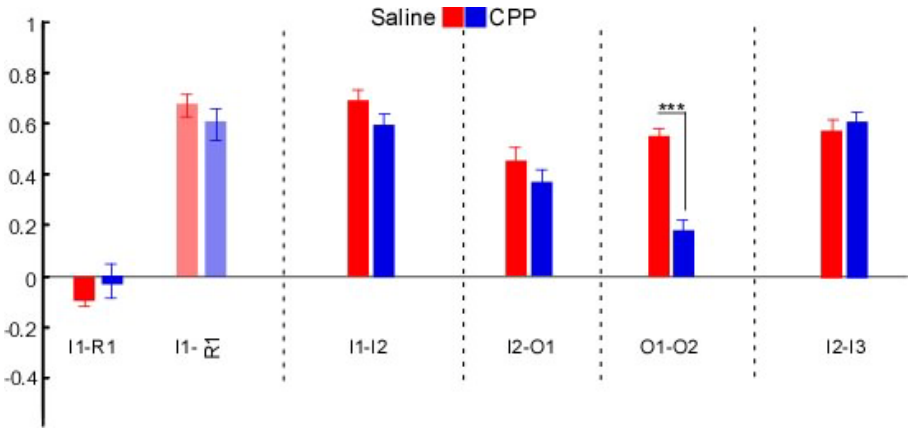

Figure A.1. Correlation scores for session pairs. Height of bars gives the mean correlation score. Error bars are SEM. Sessions being compared are given underneath the bars. Solid bars in the first group are the I1 to R1 comparison. Light bars in that same group are I1 to a clone of the R1 map rotated counter clockwise 90 deg to offset rotation of the cues. The third group shows lower means than the other groups due to the effect of barrier removal described in Supplementary Figure 2. The only significant difference between CPP and Saline was seen in the O1-O2 comparison. In contrast to the figure in the main text, the correlation score shown here is for the entire environment and not broken into inner and outer box areas

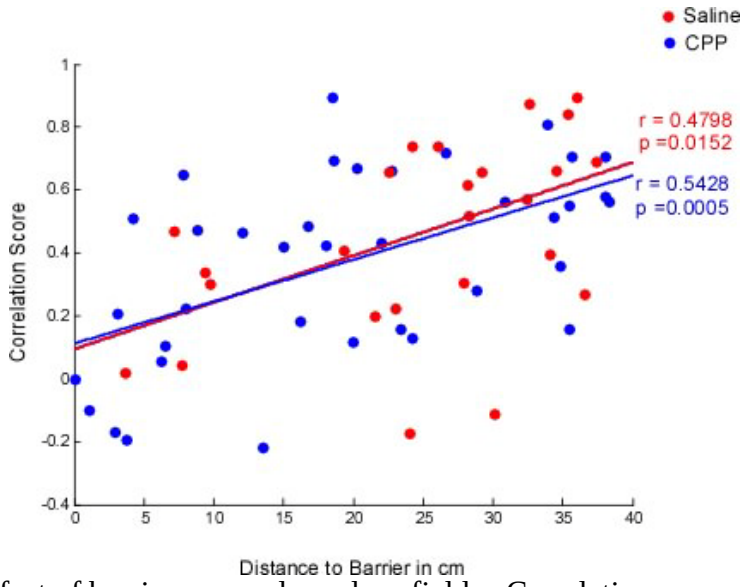

Figure A.2. Effect of barrier removal on place fields. Correlation scores for the I2-O1 comparison are plotted against the distance to the barrier. A linear relationship between the two variables suggests that place fields near the removed barrier were preferentially destabilized. No differences were seen between the Saline and CPP groups.

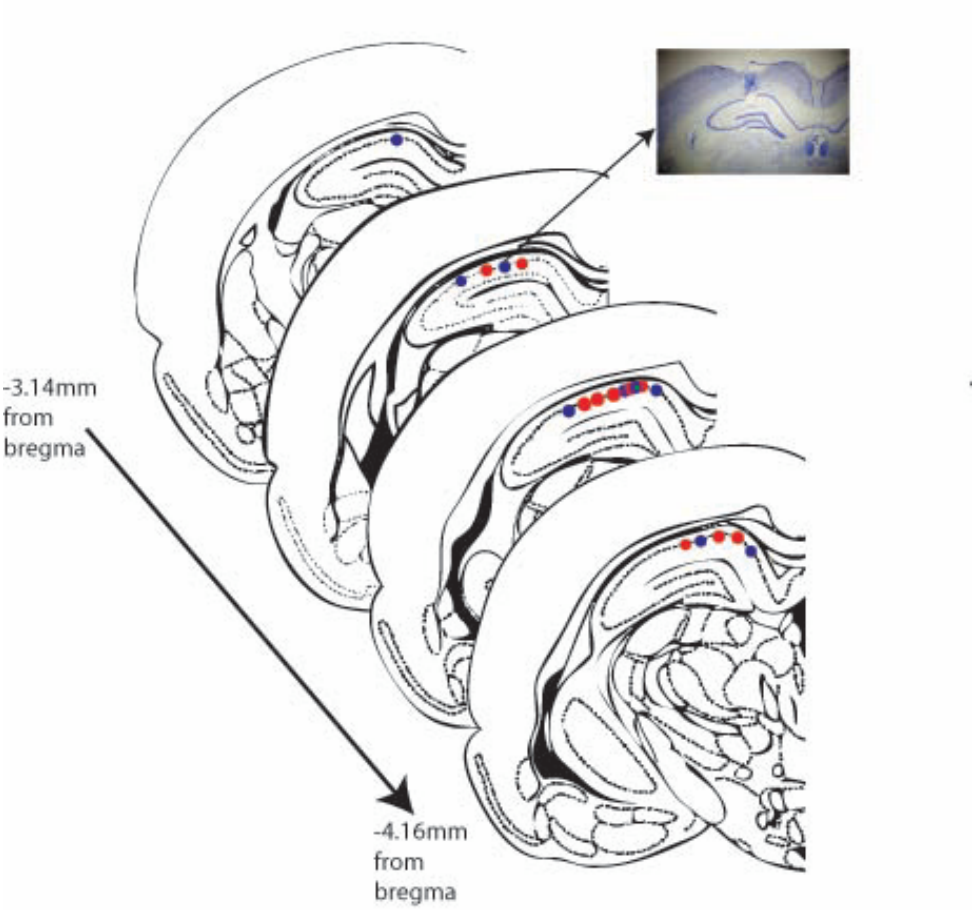

Figure A.3. Recording locations. Final electrode positions are given as red (saline) and blue (CPP) dots. Inset shows an example animal. Images were traced in from the rat brain atlas(Paxinos and Watson, 1998) using Adobe Illustrator (Adobe, San Jose, CA).

Table A.1. Single Session Statistics. Single session statistics described in the Full Methods are given for saline and CPP groups. CPP did not significantly alter any parameter in the standard environment (I2), but did significantly reduce the mean firing rate and coherence in the expanded environment, consistent with the degradation of place fields in response to novel environments seen in NMDA receptor knockout mice (Nakazawa et al., 2003).

	I1	R1	I2	O1	O2	I3
Mean Rate	0.53 ± 0.11 0.71 ± 0.09	0.56 ± 0.11 0.78 ± 0.10	0.54 ± 0.18 0.63 ± 0.08	0.48 ± 0.14 0.80 ± 0.09**	0.63 ± 0.13 0.68 ± 0.07	0.54 ± 0.11 0.51 ± 0.06
Peak Rate	6.69 ± 0.43 7.73 ± 1.09	5.99 ± 0.36 7.82 ± 0.99	5.90 ± 0.46 7.19 ± 0.95	7.64 ± 0.49 10.32 ± 0.89	11.09 ± 0.37 13.73 ± 1.18	5.81 ± 0.31 4.94 ± 0.49
Coherence	0.56 ± 0.09 0.62 ± 0.05	0.51 ± 0.07 0.62 ± 0.49	0.52 ± 0.09 0.63 ± 0.05	0.46 ± 0.08 0.57 ± 0.03*	0.45 ± 0.05 0.64 ± 0.04***	0.56 ± 0.07 0.58 ± 0.05
Field Size (pixels)	25.84 ± 5.33 30.25 ± 3.90	28.74 ± 5.54 36.12 ± 4.35	30.94 ± 7.61 35.07 ± 3.89	32.59 ± 9.19 40.22 ± 3.74	27.42 ± 5.04 34.11 ± 2.74	26.96 ± 4.25 37.47 ± 4.38
Median # Fields	1 1	1 1	1 1	2 2	2 2	1 1

CPP ± SEM
Saline ± SEM
* = P < 0.05
** = P < 0.01
***= P<0.0001

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