Rapid updating of the hippocampal representation of space

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For my family

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Abstract

Several studies suggest that rats solving spatial tasks in relation to stimulus arrays fail to use novel cues to find a goal. This is analogous to a learning phenomenon called 'blocking' where prior associations of a stimulus and an event prevent the subsequent association of a novel stimulus with the same event. Contrary to these findings, the cognitive map theory of spatial learning predicts that novel spatial cues should be attended to and rapidly incorporated into the representation of the environment. We therefore ask if blocking is seen for stimulus control over the firing fields of hippocampal place cells. Rats in two blocking groups foraged for food pellets in the presence of a white card for 40 mins. Rotations of the white card caused equal field rotations. Rats were then exposed to the white card and a novel black card for 10 mins (short compound group) or 60 mins (long compound group). Rotation of the black card alone elicited equal field rotations as well as a significant decrease in field coherence. Fields from 2 long compound rats remained in a similar position as the previous session suggesting that these rats were 'blocked'. Rats in the control group were initially exposed to both black and white cue cards for 20 mins. Each card exhibited control over field location although significant decreases in firing rate were found. The results suggest that rats tend to incorporate new spatial information in a map-like representation of the environment. However, increased compound exposure time may make spatial blocking possible.

The hippocampus is an essential component in the encoding of memory. Its unique architecture and inputs from many different sensory modalities supply an architecture that is believed to provide a mechanism for the representation of space (O'Keefe and Nadel, 1978). The strongest support for the role of the hippocampus in the formation of memories and its intimate role in the processing of space comes from neuropsychological studies demonstrating anterograde amnesia in a patient with hippocampal resection (Scoville and Milner, 1957) and by the observation that hippcampal pyramidal cells in rats have receptive fields in space that are called "place cells" (O'Keefe and Dostrovsky, 1971). At the computational level, the organization of hippocampal and extra-hippocampal circuitry has led to the theory that the hippocampus facilitates rapid autoassociative memory that allow for the underpinning of cognitive processes such as pattern completion, pattern separation and detection of novelty (Gray, 2000; Leutgeb and Leutgeb, 2007; Marr, 1971; McNaughton and Morris 1987; Rolls 1989; Treves and Rolls 1991). Finally, the neurons of the hippocampus exhibit what is considered to be the biological substrate or molecular mechanism of learning in the form of co-operative, long-term synaptic enhancement (Bliss and Lomo, 1973; McNaughton, Douglas and Goddard, 1973). Taken together, these features make the hippocampus a key structure in understanding the biological and computational mechanisms of learning, associative memory and the nature of cognitive representations. Perhaps the most studied cognitive representation supported by all of these different hippocampal processes is the cognitive map.

The nature and parameters of spatial learning, its underlying physiology and associated cognition, have been characterized by O'Keefe and Nadel (1978) in their Cognitive Map Theory, in which they separate spatial learning and navigation into 2 categories: *taxon* and *locale* learning. The theory is grounded in the discovery of place cells, or the tendency of an individual hippocampal pyramidal neuron to fire when the rats' head is in a circumscribed area of the environment called a 'firing field' (O'Keefe and Dostrovsky, 1971; O'Keefe, 1976). These firing fields are described by O'Keefe and Nadel as behaving like co-ordinates in a map. They also continue to fire in the same pattern upon repeated exposures to a given environment. O'Keefe and Nadel (1978) proposed that the hippocampus thus acts as an allocentric mapping system creating,

updating, and possibly storing maps of any given environment. Spatial navigation that was dependent upon this mapping system, and thus an intact hippocampus, fell under the banner of locale learning. Meanwhile, navigation that was dependent on route, guidance (e.g., 'walk toward the clock tower') and orientation (e.g. 'turn right on Atlantic Avenue) hypotheses, would be considered taxon strategies. While the locale system is thought to be mediated by the hippocampus, it is proposed that guidance and orientation strategies may depend on more diffuse extra-hippocampal structures.

According to O'Keefe and Nadel (1978), the strictest definition of a place cell, reinforcing the belief that the cells act as a substrate for the map of space, includes only those cells whose location specific firing can be shown to be controlled by a subset cues that help define any given environment. This proposed role of hippocampal cells in navigation is supported by lesion studies where hippocampally damaged animals tend to be impaired on tasks in which a goal can be located only in relation to a constellation of several distributed cues. However, hippocampally damaged rats perform normally or better than controls on tasks in which approach responses and body-orientation strategies can be used to locate a goal area (Jarrard, 1983; O'Keefe and Nadel, 1978; O'Keefe and Conway, 1980; Morris, Garrud, Rawlins and O'Keefe, 1982). Normal rats also tend to perform better in spatial tasks with a constellation of cues rather than a cluster of cues focused around a goal area (Suzuki, Augerinos and Black, 1980; O'Keefe and Conway, 1980). In a similar fashion, electrophysiological data have shown that place cells, in accordance with O'Keefe and Nadel's definition, have a stable firing pattern as long as a subset of experimentally controlled cues are available (Fenton, Cszizmadia, and Muller, 2000; O'Keefe and Conway, 1978; Muller and Kubie, 1987; O'Keefe and Speakman, 1987; Quirk, Muller, Kubie, 1990).

Certain features of the neural circuitry of the entorhinal-hippocampal and intrahippocampal projections are believed to be of computational importance and ultimately make cue redundancy or *'pattern completion'* possible. Specifically these features are thought to be the recurrent collaterals in region CA3, and the size of the dentate gyrus. The extensive recurrent connections between pyramidal cells in CA3 has been theorized to support an auto-associative memory (Marr, 1971; McNaughton and Morris, 1987; McNaughton and Nadel, 1990; Treves and Rolls, 1992; McClelland,

McNaughton and O'Reilly, 1995). In these models, based partially on Hebbian learning in the recurrent connections, presentation of only part of a previously learned configuration of cues can produce retrieval of an entire stored representation.

Place cell data provides evidence for both pattern completion and pattern separation (or '*orthogonalization*'). As previously mentioned, re-presentation of a subset of the original cues present during training can maintain normal place cell firing (Fenton, Cszizmadia, and Muller, 2000; O'Keefe and Conway, 1978; Muller and Kubie, 1987; O'Keefe and Speakman, 1987; Quirk, Muller, Kubie, 1990). Apart from physiological evidence suggesting recurrent connections in CA3, place cell data from mutant mice has also implicated a role of CA3 in orthogonalization (Nakazawa et al, 2002).

Leutgeb et al. (2004) further examined the function of structural differentiation in hippocampal circuitry by examining how cell ensembles in rat CA3 and CA1 generate representations of different spatial environments with common elements but in different rooms. In CA3, distinct subsets of pyramidal cells were activated in each room, regardless of the similarity of the testing enclosure. In CA1, the activated populations tended to overlap across different environments more often than CA3. Moreover, this overlap tended to increase with the similarity of the different enclosures. In addition to these findings, the results of the experiment suggest that after exposure to a novel room, ensemble activity tends to develop slower in CA3 than CA1, suggesting that the representations in the two circuits tend to emerge independently.

In further investigations of remapping in different enclosures, Leutgeb at al. (2005) again examined differences in CA1 and CA3 while altering the appearance of the recording environments. While recording from similar environments in different rooms again appeared to produce a complete remapping, still on a greater scale in CA3, alterations of shape and color to the environment in the same room tended to produce a greater change in cell firing rate than to the position of the cell's firing field. These changes in firing rate tended to be slightly greater in CA1 than CA3. This more subtle type of remapping has been referred to as "rate remapping". It is thus thought that the existence of independent population codes for locations and spatial cues implies that hippocampal cell ensembles may simultaneously convey information related to an animal's position and what is currently present at this position. However, perhaps due to

different training regimes, 'complete remapping' has also been found to occur by changing the recording environment even in the same room (Bostock, Muller and Kubie, 1991; Cressant, Muller, Poucet, 2002; Lever et al., 2005).

A classic example of this type of 'complete remapping,' due to seemingly minute changes to the environment, is a study by Bostock, Muller and Kubie (1991). In this experiment, rats were trained to chase sugar pellets in a gray cylinder with a white cue card on the wall that was later exchanged with a black cue card. Simply changing the white cue card to a black cue card was sufficient to cause stable and complete remappings in half the rats in the experiment, while there was no remapping with the remaining rats. This is thought to mean that half the rats 'judged' the 2 environments to be quite similar while the other half judged it to be quite different. That rats may show individual differences with regard to remapping strongly suggests that prediction rules for hippocampal cell activity are very complex and may be dependent on the experience of the animal.

When considering how the hippocampus functions as a cognitive map, its role in pattern completion and separation has been relatively well established. However, the role of the hippocampus, and in particular hippocampal place cells, in guiding spatial behavior has been somewhat under-explored. The notion that place cells are part of a coherent map that can be used to perform a spatial task, for many years was more of an inference than actual fact insofar as the rotation of relevant spatial cues or landmarks in a given environment would cause place fields to rotate, in unison, by an equivalent degree (Fenton, Csizmadia and Muller, 2000; O'Keefe and Conway, 1978; Muller and Kubie, 1987; Knierim, Kudrimoti and McNaughton, 1995; Jeffery, Donnet, Burgess and O'Keefe, 1997; Jeffery and O'Keefe, 1999).

The study carried out by Fenton, Csizmadia and Muller (2000) serves as a unique example of '*cue control'* over place cell firing in that it also serves as an example of pattern completion. Fenton et al. trained rats to chase sugar pellets in a gray cylinder with a black and white cue card on the wall. Fenton found that removing one of the cues and rotating the remaining cue 45 degrees relative to the standard position, resulted in stable firing fields that rotated, in unison, to an equivalent angle. In short then, the angular position of a single card on the cylinder wall accurately determines the angular position

of firing fields. When the card is rotated to a new position, the field rotates equally, showing that the card exerts a reliable form of stimulus control over field location. Though the findings warrant further study, several other studies have also linked place cell activity to spatial behavior by examining the relation of place field firing to behavioral choices in spatial tasks and found that field location can predict behavioral choices (Speakman and O'Keefe, 1990; O'Keefe and Speakman, 1987; Huxter, Thorpe, Martin and Harley, 2001; Lenck-Santini, Save and Poucet, 2001, Lenck-Santini, Muller, Save and Poucet, 2002).

A key difference in O'Keefe and Nadel's distinction between different types of spatial behavior, is that locale and taxon learning differ greatly in their underlying motivations; Taxon learning is usually carried out to fulfill a biological need such as obtaining a reward or avoiding a punishment, while Locale learning is seen as being driven by curiosity, allowing for construction and continuous updating of an environmental map through exploration. According to the theory, the novelty seeking properties of the mapping system in the hippocampus, critical to its function, have the disadvantage of 'pre-empting' the output systems whenever novelty occurs. Thus in an environment that is seen to be constantly changing, the brain may shift information processing of space to relevant brain structures outside the hippocampus. This variability could result in the interruption of the locale system and bias the organism toward other spatial problem solving hypotheses that are independent of the hippocampus (Knieirm, Kudrimoti and McNaughton, 1995; O'Keefe and Nadel, 1978).

Yet another feature that distinguishes hippocampal dependent locale processes from taxon processes, according to O'Keefe and Nadel (1978), is that the locale system is relatively free from the effects of time and repetition:

> 'Whenever an organism attends to an object it is encoded in the map. Subsequent attention to that object will have no further affect on the representation of that object in the map….By virtue of the workings of the misplace system, the locale system will act in such a way as to direct the animal's attention away from objects whose presence it can predict towards those whose presence was unexpected. In

> this sense it is…a novelty- seeking device. Incorporation of information about stimuli occurs in a non-incremental fashion. The map itself can become richer and more distinct (i.e., there is better and finer differentiation of places) but it is not altered in any fundamental sense with repeated exposures to the same environment.' (p.95)

The notion of hippocampal involvement in novelty detection has been further suggested by studies examining the immediate early gene (IEG) c-fos as a measure of neural responsiveness. C-fos is frequently used in this type of study as its expression is correlated with raised neuronal activity and learning (Herrera and Robertson, 1996; Tischmeyer and Grimm, 1999). Research studying c-fos levels has shown that novel, individual items increase FOS levels in the perirhinal cortex (Zhu, Brown, McCabe and Aggleton, 1995; Wan, Aggleton and Brown, 1999) while novel environments increase hippocampal FOS levels (Hess, Lynch and Gall, 1995; Vann, Brown, Erichsen and Aggleton, 2000). In addition, FOS levels were also found to increase in response to novel arrangements of familiar items in subfields of the hippocampus (rostral CA1, rostral CA3 and rostral Dentate Gyrus) as well as the parietal cortex and postsubiculum (Jenkins, Amin, Pearce, Brown and Aggleton, 2004; Wan, Aggleton and Brown, 1999). These results provide further support for the notion that the hippocampus is selectively involved in novelty detection in relation to arrangements of multiple spatial stimuli.

These results are further supported by the findings of Lenck-Santini, Rivard, Muller and Poucet (2005). Lenck-Santini et al. found that rotating 2 intra-maze cues in relation to the cylinder center and a static white cue card on the wall of the cylinder has different results for place fields that were near the intra-maze objects and those nearer the cylinder wall. Many of the 'near fields' stopped firing altogether while those fields that remained intact after the object rotation rotated to unexpected angles with a great deal of variation from the expected 90 degrees. Far fields appeared to be unaffected by the rotation of the intra-maze cues. Furthermore, replacing one of the intra-maze cues with a new stimulus caused the rat to investigate the new cue, as measured by the rat spending

significantly more time exploring in the vicinity of the new cue, but substituting the cue did not have any affect on the activity of firing fields either 'near' or 'far' from the cue. There was no partial remapping. Place cells did not stop firing. These results are rather difficult to interpret in the context of the aforementioned emphasis on the role of the hippocampus in novelty detection. How then was the hippocampus responding, if at all to the novel spatial cue? A potential answer may lay in the inherent differences between intra-maze and distal cue alterations and their effects on place cell firing (Cressant, Muller and Poucet, 1997; Cressant, Muller and Poucet, 1999) and spatial behavior. Clearly, substituting one of the intra-maze cues for a novel object did not have the same result as the Bostock, Muller and Kubie study (1991) that showed that merely replacing the white cue card with a black card could cause remapping in a matter of minutes. One interpretation could be that the new stimulus was readily added to the previous cognitive map and therefore the environment was seen as not being sufficiently different enough to warrant remapping. If not for the experimenters having taken a measure of the exploratory activity around the new stimulus, one might argue that the rat did not notice the changed cue or that the identity of the cue was somehow superfluous. Lenck-Santini at al suggest that it may not be the case that the identity of the object is redundant, rather that the configuration of the objects relative to the cue card is more important than their identity (Biegler and Morris, 1999; Cheng, 1986; Cheng and Gallistel, 1984; Hermer and Spelke, 1994). This explanation, however, begs the question that if the identity of the intra-maze cue is so unimportant and the overall shape of the array so informative, why should landmark identity have any influence over exploration once changed? A possible explanation could be that a landmark may be more conspicuous when initially learning about an environment, but with additional experience the geometry of a given array may become more salient and overshadow the identity of specific landmarks (Gallistel, 1990; Worden, 1992).

The notion of cue competition or cue redundancy in spatial stimulus processing is best explored in Kamin blocking (Biegler and Morris, 1999; Kamin, 1968; Sutherland and Hamilton 2004). Kamin (1968) started out with the intent of using the previously established procedure of conditioned emotional response (CER) training in Pavlovian conditioning (Estes and Skinner, 1941) to examine the role of "attention-like" processes

in conditioning. Kamin's theoretical approach, in general, was to train an animal to respond to a simple CS, consisting of element A and then train the animal to respond to a compound consisting of element A, plus a superimposed element B. In the final phase of the experiment the animal would be tested with element B alone in order to determine how much the animal had learned, if anything at all, about element B during the compound phase of the experiment. Kamin theorized that due to the prior training to element A, this element might so "engage the animal's attention" during presentation of the compound that it would not "notice" the added element B. The failure 'to notice' the superimposed element might thus preclude any conditioning to it. Over the last 27 years the phenomenon of Kamin blocking has proven to be a relatively robust phenomenon and an important stumbling block for any new theory of associative learning processes.

O'Keefe and Nadel (1978) describe spatial learning and navigation, as expressed in the hippocampus, as not subject to the same rules of cue competition as other forms of associative learning rules. As mentioned previously, the cognitive map theory suggests that through experiencing an environment there is an automatic updating of stored information about topographical relationships among cues. Spatial learning is an all or none rather than gradual process. Therefore, spatial learning should not be subject to the laws of associative learning.

If the rules governing locale learning are different from those governing associative learning, then associative learning phenomena should not be found in locale learning. If the hippocampus, presumably through its intrinsic connectivity, is able to automatically update the cognitive map when new stimuli are introduced then blocking should not be found in the spatial domain. In other words, after some investigation one should be able to encode the relationship between the newly introduced stimuli and the stimuli that had previously been present. Furthermore, if the older stimuli are removed, leaving only the newly introduced stimuli, one should be able to use these new stimuli to solve a spatial problem. Associative learning theories, such as the Rescorla-Wagner model (1972), predict that new spatial stimuli will be encoded only if the learned relationship between the pre-existing stimuli and a goal zone (e.g., for a food US) is pre-asymptotic, i.e., $\lambda_j > V_j$. To put it another way, if behaviorally performing in a spatial

task, or if the organism has not finished mapping the environment, there may be a greater likelihood of encoding the novel stimuli or updating the map. This may be comparable to Kamin's (1968) experiment in which the rats received pairings of light and shock for only 4 trials before the presentation of the light-tone compound. Given the pre-asymptotic nature of the association between the light and the US, some associativity was available for the newly introduced tone CS. The tone was therefore able to elicit some suppression of the lick response. However, the cognitive map theory proposed by O'Keefe and Nadel (1978) predicts that the new spatial stimuli will be encoded as soon as they have been sufficiently explored.

The first attempt at looking at blocking in the spatial domain was by Chamizo, Sterio and Mackintosh (1985). In this study, rats were trained on an elevated 8 arm radial maze to locate relevant arms for food reward using either intra-maze cues (floors of different texture regardless of position) or extra-maze cues (the rewarded arm was always pointed toward a particular corner of the room). The results indicate that beginning the training with one set of cues could block the learning of another set of cues in solving a spatial problem when both sets of cues were later presented in compound. Thus, learning to locate a place using proximal or intra-maze cues can block the subsequent place learning using distal or extra-maze cues and vice versa. However, this study may be irrelevant to the present discussion as the results appear to confirm O'Keefe and Nadel's distinctions between the learning differences between locale and taxon hypotheses. As in McDonald and White (1993), the association of a goal arm and a stimulus, be it a light or specific floor texture, with a goal orienting response is likely mediated through extra-hippocampal processes. By setting locale and taxon hypotheses in competition, one may expect to find blocking. A similar difficulty in interpretation can be found in a study by Roberts. & Pearce (1999). This study also looked at blocking between locale and taxon strategies using a widely used spatial task, the Morris water maze. In the first stage of the experiment rats either swam to a submerged platform that had a noticeable beacon attached while a curtain was drawn, thus preventing the room cues from being seen, or they swam to the platform and beacon that were moved from trial to trial with the curtains open and the room cues available. In the second stage, rats again escaped from the pool by swimming to the platform, now with the curtains open and room cues

available for rats in all groups. Probe trials were then conducted in the absence of the platform and the beacon in order to determine how much the rats had learned about the position of the platform in relation to the room cues. The results of the experiment suggest that learning about the room cues in the second stage of the experiment was blocked by the presence of the beacon. In addition, the blocking effect appears to be disrupted by changing the appearance of the beacon for the second stage or compound phase of training, implying a role for Kamin's (1968) notion of surprise in unblocking. Furthermore, the blocking effect was also found to be disrupted by restricting the amount of pre-exposure to the beacon during the first phase of training, thus providing further support for learning models that theorize that blocking occurs due to the existence of a limited amount of associability between a US and a given stimulus and that learning new associations to a US can only occur if learning with the previous stimulus is pre-asymptotic (i.e., Rescorla-Wagner, 1972). A dominant theme throughout this study is that spatial learning, or at least taxon processes, may be governed by a similar error-correcting rule to that on which the Rescorla-Wagner model is based. Since the rats in the study were able to use individual stimuli to locate the submerged platform, most likely using a taxon or beacon strategy, it is possible that they could learn to use a heading vector to accurately find the goal area instead of learning about additional stimuli. This type of learning may create a situation in which individual stimuli compete for the degree to which they signal where a goal is located, in the same sense that stimuli in Pavlovian conditioning must compete for associative strength. However, it has not yet been determined whether or not associative learning processes, or blocking in particular, occurs in locale learning.

In an attempt to address the question of whether or not blocking can be seen using a locale strategy, Rodrigo, Chamizo, McLaren and Mackintosh (1997) introduced a novel distal cue into a previously learned constellation of cues used to locate a submerged platform in a Morris water maze task. The experiment was carried out in a typical blocking design with 3 different phases and included 2 groups of rats, one given initial training in phase 1 with cues A, B, and C, and both then trained in phase 2 with cues A, B, C and X. During the 80 trials of the first phase rats were placed on the submerged platform for 30 seconds. It was only at the at the end of this 'placement' training that the

rats received 8 escape trials where they were permitted to swim through the maze and find the platform. During the compound training in phase 2 of the study, cue X was added to the constellation of cues and the animals again received 40 placement trials before they were given an additional 16 escape trials in which they were allowed to swim to the platform themselves. This was followed by a single probe session on 2 different days with cues A,C and X after both cue B and the submerged platform had been removed. In the first test trial, the blocking and the control group only differ marginally in terms of the amount of time they spend searching in the correct quadrant. This is due to the fact that the performance of the control group is slightly disrupted by the removal of cue B. However, the performance of the control recovers by the second probe trial while the performance of the blocking group remains slightly poorer. In the final 2 probe trials the rats were tested with cues A, B and C and both groups of rats spent more time in the correct quadrant. This difference in performance in the second probe was found to be statistically significant. The results of the final 2 probe trials reinforced the authors interpretation of the results as an indication that the rats in the blocking group were unable to learn about the spatial relationship of cue X to cues A, B and C. This is seen as evidence of blocking in the spatial domain. However, it is not clear whether or not the training procedures may have biased the rats toward a locale or taxon strategy which makes the author's claims regarding spatial blocking rather tenuous.

In an effort to truly test the notion of blocking in locale learning Biegler and Morris (1999) took great pains to develop an original task that could take into account the pitfalls and confounds associated with such studies, and would allow for clearer interpretations of the results. Biegler and Morris wanted to design a procedure that would correct for some of the more problematic procedures in the Rodrigo et al. (1997) study such as the lack of exploration of cues, performance decrement after switching from phase 1 to phase 2, poor determination of strategy used by the rats to solve the spatial problem, and presenting the data in terms of ranking instead of time spent in investigating the goal location. Biegler and Morris (1999) selected a spatial task in which the rats had to ultimately spend 2.5 seconds in a reward zone specified by 2 ambiguous cues. In the first phase of training, one of 2 disambiguating cues was introduced in either the East or West part of the apparatus for the experimental groups. A third stimulus was introduced

for the control group, again in the East or West positions. This was done to correct for any possible differences in stimulus saliency or geometry of the array. There was also a correction here to guarantee that the cues in the East and West side of the apparatus were equidistant from the goal area. In phase 2 of the study all groups were exposed to both cues in either the East or West position. There were then several tests of performance with individual landmarks, both landmarks, interchanged landmarks (i.e., where the cue in the West position was moved to the East position and Vice Versa), and with both cues removed, leaving only the ambiguous cues. When the performance of the experimental group was compared to the control group for the relevant cue and position arrangements, preference ratios were found to be significantly different. The rats in the blocking group did not search in the correct goal area when only the 'blocked' cue was available, even though a significant amount of exploration had occurred around the newly introduced cue at the beginning of phase 2. This result is of critical importance in interpreting the results, as exploratory behavior is a supposed hallmark indication of the updating of a locale representation of space. That this exploratory behavior did not result in the acquisition of the added cue is in direct opposition to O'Keefe and Nadel's (1978) cognitive mapping theory. Analyses of spatial behavior in phase 1 and phase 2 with the pre-exposed cue indicate that there was no change in performance, implying that associative strength of the pretrained landmark had reached asymptote by the end of phase 1. This lends further support to the Rescorla-Wagner model (1972) and its notions of asymptotic learning as an explanation of blocking. Furthermore, changing the location of both the East and West cues had no effect on performance. This finding removed the possibility that rats were using heading vectors to locate the goal location, lending further support to the idea that rats were using a locale strategy in this task. The rats were therefore able to determine the goal location by reference to the shape of the array alone, regardless of the identity of the landmarks. Interestingly, the rats were 'aware' of the switching of the position of the landmarks as there was a small but significant increase in exploration around both cues. The authors conclude that this result may point to several complications regarding array processing and stimulus generalization within the spatial domain. In any case, these results are contrary to O'Keefe & Nadel's (1978) cognitive mapping theory and inconsistent with the Hebbian notion that spatial learning might be associative but

sensitive only to correlations (Biegler & Morris, 1999). Moreover, these findings suggest that the conditions of spatial learning may comply with error correcting learning rules of the kind developed to account for a wide variety of conditioning phenomena.

The purpose of the present study is not to serve strictly as an analysis of blocking or its parameters per se, but rather it is also an attempt to understand the role of hippocampal place cells in the processing of environmental stimuli. Moreover, the study attempts to elucidate the flexibility and dynamics of the mapping system by making subtle alterations to a given spatial environment. How does the hippocampal network, as measured by the activity of place cell firing fields, create a representation of a novel environment and then modify that environment when a novel spatial cue is added? Will this novel cue be sufficient to anchor the hippocampal representation of space?

Methods

Many of the methods are adapted from previous work in this laboratory (Muller, Kubie and Ranck, 1987; Fenton, Csizmadia and Muller, 2000) and are only summarized here. Methods developed specifically for this work are stated in detail.

2.1 Subjects and initial training

All animal procedures in the current experiment were carried out in accordance with NIH and institutional guidelines (IACUC# 11-193-05). The subjects were 16 male Long Evans rats (300-350 g). The rats were housed one per cage and maintained on a 12:12 h light:dark cycle in the departmental animal quarters. The first week of training consisted of: 1) Handling the rats daily; 2) Reducing their weight to 85% of the ad lib value; 3) Acclimating the rats for ~30 minutes per day to a square apparatus used to teach them the behavioral task and for single cell screening.

In the "pellet chasing" task, 25 mg sugar pellets are randomly scattered into the apparatus at a rate of ~3 per min. The hungry rats learn to forage for the pellets and consequently run nearly continuously over the entire apparatus floor, making it possible to measure place cell firing rates everywhere. By the end of the first training week the rats were tame, at the desired body weight and spent at least 75% of their time covering the full apparatus area.

2.2 Recording environment

Recordings were made in a 2.5 x 2.5 m sound-proof room. For convenience, the layout of the room and enclosed apparatuses will be described from the viewpoint of the overhead TV camera used to track rats. The soundproof room in which the experiment took place can be thought of as a large square with the usual clock coordinates. Thus, the middle of the top wall was at 12:00 o'clock and the middle of the wall containing an entry door was at 6:00 o'clock. Centered in the room was a black circular curtain 2 m in diameter. Suspended from the ceiling, 2 m above the floor was a 2.0 m diameter black disk on which were mounted 4 light-bulbs whose intensity could be controlled with a Variac. A 2 m diameter black circular curtain was suspended from the disk.

Two apparatuses were used at different stages of the experiment. The first was a white square 76 cm at a side and 66 cm high. The 3:00 o'clock wall position was decorated with a 45 cm wide, 60 cm high black-and-white striped card. The white box was used for pellet chasing training and cell screening. During training and screening, the black curtains were opened by ~ 30° at their top in the middle of the 3:00 room wall and brought together at their bottom against the corresponding box wall. The white box was set on top of a piece of gray photographic paper that was replaced after several sessions.

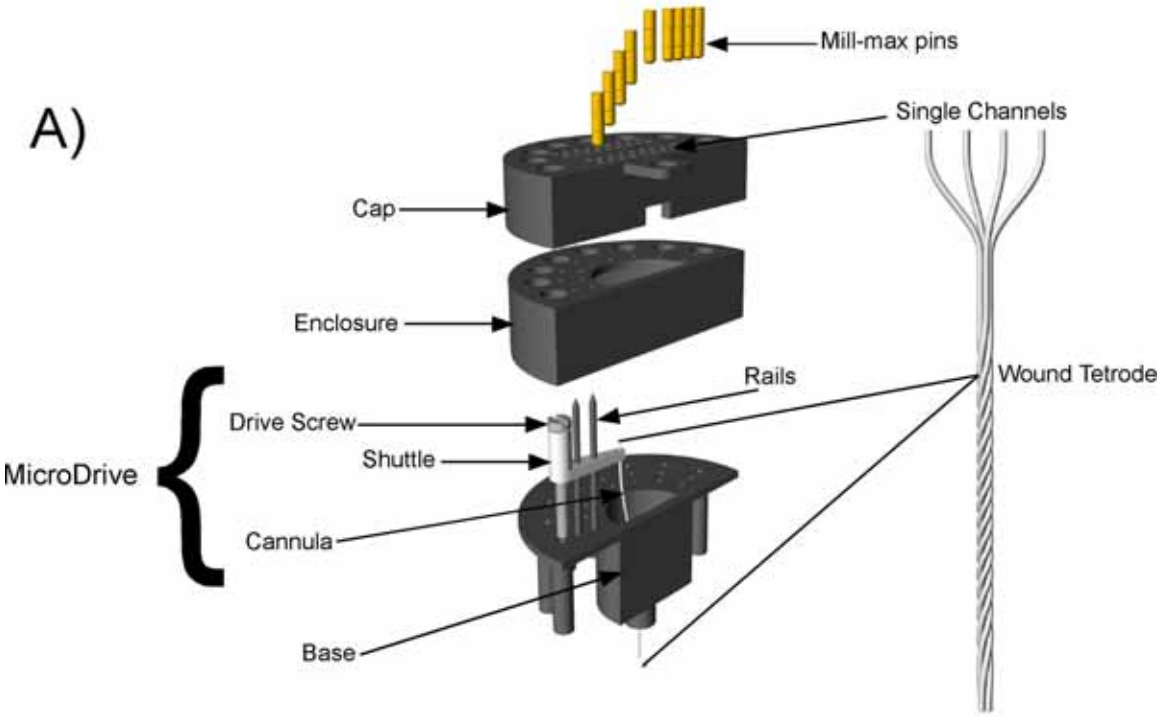
The second apparatus, used for the experiment proper, was a 76-cm diameter, 51-cm high, gray cylinder. During experimental recordings, the curtain was closed to completely surround the cylinder. The cylinder was placed on gray photographic backdrop paper (Setpaper, Thunder Gray) that was changed after each recording session. Polarizing information was provided at different times by one or two cue cards, which in use occupied 45° of the cylinder wall. The white card was made from Color-aid GRAY 9.5 paper (Color Aid Corp, NY); the black card from Color-aid GRAY 2.5. The Color-aid shades were chosen so that their reflectance contrasted equally with the gray cylinder wall (Fenton et al, 2000a). This was done in an attempt to equalize the salience of the two cards. The standard position for the center of the white card was the 12:00 o'clock; alterations in its position and positioning of the black card are described below.

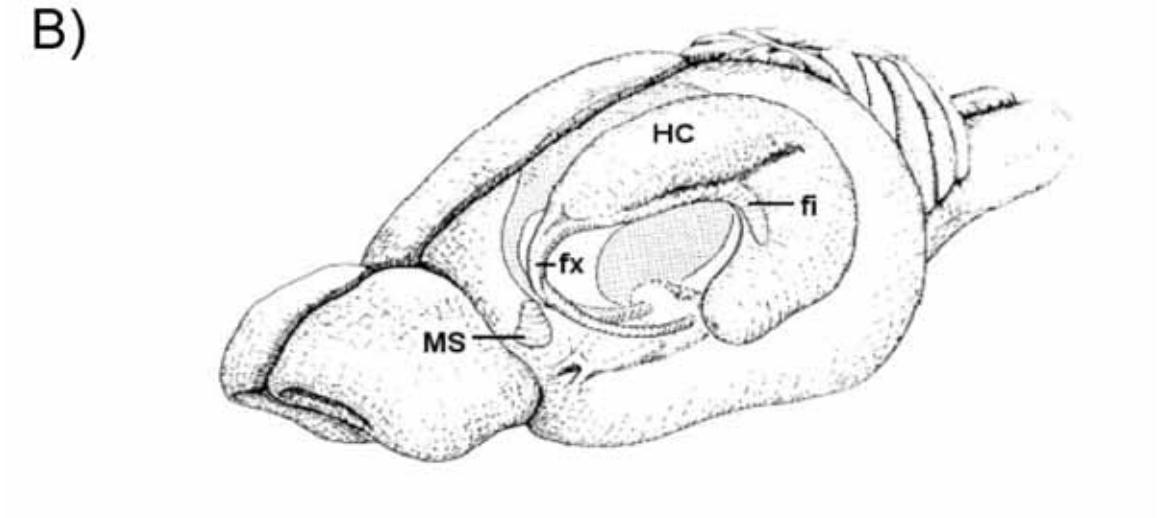
2.3 Recording Implant and Surgery

To permit electrical recordings of single cell activity and the hippocampal EEG a multidrive microelectrode implant (figure 1A) was surgically attached to the rat's skull. The implant contained 8 tetrodes that could be independently lowered into the brain from their initial position. Prior to use, each tetrode wire was gold plated to lower its resistance to ~100kΩ.

For implantation, the rat was anesthetized (Nembutal, 50 mg/kg ip) and placed in a stereotaxic frame. The skull was exposed and holes drilled for anchoring screws over the left and right olfactory bulbs and the left and right cerebellar hemispheres. The screws were inserted and a 2.0 mm diameter trephine hole made above the dorsal hippocampus (3.8 mm posterior to bregma and 3.0 mm lateral to the midline) on the left

Figure 1: Exploded view of recording implant relative to rat brain. A) The cranial implant consisted of 4 principle components constructed from delrin plastic: 1) the base; 2) the microdrive; 3) the enclosure; and 4) the cap. The base supports the implant on the rat's skull and contains several microdrives. Each of the drives allows a separate tetrode to be lowered into the brain. A drive screw moves into a hollow column on the periphery of the base. The shuttle is fitted under the head of the screw and is pushed down when the screw is turned. The shuttle is stabilized by two rails extending through the base. Each cannula contains a tetrode (25 mm nichrome wire) and is attached to the end of the shuttle opposite the drive screw. When surgically attached, the cannula extends ~0.5 mm below a hole in a peg at the bottom of the base with the tetrode tips extending ~1.5 mm lower to enter the brain. The tetrode tips are gold plated to lower the impedance of each wire to ~100 kW. The upper, unwound tetrode wire ends travel from the cannula top through the enclosure to the cap. The enclosure also houses the drive screw and shuttles. At the cap each tetrode wire connects to a Mill-Max pin. B) A three-dimensional diagram of the rat septo-hippocampal system. The hippocampus (HC) is a C-shaped structure whose dorsal region is the target of the tetrodes. The implant (A) is drawn to be above the position at the tetrodes penetrate the cortex in preparation for lowering into hippcampus. Abbreviations: fx = fornix; fi = fimbria; MS = medial septum (modified from Amaral and Witter 1995).


A)
Mill-max pins
Single Channels
Cap
Enclosure
Rails
Wound Tetrode
Drive Screw
Shuttle
MicroDrive
Cannula
Base


B)
HC
fi
fx
MS

side. The dura was removed and the 8 tetrodes inserted in the brain as a bundle with the stereotaxic micromanipulator. The initial depth of the tetrode tips was 2.0 mm below the skull surface; with this placement, they are ~ 1mm above the CA1 cell layer. Once the implant was at the correct depth, the hole was covered with a light coat of vaseline. A ground wire from the implant was connected to one of the cerebellar screws. The implant was fixed to the head by applying Grip Cement to bond the implant to the anchor screws. The wound was closed with surgical clips and a topical antibiotic (Triple antibiotic ointment, Duane Reade) put on the exposed tissue. Rats were given a week to recover from surgery before screening for cells was started.

2.4 Electrophysiological Recordings

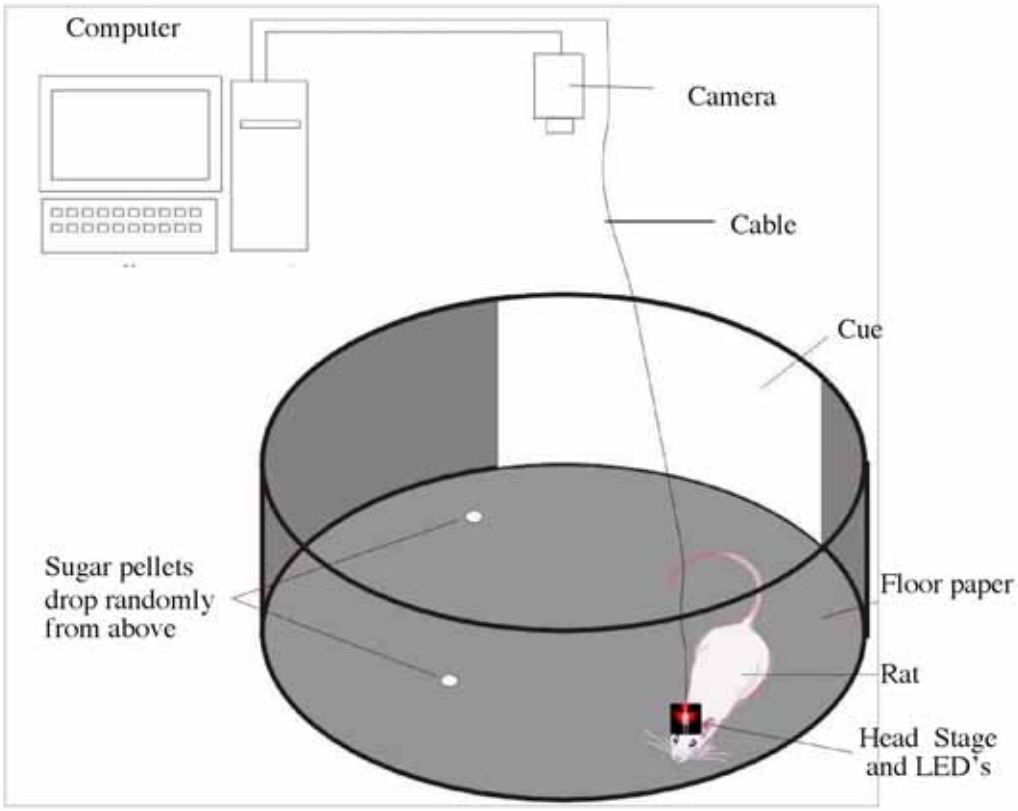
Electrical signals from the tetrodes were amplified X1 at the rat's head (See figure 2A). The signals were led to a commutator (Dragonfly Inc., Ridgeley, West Virginia) via a cable. Although 8 tetrodes were implanted, the commutator could only accommodate 29 channels. Therefore, one tetrode was left in neocortex and one of its wires used as a reference electrode. After the commutator, all signals were amplified X10,000 - 20,000 and band-pass filtered between 300 and 6000 Hz (Neuralynx, Montana). Each signal was digitized at 30 kHz with a 12 bit A/D converter whose outputs were monitored in the computer. A spike-event was stored on disk each time any of the 4 wires of a tetrode exceeded a threshold between 60 and 100 μV. The rat's position was tracked at 30 Hz with a TV/computer system. The signal from a TV camera mounted 2.1 m above the apparatus floor was led to a frame grabber (Data Translation, DT3120) and the digitized intensity values scanned for the images of 2 red light-emitting diodes (LEDs) that were the brightest objects in the video field. The LEDs were mounted on the implant 50 mm above the head and 7.5 mm behind the rat's eyes.

2.5 Cell Screening

Following recovery, screening for single unit activity was started. Screening sessions lasted between 10 and 45 minutes. If the number of well-isolated cells was less than ~10, inactive tetrode drives were advanced by ~60 μm and the rat was returned to its

Figure 2: Schematic of the recording process. A) Electrical signals from the tetrodes were amplified X1 at the headstage. The signals were led to a commutator via a cable. All signals were amplified X 10,000 - 20,000 and band-pass filtered between 300 and 6000 Hz and digitized at 30 KHz with a 12 bit A/D converter whose outputs were monitored in the computer. A spike-event was stored on disk each time any of the 4 wires of a tetrode exceeded a threshold between 60 and 100 μV. The rat's position was tracked at 30 Hz with a TV/computer system. The signal from a digital camera mounted 2.1 m above the apparatus floor was led to a frame grabber and the digitized intensity values scanned for the images of 2 red light-emitting diodes (LEDs) that were the brightest objects in the video field. The LEDs were mounted on the implant 50 mm above the head and 7.5 mm behind the rat's eyes.

A)


Computer
Camera
Cable
Cue
Sugar pellets
drop randomly
from above
Floor paper
Rat
Head Stage
and LED's

home cage for at least two hours or overnight. Once >10 place cells were isolated the experiment began.

2.6 Experimental Protocol

For each rat, the entire experimental protocol was carried out in a single day. During this time, the animal was never detached from the recording cable, enhancing the likelihood that the same cells could be continuously recorded.

To start the experiment proper, the rat was removed from the square and placed in its home cage on a stool outside the curtains. The square chamber was removed and replaced by the cylinder. Intervals between trials lasted ~8 min during which time the floor paper was renewed, the cylinder re-centered and cue card manipulations made. All such manipulations were done with the rat in its home cage outside the curtain.

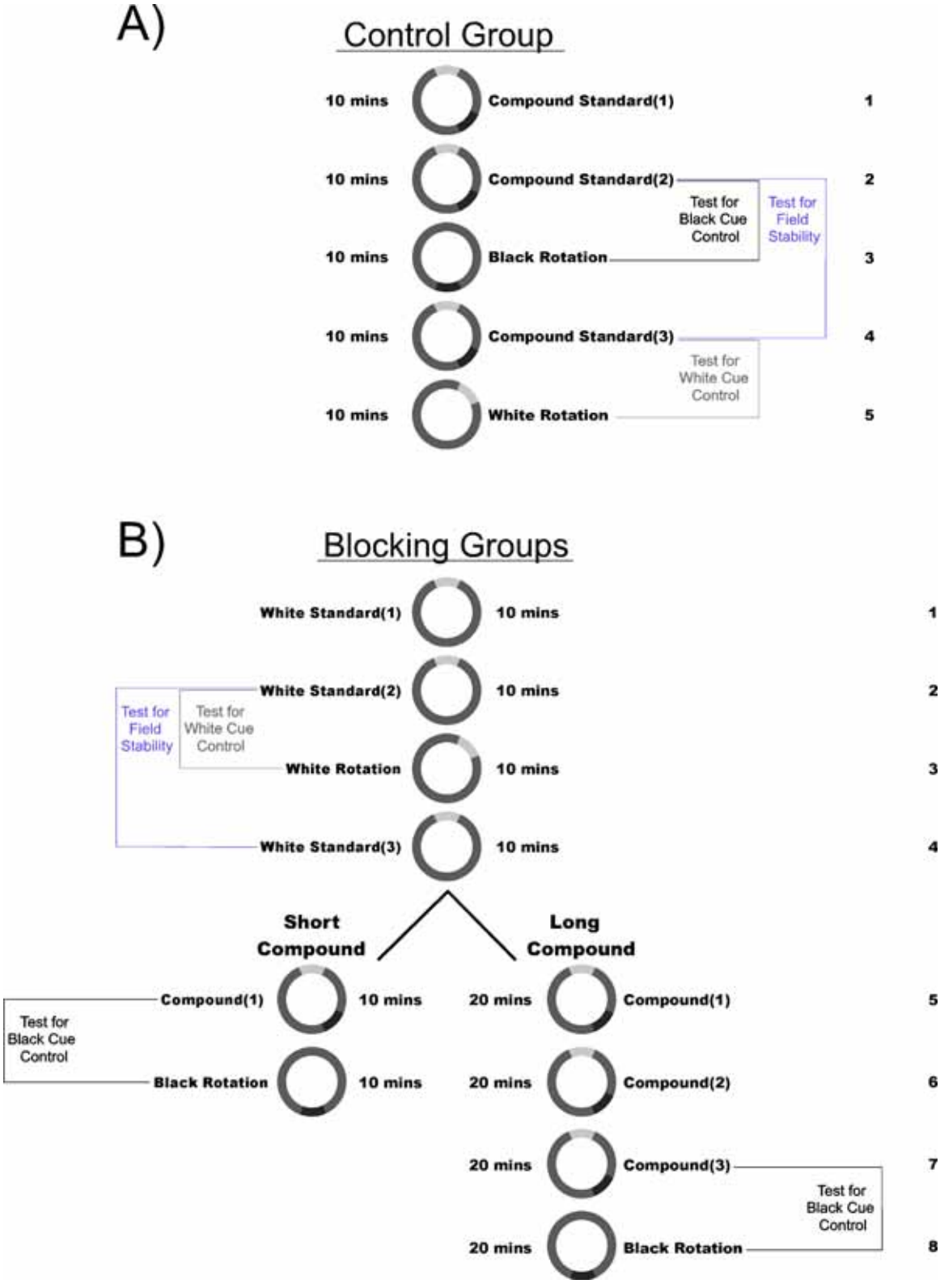
The rats were divided into 3 groups (see figure 3): 1) The control group; 2) The short compound stimulus exposure group; and 3) The long compound stimulus exposure group. For brevity, groups 2 and 3 are called the short and long compound groups; together they are called "blocking groups". The protocol for each group is summarized in figure 3.

Control Group:

The control protocol consisted of 5 sessions that lasted 10 min each. It was a modified version of the cue removal manipulations in Fenton et al. (2000a) but on a timescale more relevant to the blocking design. Sessions 1 and 2 were done with the white and black cards at their standard positions; the white card was centered at 12:00 o'clock and the black card was placed 135° clockwise, centered at 4:30 o'clock. During session 3 the white card was removed and the black cue card was rotated 45° CW to 6:00 o'clock. In session 4 both cues were present at their standard positions. In Session 5 the black card was removed and the white card rotated 45° CW to 1:30 o'clock. The control group was composed of 4 rats.

Short compound group:

Figure 3: The experiment started after the square chamber was removed and replaced by the cylinder. Intervals between trials lasted ~ 8 min. The rats were divided into 3 groups: A) The control group which consisted of 5 sessions that lasted 10 min each. Sessions 1 and 2 were done with the white and black cards at their standard positions; the white card was centered at 12:00 o'clock and the black card was placed 135° clockwise, centered at 4:30 o'clock. During session 3 the white card was removed and the black cue card was rotated 45° CW to 6:00 o'clock. In session 4 both cues were present at their standard positions. In Session 5 the black card was removed and the white card rotated 45° CW to 1:30 o'clock; B) The short compound stimulus exposure group which consisted of 6 sessions; sessions 1 to 5 were 10 min long whereas session 6 was 20 min long. In sessions 1 to 4, only the white card was present. It was at its standard 12:00 o'clock position in session 1, 2 and 4 and was rotated 45° CW during session 3. During session 5 the rat was exposed to the compound stimulus with the white card at 12:00 o'clock and the novel black card at 4:30 o'clock. Finally, during session 6, cue control by the black card over firing field firing was tested by removing the white card and rotating the black card 45° CW to 6:00 o'clock; and 3) The long compound stimulus exposure group which consisted of 8 sessions; sessions 1 to 4 were 10 mins long whereas sessions 5 to 8 were 20 min long. The first 4 sessions were identical to those for the short compound protocol. In sessions 5 to 7, the rat was exposed to the compound stimulus with the white card at 12:00 o'clock and the black card at 4:30 o'clock. Finally, during session 8, cue control by the black card over firing field firing was tested by removing the white card and rotating the black card 45° CW to 6:00 o'clock. Field Rotation analyses serve to measure field displacements in: 1) The first two standard sessions; 2) White card rotation session in comparison to the preceding standard session; and 3) Black card rotation session in comparison to the preceding standard session.


A)
Control Group
10 mins
Compound Standard(1)
1
10 mins
Compound Standard(2)
2
Test for Black Cue Control
Test for Field Stability
10 mins
Black Rotation
3
10 mins
Compound Standard(3)
4
Test for White Cue Control
10 mins
White Rotation
5
B)
Blocking Groups
White Standard(1)
10 mins
1
White Standard(2)
10 mins
2
Test for Field Stability
Test for White Cue Control
White Rotation
10 mins
3
White Standard(3)
10 mins
4
Short Compound
Long Compound
Compound(1)
10 mins
20 mins
Compound(1)
5
Test for Black Cue Control
Black Rotation
10 mins
20 mins
Compound(2)
6
20 mins
Compound(3)
7
Test for Black Cue Control
20 mins
Black Rotation
8

The short compound protocol consisted of 6 sessions; sessions 1 to 5 were 10 min long whereas session 6 was 20 min long. In sessions 1 to 4, only the white card was present. It was at its standard 12:00 o'clock position in session 1, 2 and 4 and was rotated 45° CW during session 3. During session 5 the rat was exposed to the compound stimulus with the white card at 12:00 o'clock and the novel black card at 4:30 o'clock. Finally, during session 6, cue control by the black card over place field firing was tested by removing the white card and rotating the black card 45° CW to 6:00 o'clock. The short compound group was composed of 6 rats.

Long Compound Group:

The long compound protocol consisted of 8 sessions; sessions 1 to 4 were 10 mins long whereas sessions 5 to 8 were 20 min long. The first 4 sessions were identical to those for the short compound protocol. In sessions 5 to 7, the rat was exposed to the compound stimulus with the white card at 12:00 o'clock and the black card at 4:30 o'clock. Finally, during session 8, cue control by the black card over place field firing was tested by removing the white card and rotating the black card 45° CW to 6:00 o'clock. The long compound group was composed of 6 rats.

2.7 Unit isolation and characterization

All recordings were made from the CA1 hippocampal region. Based on electrophysiological properties (Fox and Rank, 1975; Fox and Rank, 1981), single neurons are divided into interneurons (theta cells) and pyramidal cells (complex-spike cells). Interneurons discharge at high rates (> 20 Hz), have short duration waveforms (initial negative peak < 250 μS), never show complex spike bursts and discharge everywhere in the environment. Pyramidal cells discharge at lower rates (< 2 – 3 Hz), have wider waveforms (negative peak duration ~500 μS) and occasionally fire complex-spike bursts. Pyramidal cells may act as "place cells" whose activity is typically confined to a small, cell-specific region called the "firing field".

The most commonly recorded interneuron types are recorded from stratum oriens and dorsal stratum pyramidale. Complex-spike cells are always recorded from stratum pyramidale itself. Only complex-spike cell actvity was analyzed to determine the effects

of stimulus manipulations.

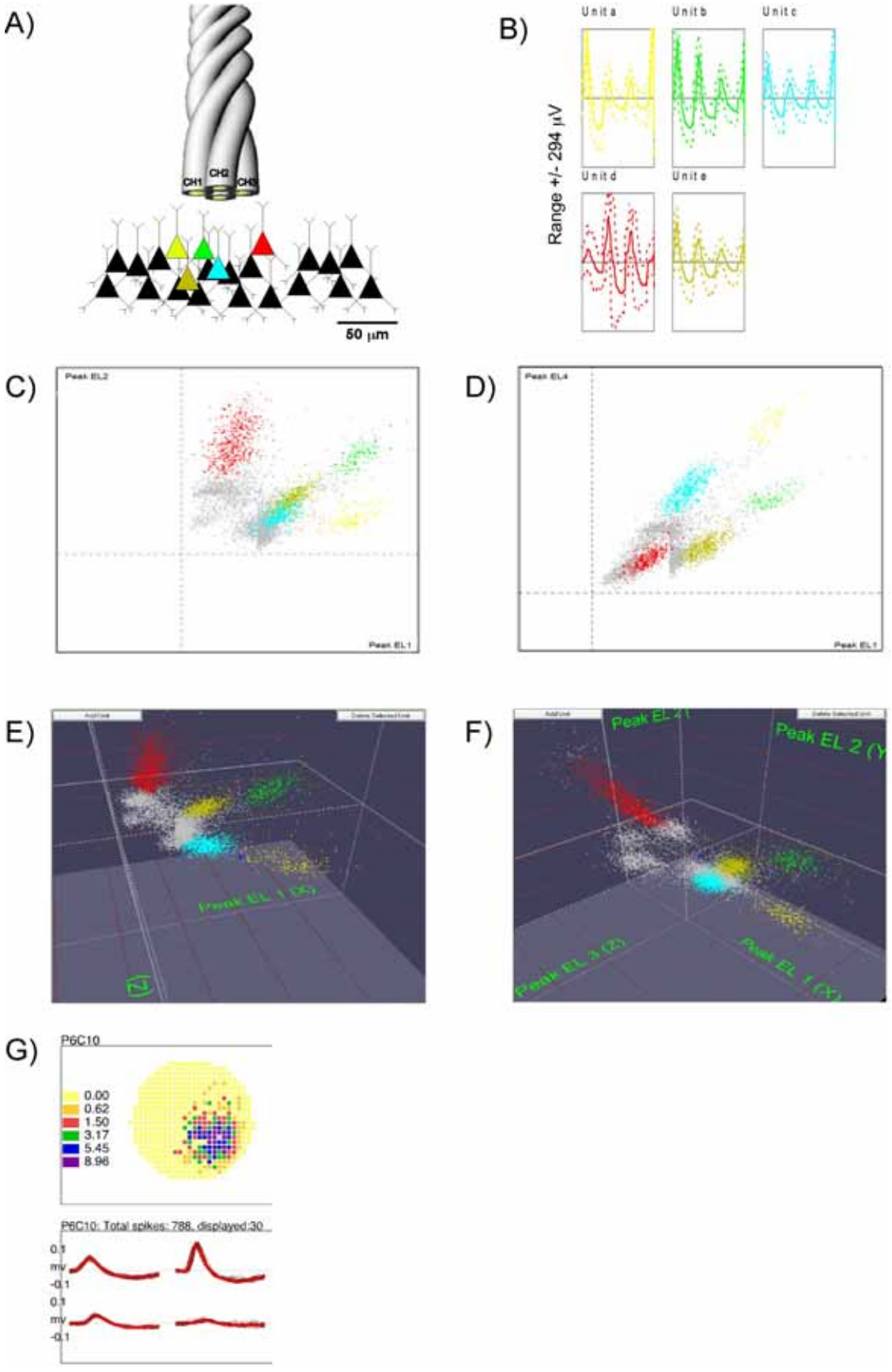
Recording was done with tetrodes (figure 4A). Tetrodes consist of four 25 μm diameter wires. The tips of the twisted wires were close enough to record activity from neighboring neurons, but separated enough so that the waveform generated by a given cell would be different on each wire (figure 4B). Waveform differences were used to distinguish among neurons with clustering methods provided by Offline Sorter (Plexon, Dallas, TX). Numerical properties of each waveform, including peak amplitude as seen on each tetrode wire, minimum amplitude and principle components are calculated and stored. To separate waveform groups into clusters presumably generated by single cells, a three-dimensional scatter plot of selected properties is drawn and rotated so that boundaries between groupings become clear. To ensure that the same waveforms were selected across multiple recording sessions a waveform "template" was created in the initial recording session. This template was then imposed on all of the subsequent recording sessions. Cells that emerged in later recordings were added to the template while cells that dropped out were eliminated from analysis by firing rate criteria. Typically 3 to 6 clusters could be isolated per tetrode, although some had yields approaching 20 clusters.

2.8 Data Analysis

The raw data consisted of series of action potential time stamps for each separated cluster and a 30 Hz series of time stamped positions for the tracking headlights. The initial step was to construct a 64 by 64 element firing rate array for each cell cluster; each element corresponded to a square pixel ~3.0 cm on a side. To make the spatial rate array, the number of spikes fired by each cell was counted for each element. Next, the total time spent by the rat was accumulated for each element. Finally, for each cell, the spike array was divided on an element-by-element basis to form the rate array.

The firing rate array was used in 3 ways. First, it was used to construct color-coded firing rate maps to allow visualization of the positional firing rate distribution. Second, it served as the basis for characterizing several features of the positional distribution. Finally, by comparing the discharge angular locus in recording session

Figure 4: The unit isolation process. A wound tetrode (A) composed of 4 wires were spaced close enough to each other to record the activity of overlapping populations of neurons, but wide enough so that the exact waveform of a neuron's signal would be different on each of the wires (B). Each action potential is represented as a point in a scatterplot that can be set to different projections using the Plexon offline spike Sorting software (C-F). These differences in the shape of the resulting clusters of spikes were then used to distinguish the contributions of different neurons. Two dimensional scatter plots are shown for a number of spike projections in C (Peak electrode 1 vs peak electrode 2) and D (Peak electrode 1 and peak electrode 4). A boundary was drawn around putative clusters until no other separable clusters were visible. Multiple combinations of spike parameters were often taken from each of the 4 tetrode wires and used to isolate units across each of the X, Y, Z axes. Examples are shown in E (Peak electrode 1 vs peak electrode 2 and peak electrode 4) and F (Peak electrode1 vs peak electrode 2 and peak electrode 3). The separated spike data are merged with position tracking data and dwell time to form a firing rate map (G) to visualize the positional firing-rate distribution for each place cell.


A)
CH1
CH2
CH3
50 µm
B)
Unit a
Unit b
Unit c
Unit d
Unit e
Range +/- 294 µV
C)
Peak EL2
Peak EL1
D)
Peak EL4
Peak EL1
E)
F)
Peak EL 2 (Y
Peak EL 3 (Z)
Peak EL 1 (X)
G)
P6C10
0.00
0.62
1.50
3.17
5.45
8.96
P6C10: Total spikes: 788, displayed:30
0.1
mv
-0.1
0.1
mv
-0.1

pairs, the degree of control exerted by the white and black stimuli could be determined for each cell.

Rate maps:

In firing rate maps (e.g., figure 4G), yellow pixels represent square regions in which the firing rate was exactly zero. Increasing firing rates are represented in the color order: orange, red, green, blue, purple. The number key for each map shows the median firing rate for each color category. Pixels never visited by the rat (where the firing rate cannot be estimated) are white.

Field properties:

The firing rate array was used to measure features of the positional firing distribution for each cell including:

1) Overall firing rate (grand rate): The total number of spikes fired by the cell divided by session time.

2) Field rate: The number of spikes in the firing field divided by the time spent in the field. A firing field is defined as a group of > 9 contiguous pixels each of which has a firing rate > 0.1.

3) Coherence: Nearest-neighbor 2-D firing rate autocorrelation (Muller and Kubie, 1989). Coherence is calculated in three steps. First, parallel lists are constructed for the firing rate in each pixel and the average firing rate in the eight nearest neighbors. Next, the product–moment correlation between the two lists is calculated. Coherence itself is defined as the z-transform of the correlation; it estimates the local smoothness of the spatial firing distribution. According to previous work (Rotenberg et al, 1996; Rotenberg et al, 2000), cells with coherence values > 0.3 are taken by trained observers to be place cells.

4) Information content: The reduction of uncertainty of the rat's location (bits/spike) produced by a single spike (Skaggs et al., 1993).

$$IC = \Sigma P_i (r_i/R)\log_2(r_i/R)$$

where r_i is the rate in pixel I, R is the overall rate and P_i is the probability of finding the rat in pixel i.

5) Field area: The fraction of the apparatus area occupied by the firing field.

Place cell selection; To be considered a place cell, a pyramidal cell had to meet the following criteria:

1) Overall rate > 0.1 spk/sec and field rate > 1.0 spk/sec.
2) Coherence > 0.3. Trained observers judge such firing patterns to be place cells.
3) Field area < 0.6.

Field rotation:

The third use of the firing rate array was to quantify angular changes in firing field location between pairs of sessions. Angular field comparisons were made in 2 ways. In the first method, referred to as the "disk method", the entire positional firing pattern for one session was rotated in 1° steps. After each step, the pixel-by-pixel correlation between the stationary and rotated firing patterns were computed. After 360 steps, the maximum correlation was found. The angle of the maximum was taken as the magnitude by which the field rotated. In the second method, referred to as the "wedge method", the firing rate in two sessions was calculated for forty-eight 7.5° wedges. The wedge sequence for one session was rotated in 7.5° steps and the correlation between the two patterns was calculated at each step. The angular position of the maximum correlation was taken as the magnitude of field rotation.

In addition to the selection for place cells according to field criteria (see above) cells were eliminated from consideration if the nature of their firing patterns precluded the measurement of their rotation. Cells were removed from analysis if the center of their firing fields fell within a 7.5 cm diameter of the apparatus center, had two or more fields or had an annular firing pattern. Finally, cells determined by the wedge analysis program to have a maximum rotational correlation between sessions < 0.2 were also dropped from consideration.

There were three aims for the field rotation analysis: 1) To establish field stability; 2) To examine cue control following rotation of the white cue card; and 3) To examine cue control following rotation of the white cue card. The sessions that were compared in order to achieve these aims are shown in figure 6.

Results

3.1 Place cell sample

Recordings were made from 3 groups of rats; the treatment for each group is summarized in figure 3. The control group consisted of 4 rats, the short compound group consisted of 6 rats and the long compound group consisted of 6 rats. Firing field stability was estimated by measuring field rotation between two sequential standard sessions. Control over the angular location of firing fields by cue cards was estimated by measuring field rotation after separate rotations of the white and black cards (see figure 3). The number of cells for each comparison for each group is summarized in Table 1.

	Control	Short Compound	Long Compound
Stability	39	54	60
White card control	39	51	59
Black card control	31	54	47

Table 1: The number of firing fields involved in each session comparison.

3.2 Comparison of analysis methods

For each session comparison, angular rotations for all cells were calculated by both the wedge and disk methods. The results were in very good agreement but within-animal variances were somewhat smaller for the wedge method. Accordingly, all rotation results are based on the wedge method.

3.3 Firing field locations are stable under constant conditions

A great deal of earlier work has shown that firing fields are reproducible if the rat is put back into the recording cylinder with the standard stimulus configuration. Examples of this stability are illustrated in figure 5 for a rat in the control group (figure 5A), the short compound group (figure 5B) and the long compound group (figure 6C). The top row for each part of figure 5 shows the firing rate maps in a standard session for 8 simultaneously recorded cells; the bottom row is a firing rate map for the same cells in a second standard session. The differences between the two maps for each cell are due to

Figure 5: Examples of field stability for 8 simultaneously recorded place cells are shown for rats in each group: A) Control group (Rat39); B) Short compound group (Rat36); and C) Long compound group (Rat28). Firing rate maps for an initial standard session are shown in the top row for each part of the figure; corresponding maps are shown for the same cells in a later session. Differences between map pairs are due to random fluctuations in cell discharge. In particular, there is no tendency for firing fields to systematically rotate in one direction. The angle of peak correlation between standard sessions is near 0° and can be compared with the angle of peak correlation between standard and cue rotation sessions. If the average field rotation caused by card rotation is significantly greater than the nearly zero rotation between standard session pairs one can conclude that the rotated card had stimulus control over firing fields.

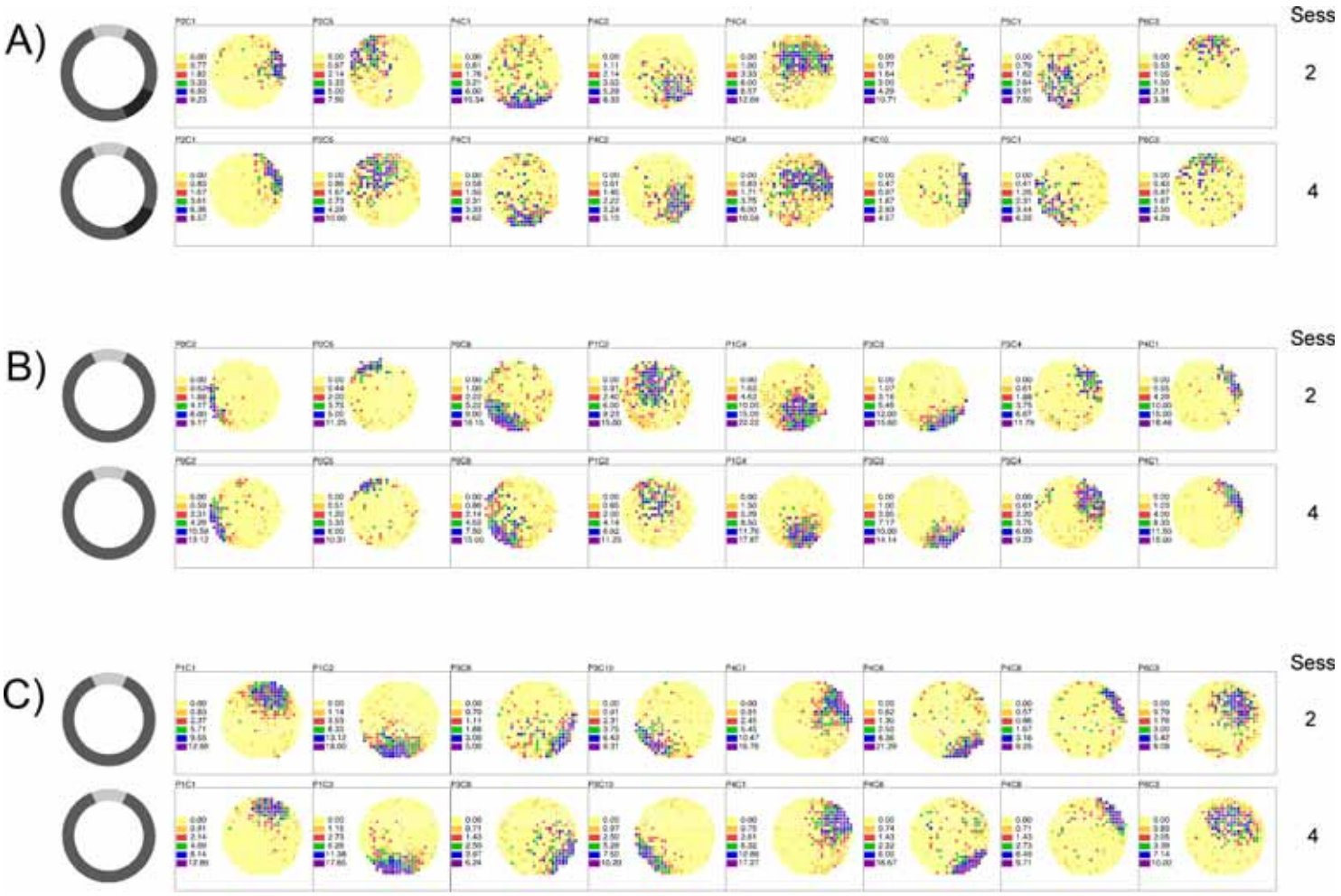
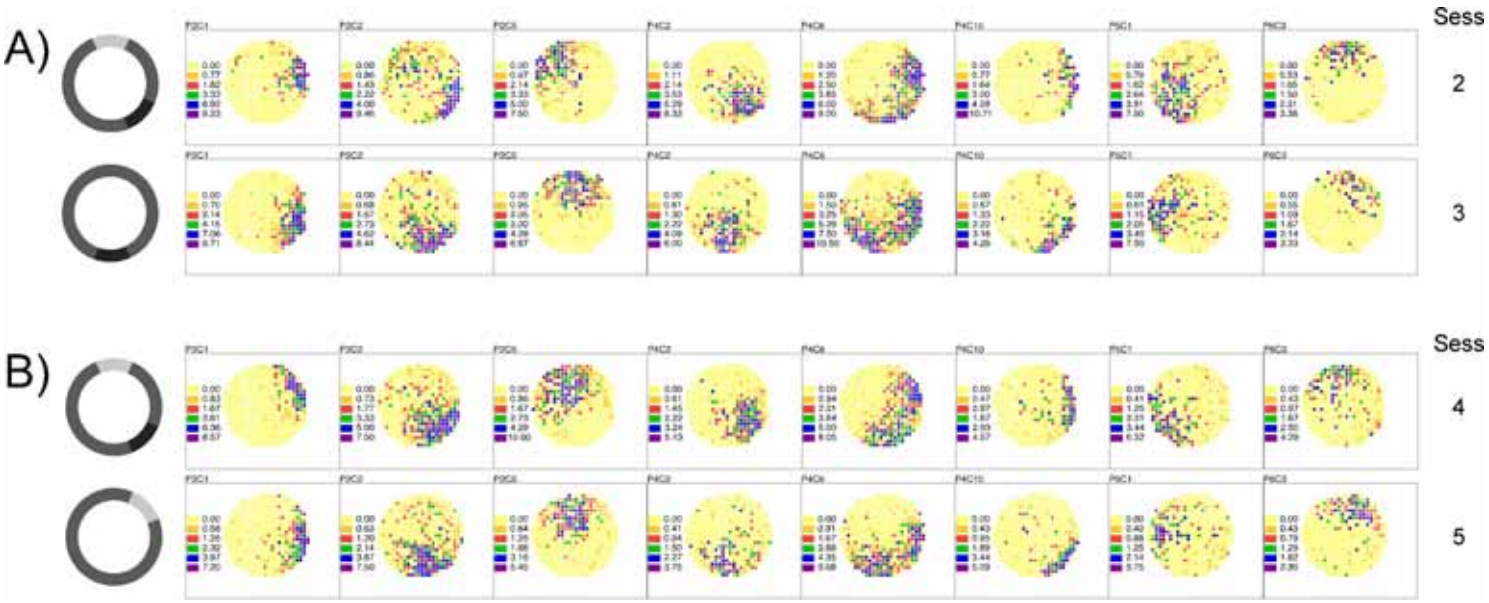

A)
Sess
2
4
B)
Sess
2
4
C)
Sess
2
4

Figure 6: Sample firing rate maps from a control rat (Rat39) showing stimulus control over firing position by white and black cue cards. Eight simultaneously recorded place cells from Rat39 are shown across 4 sessions: A) Firing rate maps from standard session 2 are shown in the top row while the bottom row shows the same cells during a black card rotation session. The black card had clear control over firing field position; B) Firing rate maps from the third compound session are shown in the top row while the bottom row shows the same cells during a white card rotation session. The white card had clear control over firing field position. Differences between map pairs in the two standard sessions are due to random fluctuations in cell discharge. There was no tendency for firing fields to systematically rotate in one direction unless one of the cue cards is rotated. White and black cue cards showed equivalent cue control when an animal was initially exposed to the cylinder with both cards.


A)
Sess
2
3
B)
Sess
4
5

T-tests do not reject the hypothesis that the average rotation was 0° for the control group ($t_3 = 0.37$; $p = 0.73$), for the short compound group ($t_5 = 0.97$; $p = 0.38$) or for the long compound group ($t_5 = -0.15$; $p = 0.89$).

3.4 Stimulus control by cue cards: Control group

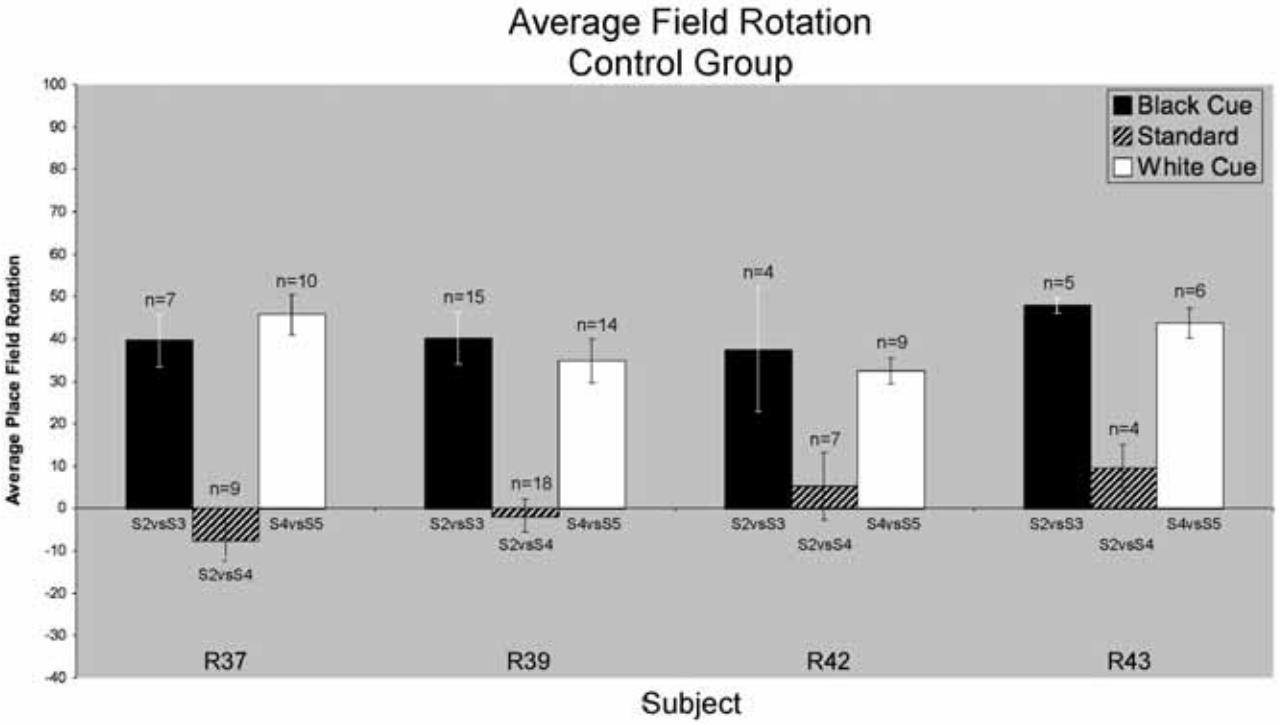
Control rats were exposed to the cylinder for two ten-minute sessions with both the black and white cards present. In session 3, the white card was removed and the black card was rotated. Session 4 was another standard session. Finally, in session 5, the black card was removed and the white card was rotated.

Comparing session 3 to session 2 showed that the black card controlled the angular positions of firing fields. This control is visible in the firing rate maps of figure 6A where the top row of maps shows the spatial firing patterns of the cells from a single rat in a white+black card standard session and the bottom row shows the spatial firing patterns for the corresponding cells in the rotated black card-only session. Similarly, comparing firing patterns in standard session 4 to the same cells in the following white card only session (figure 6B) demonstrates control by the white card.

For the 4 rats in the control group, the average field rotation was 41.3° ± 2.30° after 45° rotation of the black card and 39.2° ± 3.26° after 45° rotation of the white card. T-tests do not reject the hypothesis that the average field rotation was 45° after either card was rotated, but the trend for under-rotation persists in the blocking groups. Paired t-tests indicate that the average field rotation following black card rotation ($t_3 = 12.7$, $P < 0.01$) and white card rotation ($t_3 = 6.85$, $P < 0.01$) are both greater than the negligible rotation between the pair of standard sessions. Another t-test indicates there was no difference between the average field rotation following rotation of the black and white cards ($t_3 = - 0.77$, $P = 0.50$). Thus, when rats were initially exposed to the compound stimulus both cards were salient and the degree of control exerted by each was equal. This equal degree of cue control for the black and white cards can also be seen for individual animals in figure 7. The average angular rotation between a pair of standard sessions is also shown.

3.5 Stimulus control by cue cards: Short compound blocking group

Figure 7: Average angle of peak correlation between firing field maps during the standard sessions or standard and card rotation sessions for individual rats in the control group. The average field rotation between standard sessions and the black and white card rotation sessions was similar to the 45° card rotations. Both the black and white cue card rotations elicited an equivalent amount of field rotation suggesting that either cue was sufficient to drive the positional firing of place cells. The average angle of peak correlation between card rotation sessions was also significantly greater than that between standard sessions. The standard error of the mean and the number of cells for each session comparison is also shown for each rat. While the experimental protocol demanded that cells meet inclusion criteria between standard sessions as well as standard and rotation sessions, cells were not required to meet criteria across all sessions. Cells could therefore be included or excluded over the course of recording, accounting for the different number of cells in each session comparison.


Average Field Rotation
Control Group
Black Cue
Standard
White Cue
Average Place Field Rotation
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
n=7
n=9
n=10
n=15
n=18
n=14
n=4
n=7
n=9
n=5
n=4
n=6
S2vsS3
S2vsS4
S4vsS5
R37
R39
R42
R43
Subject

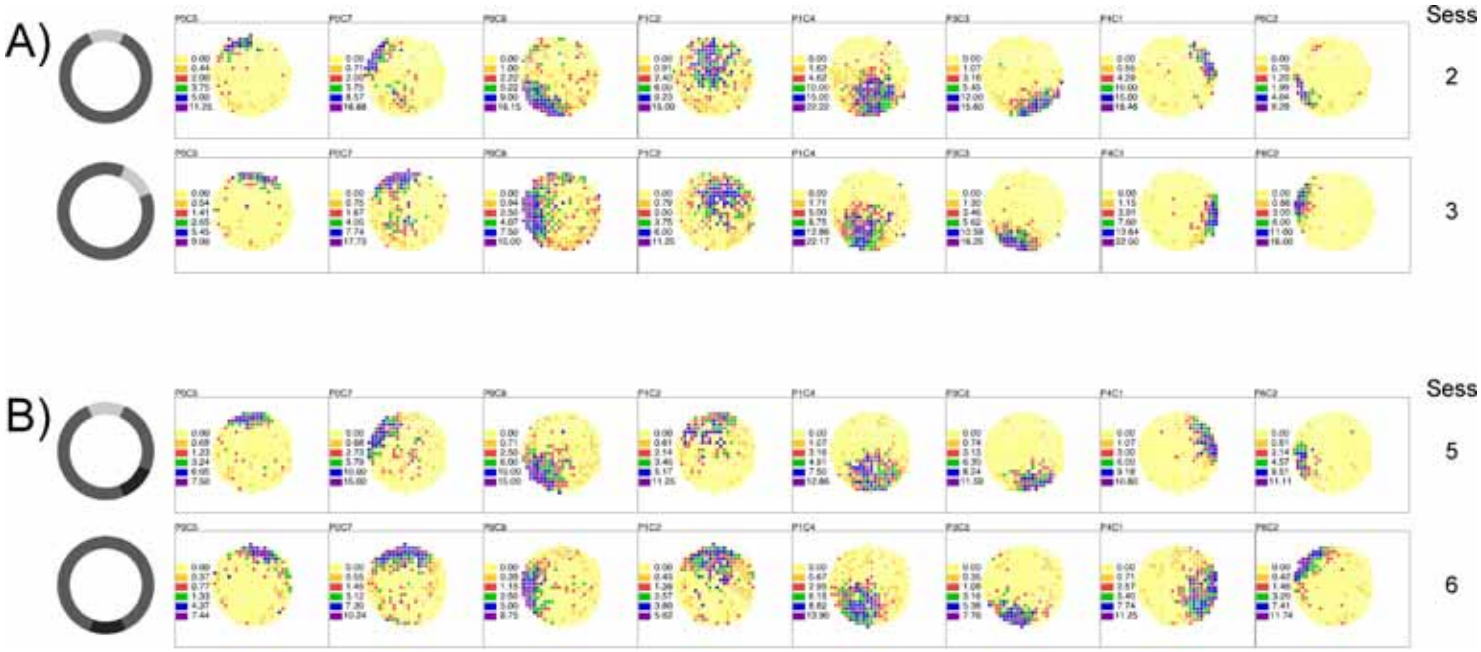
Short compound rats were exposed to the cylinder for 6 consecutive 10-minute sessions. In sessions 1 to 4 only the white card was present. In session 3, the white card was rotated by 45°; in Session 4 it was returned to its standard position. In session 5 (single compound session) the black card was present at the same time as the white card. In session 6 the white card was removed and the black card rotated by 45°.

Comparing session 3 to session 2 showed that the familiar white card controlled the angular positions of firing fields for short compound rats as illustrated seen in figure 8A where the top row of maps is for the standard session and the bottom row is for the rotated white card session. The average field rotation of 40.8° ± 2.63 caused by white card rotation is much greater than the nearly zero rotation in angular field position that occurred between standard session pairs (paired $t_5 = 14.9$; $p < 0.001$). A t-test for the short compound group did not reject the hypothesis that the mean field rotation of 40.8° after white card rotation is equal to the 45° card rotation ($t_5 = -1.61$; $p = 0.17$). Nevertheless, there is a tendency for under-rotation, suggesting that control over fields by the white card is shared with static background cues whose angular position is not altered by card manipulations.

Following a single, brief exposure to the compound stimulus, a black card probe session was run to determine if the newly introduced card had acquired stimulus control. Fields in the black card-only session were compared to the preceding compound-stimulus session. A typical outcome for multiple simultaneously recorded cells is shown in figure 8B where by comparing the compound session (top row) to the black card session (bottom row) it is evident that 45° rotation of the new card caused firing fields to rotate in the same direction by about the same amount. The example of figure 8B therefore suggests that acquisition of stimulus control by the white card did not block acquisition of stimulus control by the black card; the new card gained stimulus control even though it added no new information regarding the orientation of the recording cylinder.

A quantitative treatment leads to the same conclusion; the mean field rotation after black card rotation of 34.0° ± 2.93° was reliably greater than the very small rotation seen between a pair of standard sessions (paired $t_5 = 7.33$; $p < 0.001$) but there was no significant difference between field rotations caused by the white and black cards (paired $t_5 = 1.76$; $p = 0.14$). Nevertheless, the mean rotation of the black card was detectably

Figure 8: Sample firing rate maps from a short compound rat (Rat36) showing stimulus control over firing position by white and black cue cards. Eight simultaneously recorded place cells from Rat36 are shown across 4 sessions: A) Rats in the short compound group were initially exposed to the gray cylinder with a single white cue card on the wall. Firing rate maps from standard session 2 are shown in the top row while the bottom row shows the same cells during a white card rotation session. The white card had clear control over firing field position; B) Firing rate maps from standard session 3 are shown in the top row. It was in this 10 minute compound session that the novel black cue card was added to the environment. Comparison with the top row shows that the introduction of the novel spatial stimulus produced no obvious change in the spatial firing patterns of the place cells. The bottom row shows the same cells during the black card rotation session. The black card had clear control over firing field position. Moreover, both cue cards exhibited an equivalent amount of stimulus control. Differences between map pairs in the two standard sessions were due to random fluctuations in cell discharge. In a single 10 minute session the hippocampal representation of the cylinder was updated to include the black cue card. This occurred without the performance of a spatial task or reward contingency and can therefore be described as incidental learning.


A)
Sess
2
3
B)
Sess
5
6

smaller than 45° (t_5 = -3.74; p = 0.013) again suggesting an under-rotation that may be due to the partial control of static background cues over angular field position.

In addition to conclusions drawn from average group values, it is useful to look at variations of control in individual rats by the white and black cards, as illustrated for the short compound group in figure 9; shown also for each rat is the average angular rotation seen between a pair of standard sessions. It is evident from figure 9 that the same pattern of strong control by the black and white cards is seen for each animal in the short compound group.

3.6 Stimulus control by cue cards: Long compound blocking group

Rats in the long compound group received four 10-min sessions in the presence of the white card only; session 3 was done with the white card rotated by 45°. Sessions 5 through 7 were each done in the presence of the compound stimulus and were 20 min in duration. In session 8, which lasted 20 min, the white card was removed and the black card rotated by 45°.

Control by the familiar white card for long compound rats is visible in figure 10A where the top row is for cells recorded in standard session 2 and the bottom row is for following the 45° white card rotation session. The average field rotation was 36.1° ± 2.33°, a value reliably greater than the angular field shift between a pair of standard sessions (paired t_5 = 7.59; $p < 0.001$). The mean field rotation was, however, significantly lower than 45° (t_5 = -3.82; p = 0.012). Again, this under-rotation is ascribed to partial control by static background cues.

The most common effect of rotating the black card for the long compound group is shown in the example of figure 10B where the top row is for cells recorded during the compound session and the bottom row is for the corresponding cells recorded in the black card rotation session; for each cell its field rotated in the same direction as the black card by about the same amount. Thus, for this animal, pre-exposure to the white card did not block acquisition of stimulus control by the novel black card.

The mean black card induced rotation for long compound rats was 31.4° ± 9.16° where the standard error of the mean (SEM) is considerably greater than for any other pair-wise comparison in the complete data set. On average, there is indeed a trend for the

Figure 9: Average angle of peak correlation between firing field maps during the standard sessions or standard and card rotation sessions for individual rats in the short compound group. The average field rotation between standard sessions and the white and black card rotation sessions was similar to the 45° card rotations. Both the black and white cue card rotations elicited an equivalent amount of field rotation suggesting that either cue was sufficient to drive the positional firing of place cells. In addition, the average angle of peak correlation between card rotation sessions was significantly greater than that between standard sessions. The standard error of the mean and the number of cells for each session comparison are also shown for each rat. The black card attained an equivalent degree of stimulus control as the white card after a single 10 minute compound exposure.

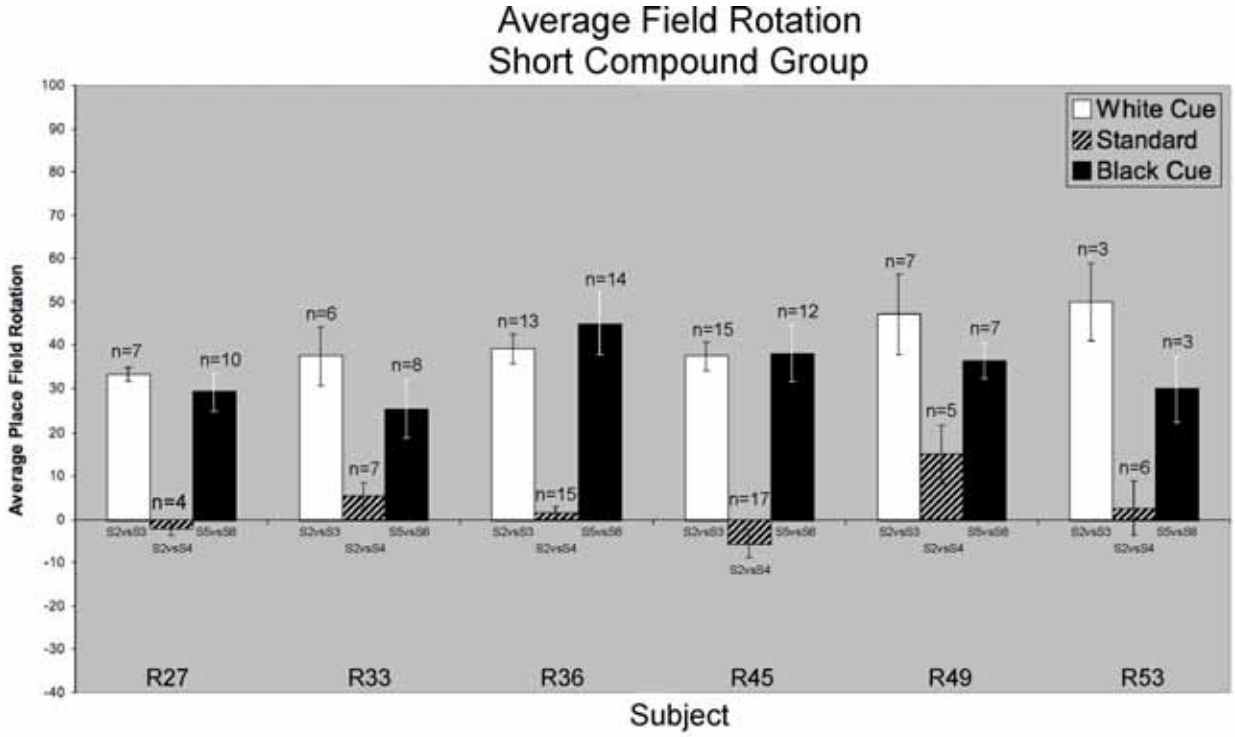
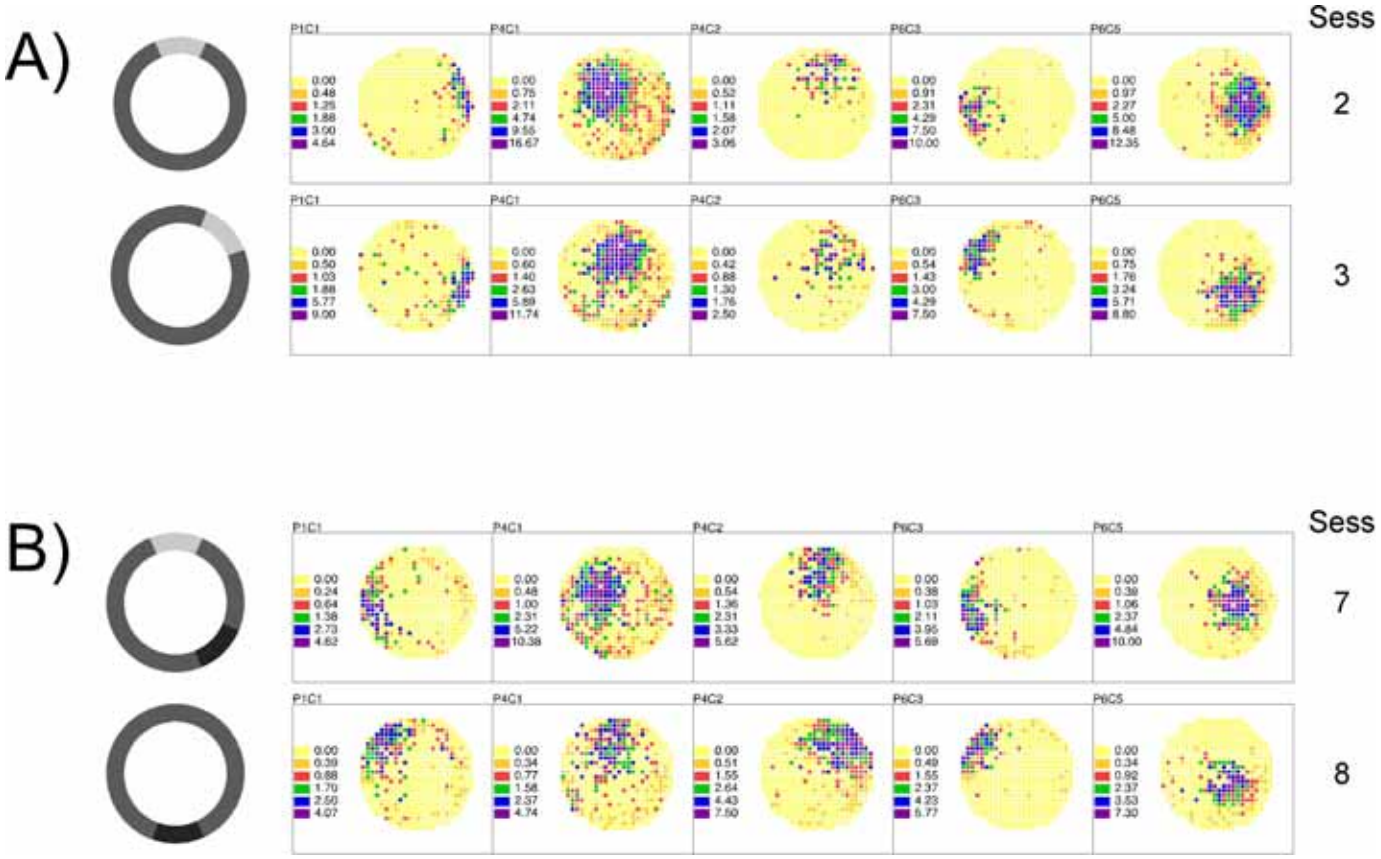

Average Field Rotation
Short Compound Group
White Cue
Standard
Black Cue
Average Place Field Rotation
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
n=7
n=4
n=10
n=6
n=7
n=8
n=13
n=15
n=14
n=15
n=17
n=12
n=7
n=5
n=7
n=3
n=6
n=3
S2vsS3
S5vsS6
S2vsS4
R27
R33
R36
R45
R49
R53
Subject

Figure 10: Sample firing rate maps from a long compound rat showing stimulus control over firing position by white and black cue cards. Five simultaneously recorded place cells from Rat31 are shown across 4 sessions: A) Rats in the long compound group were initially exposed to the gray cylinder with a single white cue card on the wall. Firing rate maps from standard session 2 are shown in the top row while the bottom row shows the same cells during a white card rotation session. The white card had clear control over firing field position; B) Firing rate maps from compound standard session 6 are shown in the top row. This was the final of three 20 minute sessions in which the black cue was presented at the same time as the white cue. Apart from the 225° rotation of cell P1C1, the introduction of the novel spatial stimulus produced no obvious change in the spatial firing patterns of the place cells. The same cells are shown in the bottom row during the black card rotation session. The black card had clear control over the firing field position. Moreover, both cue cards exhibited an equivalent amount of stimulus control. Differences between map pairs in the two standard sessions were due to random fluctuations in cell discharge. Three 20 minute exposures to the novel cue card appeared to have produced the same result as a single 10 minute exposure. The hippocampal representation of the cylinder was updated to include the black cue card.


A)
B)
Sess
2
3
7
8
P1C1
P4C1
P4C2
P6C3
P6C5

black card to effectively control angular field location for long compound rats; the probability of 0.052 that the rotation caused by the black card is greater than the angular field shift between a pair of standard stimulus sessions is just slightly greater than alpha = 0.05 (paired $t_5 = 2.31$). This result is apparently contradicted by the finding that a t-test does not reject the hypothesis that the mean field rotation was 45° ($t_5 = -1.48$; $p = 0.20$).

The large SEM for black card rotation, the lack of a clear difference between the effect of black card rotation and the angular change between pairs of standard sessions and the inability to reject the notion that the correct black card rotation is 45° all stem from a single source that is visible in results for individual long compound rats seen in figure 11. For all rats white card rotation caused a mean field rotation about equal to the 45° card rotation. In contrast, the field rotation caused by the black card was approximately 45° for 4 rats but virtually zero for the other 2 rats. An illustration of fields that track the white card but dot not track the black card can be found in figure 12.

Separate t-tests for the cells in these 2 rats confirm the lack of rotation. Thus, comparing mean field rotation between a compound stimulus session and the black card session versus a pair of standard sessions shows there was no difference for either Rat 28 ($t_{26} = 0.56$, $p = 0.58$) or Rat 32 ($t_{13} = 0.34$, $p = 0.74$). On the other hand, testing the average black card-induced rotation for the remaining 4 rats (43.4°) against corresponding rotations for a pair of compound stimulus sessions (-7.6°) yields a reliable difference (paired $t_3 = 7.05$; $p = 0.0059$). We believe, therefore, that blocking acts in an all or none way for individual rats; pre-exposure to the white card may eliminate or not interfere with acquisition of stimulus control by the black card. Moreover, even when 2 rats for the long compound group were blocked, the average field rotation for each group indicated a strong tendency for equivalent cue control for the white and black cue cards (figure 13).

3.7 Aberrant fields

Aberrant fields from 2 different rats that shifted their firing fields 225° between a black and white compound session and a black card rotation session were found. Two simultaneously recorded cells from Rat42 (figure 14 A and B) appear to have symmetrical firing fields (180° apart) in the standard session with the black and white cue

Figure 11: Average angle of peak correlation between firing field maps during the standard sessions or standard and card rotation sessions for individual rats in the long compound group. For 4/6 rats (rats 52, 48, 51 and 31) the average field rotation between standard sessions and the white and black card rotation sessions was similar to the 45° card rotations. For these animals, both the white and black cue card rotations elicited the same amount of field rotation suggesting that either cue was sufficient to drive the positional firing of place cells. The average angle of peak correlation between card rotation sessions was significantly greater than that between standard sessions. In contrast, rats 28 and 32 do not fit this general trend. The average field rotation following rotation of the black card is closer to 0° than 45°. This suggests that the black card did not acquire clear stimulus control in these 2 cases. The standard error of the mean and the number of cells for each session comparison are also shown for each rat. Since blocking was only found in 2 animals in the long compound group, this suggests that increased exposure to the white and black cue cards together may allow blocking to occur.

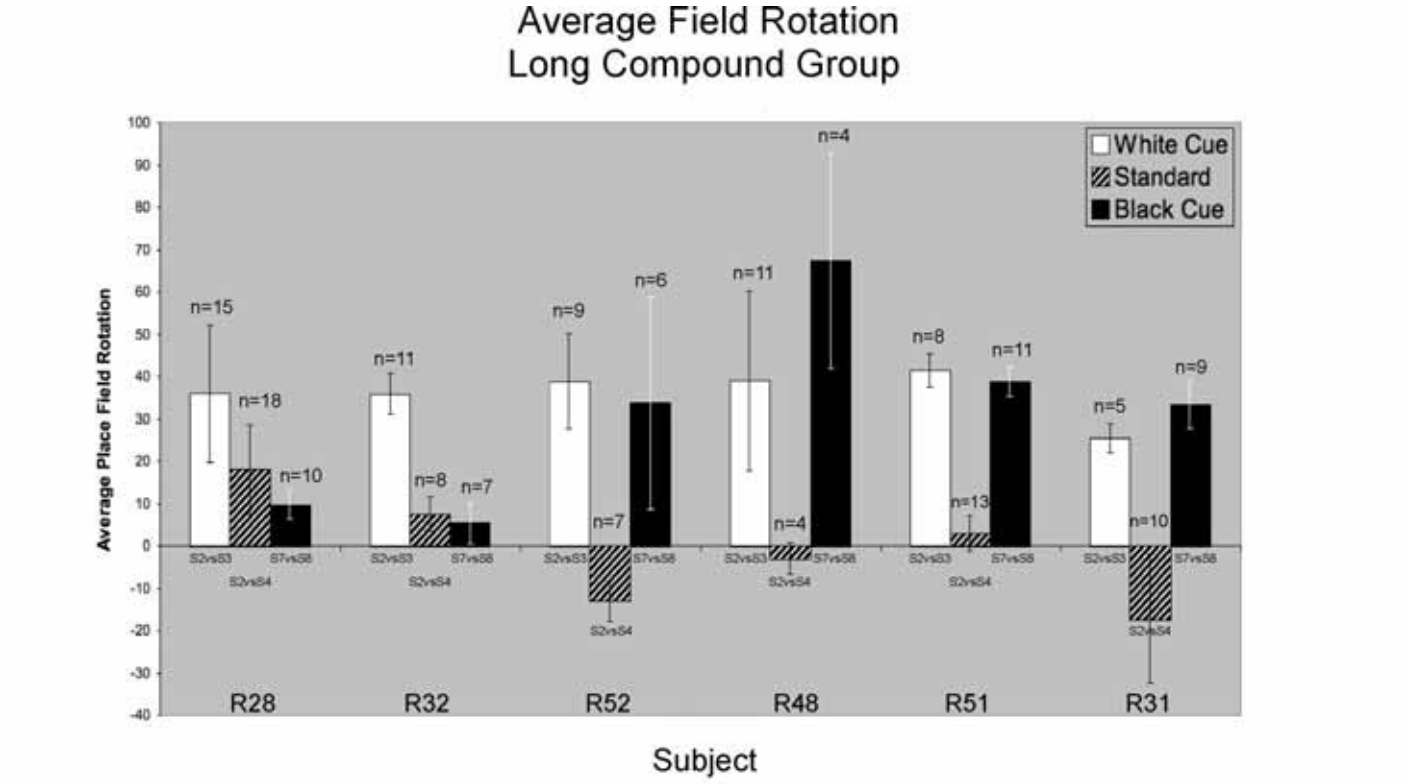

Average Field Rotation
Long Compound Group
White Cue
Standard
Black Cue
Average Place Field Rotation
Subject
R28
R32
R52
R48
R51
R31
n=15
n=18
n=10
n=11
n=8
n=7
n=9
n=7
n=6
n=11
n=4
n=4
n=8
n=13
n=11
n=5
n=10
n=9
S2vsS3
S7vsS8
S2vsS4

Figure 12: Sample firing rate maps from a long compound rat (Rat28) showing stimulus control over firing position by the white but not the black cue cards. Five simultaneously recorded place cells from Rat28 are shown across 4 sessions: A) Rats in the long compound group were initially exposed to the gray cylinder with a single white cue card on the wall. Firing rate maps from standard session 2 are shown in the top row while the bottom row shows the same cells during a white card rotation session. The white card had clear control over firing field position; B) Firing rate maps from compound standard session 6 are shown in the top row. This was the final of three 20 minute sessions in which the black cue was presented in compound with the white cue. The introduction of the novel spatial stimulus produced no obvious change in the spatial firing patterns of the place cells. Differences between map pairs in the two standard sessions were due to random fluctuations in cell discharge. The same cells are shown in the bottom row during the black card rotation session. While several firing fields appear to advance a few degrees in register with the cue card, the degree of rotation was not as robust as during the white card rotation. The rat is therefore blocked with respect to the black cue card. In that no blocking was found in the short compound group, the three 20 minute exposures to the novel cue card may have somehow made blocking possible. In this case, as in rat32, the hippocampal representation was not updated to include the black cue card.

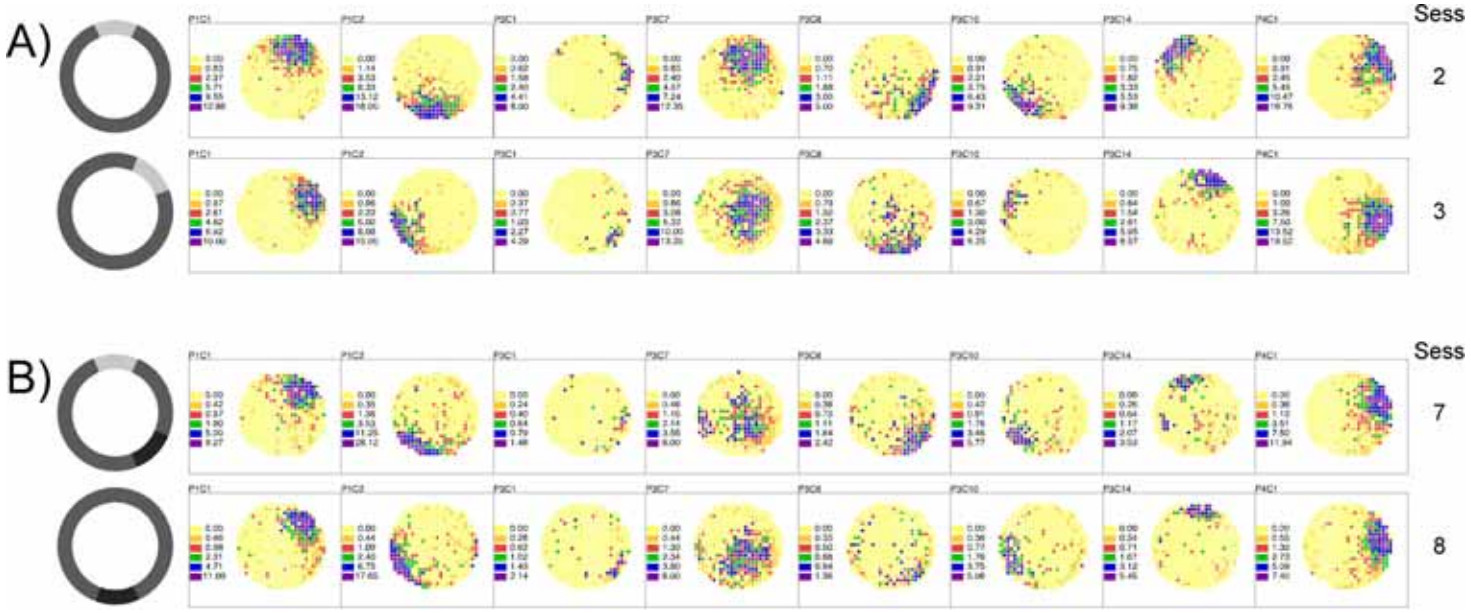

A)
Sess
2
3
B)
Sess
7
8

Figure 13: The average degree of firing field rotation from rats in each group as well as the standard error of the mean are shown for each of the three key sessions comparisons (standard session/white card rotation session; standard session/standard session; and standard session/black card rotation session). Using the average firing field rotation as an indication of the degree of stimulus control, it is clear that the white and black cue cards generally exerted an equivalent degree of control over firing field position. The degree of field rotation resulting from card rotation was in the same direction and of a similar amount as the card rotation. The degree of rotation for both cue cards was significantly greater than the 0° rotation of place fields between standard sessions for the short compound and control group. The average black card induced rotation for long compound rats was similar to the black card rotation for the short compound and control groups but the standard error of the mean is considerably greater than for any of the other pair-wise comparisons. While there is a trend for the black card to effectively control angular field location for long compound rats, the probability of 0.052 that the rotation caused by the black card is greater than the angular field shift between a pair of standard stimulus sessions is just slightly greater than alpha = 0.05. This result is apparently contradicted by the finding that a fixed value t-test does not reject the null hypothesis that the mean field rotation was 45°. The large SEM for black card rotation in the long compound rats, the lack of a clear difference between the effect of black card rotation and the angular change between pairs of standard sessions stem from the 2 blocked rats in this group. For these particular rats, the average firing field position was similar to that between two standard sessions.

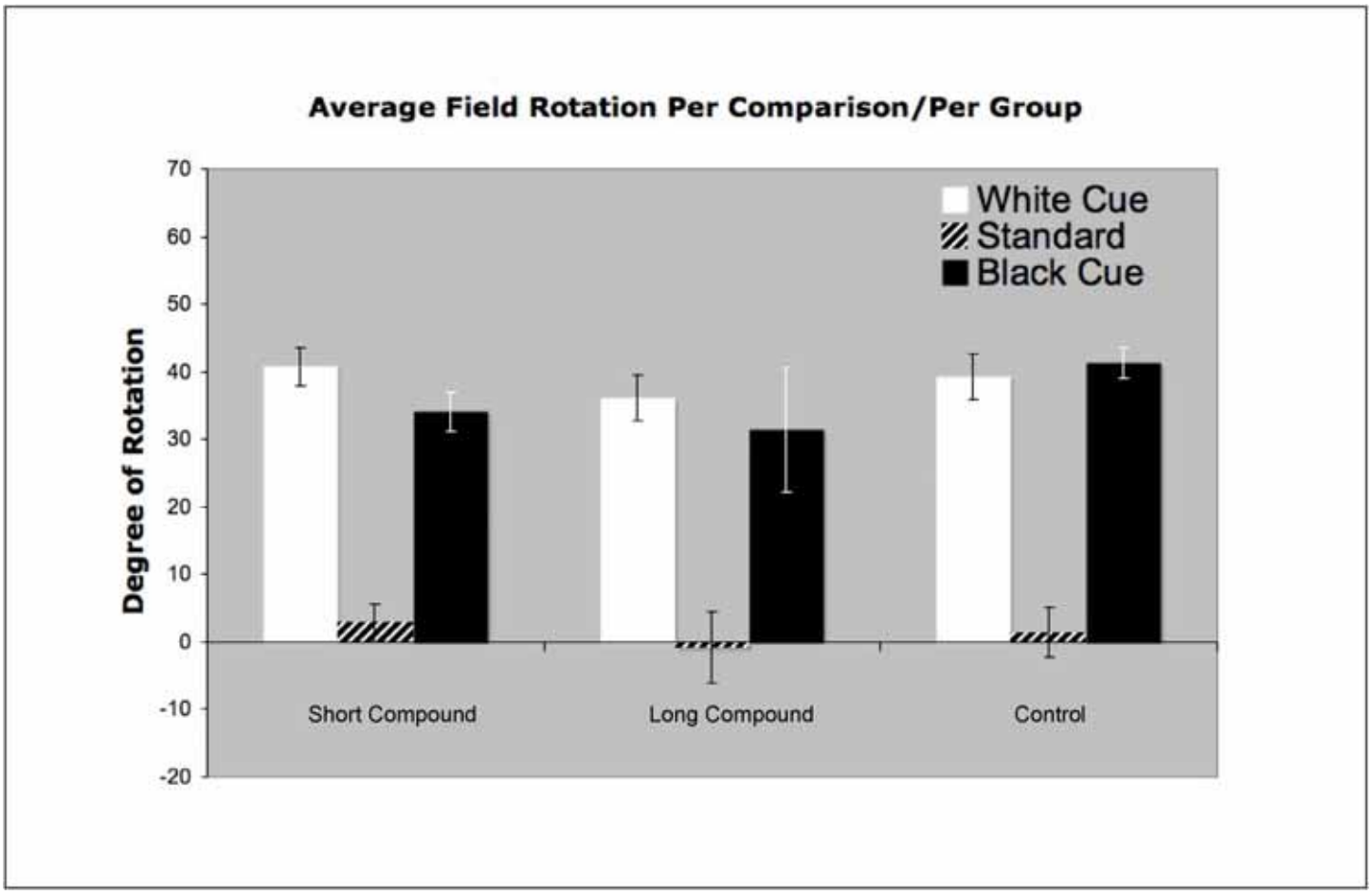
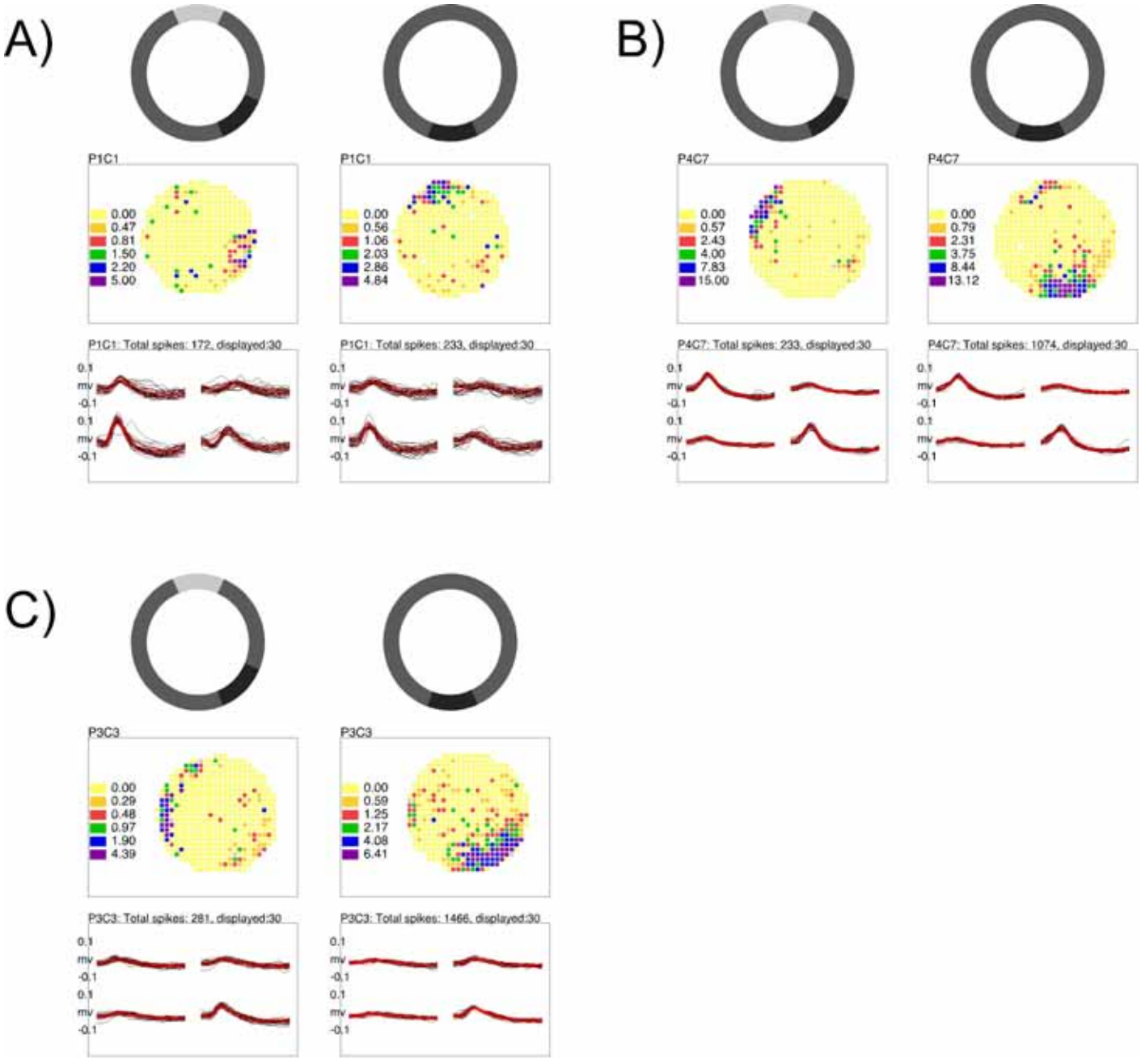

Average Field Rotation Per Comparison/Per Group
Degree of Rotation
70
60
50
40
30
20
10
0
-10
-20
White Cue
Standard
Black Cue
Short Compound
Long Compound
Control

Figure 14: Aberrant fields from 2 different rats that shifted their firing fields 225° between a black and white compound session and a black card rotation session. The two simultaneously recorded cells from Rat42 (A and B) appear to have symmetrical firing fields (180° apart) in the standard session with the black and white cue and then switch position and rotate ~ 45° in the black card rotation session. A cell from Rat28 is shown in C that also appears to rotate 225° between the standard session with the black and white cues and the black card rotation sessions.


A)
P1C1
0.00
0.47
0.81
1.50
2.20
5.00
P1C1
0.00
0.56
1.06
2.03
2.86
4.84
P1C1: Total spikes: 172, displayed:30
P1C1: Total spikes: 233, displayed:30
B)
P4C7
0.00
0.57
2.43
4.00
7.83
15.00
P4C7
0.00
0.79
2.31
3.75
8.44
13.12
P4C7: Total spikes: 233, displayed:30
P4C7: Total spikes: 1074, displayed:30
C)
P3C3
0.00
0.29
0.48
0.97
1.90
4.39
P3C3
0.00
0.59
1.25
2.17
4.08
6.41
P3C3: Total spikes: 281, displayed:30
P3C3: Total spikes: 1466, displayed:30
0.1
mv
-0.1

and then switch position and rotate ~ 45° in the black card rotation session. A cell from Rat28 is shown (figure 14 C) also appears to rotate 225° between the standard session with the black and white cues and the black card rotation sessions. These cells were eliminated from analysis because they contain subfields in at least one session.

3.8 Property changes for firing fields: Effects of card manipulation and time

The field rotation analyses suggest that cue manipulations in a novel environment caused little change in firing patterns across sessions. While many studies show that the firing field of a place cell may change in intensity, shift in position or appear or disappear under different circumstances, fields in the present study tended to remain stable and, in general, rotate smoothly with either cue card. Even in the case of the 2 "blocked" rats, there did not seem to be any changes in the firing pattern of place cells; cells maintained the same firing patterns as in the previous two-card session. Inspection of figures 6, 8, 10 and 12 confirms these observations.

Fenton et al (2000) also saw no obvious changes in the firing pattern of place cells through the direct inspection of firing rate maps. Nevertheless, application of numerical methods to examine quantitative firing field properties revealed significant changes in field properties. Thus, both firing rate and coherence dropped significantly after reconfiguring the black and white cue cards by rotating them together or apart. The resulting "topological distortion" was thought to reflect a conflict between the expected and actual stimulus patterns. In much the same way, Leutgeb et al (2005) introduced the concept of "rate remapping" in which firing field locations remain unaltered but the firing rates change significantly.

Given these precedents, we used the same numerical methods as Fenton et al (2000) to measure fine details of place cell firing. Although the rotational analysis indicated that the relative locations of firing fields remained constant throughout the session sequence, it was anticipated that card manipulations such as removal of a familiar cue card or introduction of a novel cue card might perturb firing pattern. The comparison of field properties across group protocols thus allows for interpreting the results in terms of either cue manipulation or temporal changes. In distinct contrast to the protocol for the Blocking Groups, the compound group protocol allowed for an examination of the effects

of cue removal on field properties after the rats had been initially exposed to both cue cards. In essence, the challenge to the hippocampal network caused by cue removal in the compound group protocol is more one of simple pattern completion. However, a possibly greater challenge is presented to the network in the blocking group protocols in that the network must first incorporate a novel spatial stimulus and then, if the stimulus is incorporated, carry out the process of pattern completion when the more familiar cue is removed.

Since some cells were previously excluded because their properties were inappropriate for the rotation analysis (e.g., central fields or two fields) relaxed criteria will be applied to select cells for quantitative analysis:

1) Field size: A field consists of at least 9 contiguous pixels.

2) Field rate. The firing rate in each pixel of a field must be > 0.1 spks/sec.

3) Pairwise analysis: If a cell fails to meet either criterion 1 or criterion 2 in one of the session it is s excluded from analysis.

4) Multiple fields. Only properties of the largest field are measured if there is more than one field.

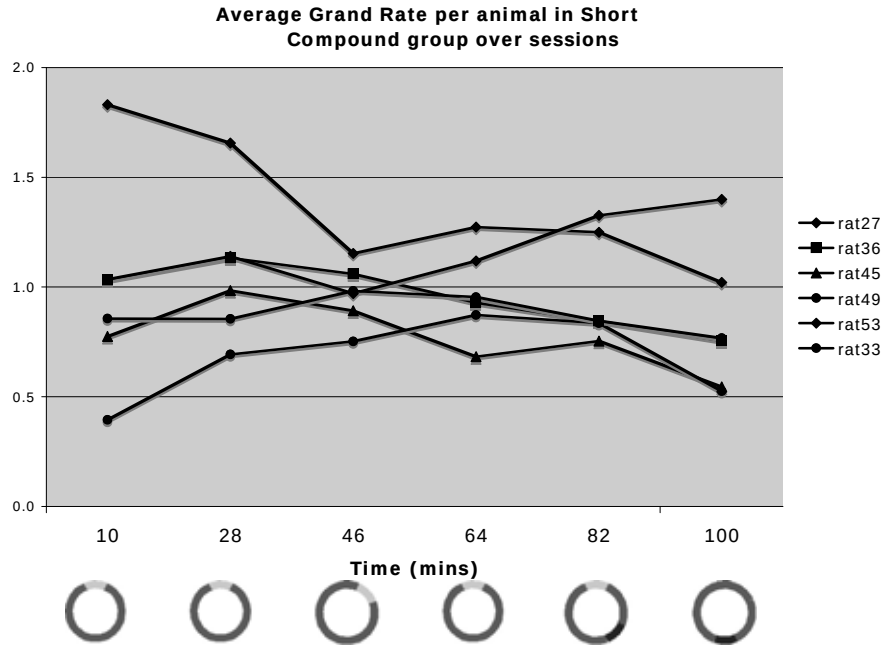
4) Cells determined to be interneurons were excluded from the analysis.

For each group, grand rate, coherence, and field rate were averaged across cells and plotted for each individual animal against the cumulative time for each session (figures 15 – 17 respectively). No obvious relationships were detected between either of the field properties and the degree to which the rat's firing fields rotated in register with the novel cue card.

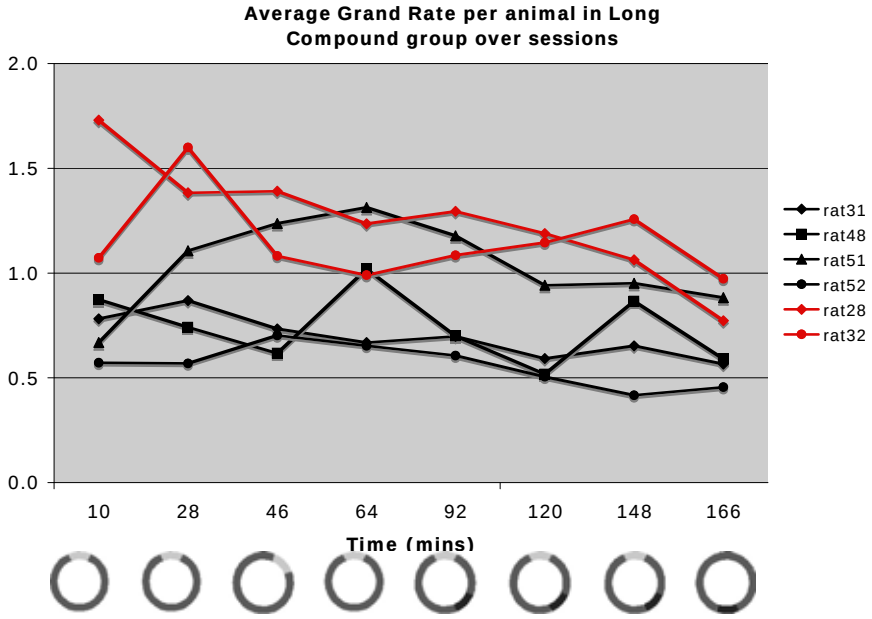
To detect changes cell firing caused by car card manipulations or cumulative recording time, repeated measures analysis of variance was carried out for the average field property values for each rat per group. If there was a significant effect across sessions, pairwise comparisons were carried through Newman-Keuls post hoc tests to find ay differences between each recording epoch. The average and standard error for coherence, field rate, grand rate, and field size as well as the average difference between

Figure 15: Average grand rate for individual animals per session for firing fields from short compound rats (A), the long compound group (B) and control group (C) plotted against cumulative recording time. Black lines indicate data from animals whose firing fields tended to rotate with the black cue card while red lines indicate data from animals whose firing fields did not tend to rotate with the black card. The figures do not suggest a relationship between grand rate and the degree of stimulus control by the novel black cue card. However, there was a tendency for the two blocked long compound rats to have a slightly higher grand rate. There was also a tendency for grand rate to decrease over cumulative recording time.

A)


Average Grand Rate per animal in Short Compound group over sessions
2.0
1.5
1.0
0.5
0.0
10
28
46
64
82
100
Time (mins)
rat27
rat36
rat45
rat49
rat53
rat33

B)


Average Grand Rate per animal in Long Compound group over sessions
2.0
1.5
1.0
0.5
0.0
10
28
46
64
92
120
148
166
Time (mins)
rat31
rat48
rat51
rat52
rat28
rat32

C)

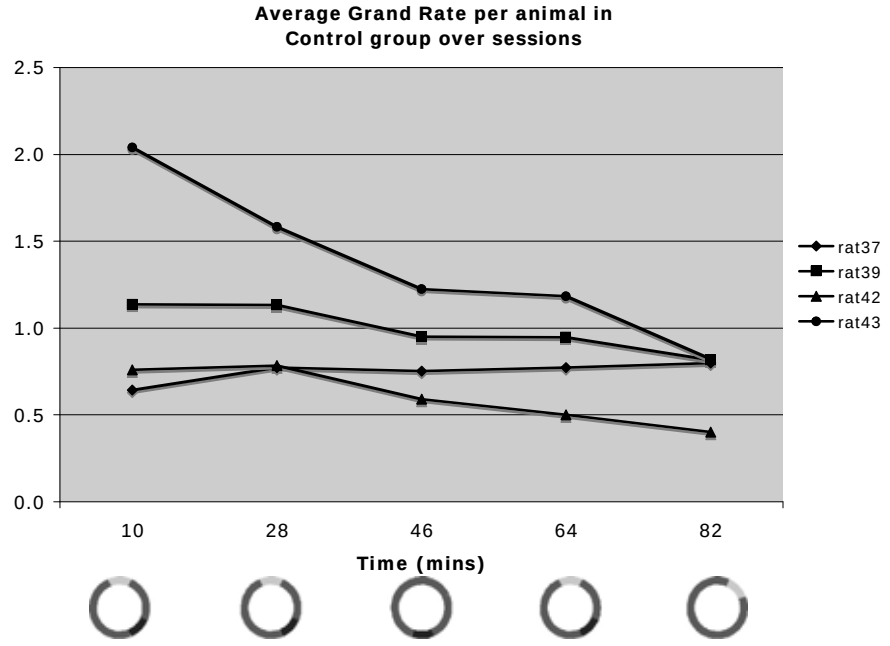
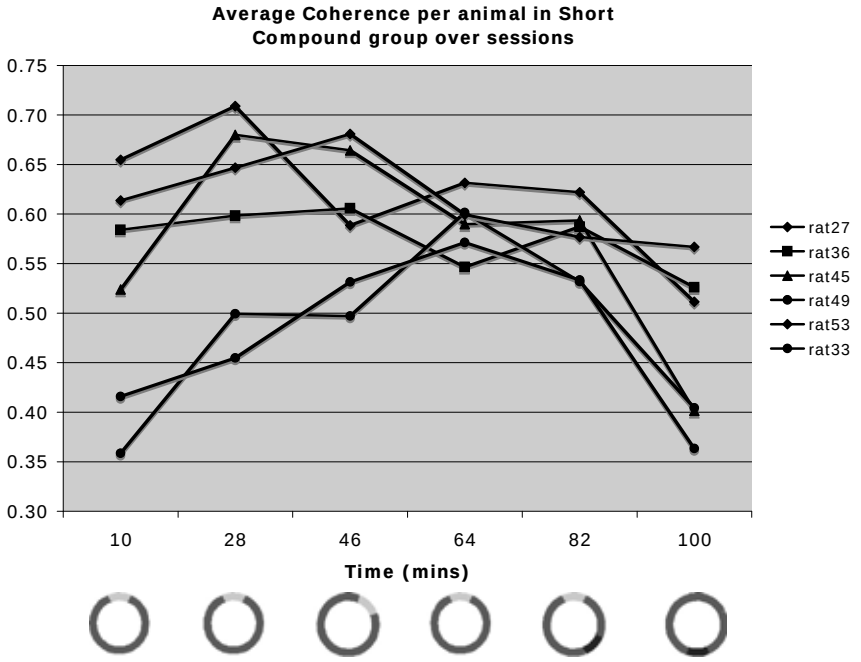
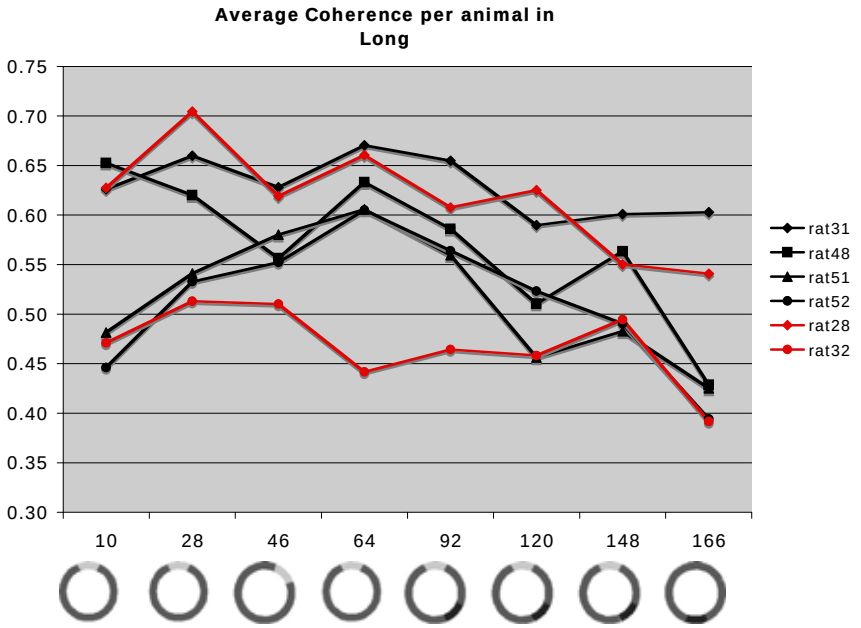

Average Grand Rate per animal in Control group over sessions
2.5
2.0
1.5
1.0
0.5
0.0
10
28
46
64
82
Time (mins)
rat37
rat39
rat42
rat43

Figure 16: Average coherence for individual animals per recording session for place fields from the short compound group (A), the long compound group (B) and control group (C) plotted against cumulative recording time. Black lines indicate data from animals whose place fields tended to rotate with the novel black cue card while red lines indicate from animals whose place fields did not tend to rotate with the black card. The figure suggests no clear relationship between coherence and stimulus control by the black cue card. However, one of the blocked long compound rats tended to have lower coherence values than the other rats throughout recording. There was an overall tendency for a sharp increase in coherence between the first and second recording sessions. The coherence values leveled off by the fourth and fifth time points for most animals in the blocking groups. There was no significant change in coherence for either of the control animals. The coherence then drops off sharply between the fifth and sixth time points for short compound rats. This is most likely due to the removal of the white cue card and rotation of the novel black card. The more gradual decline seen in the long compound rats may be an effect of cumulative recording time. However, the influence of card removal in the final recording session cannot be ruled out.

A)


Average Coherence per animal in Short Compound group over sessions
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
10
28
46
64
82
100
Time (mins)
rat27
rat36
rat45
rat49
rat53
rat33

B)


Average Coherence per animal in Long
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
10
28
46
64
92
120
148
166
rat31
rat48
rat51
rat52
rat28
rat32

C)

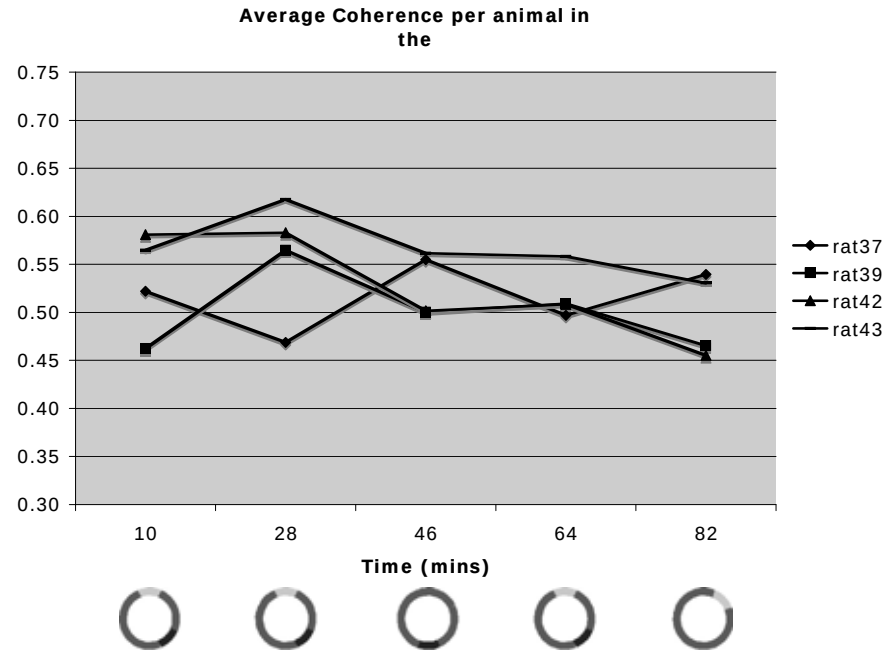
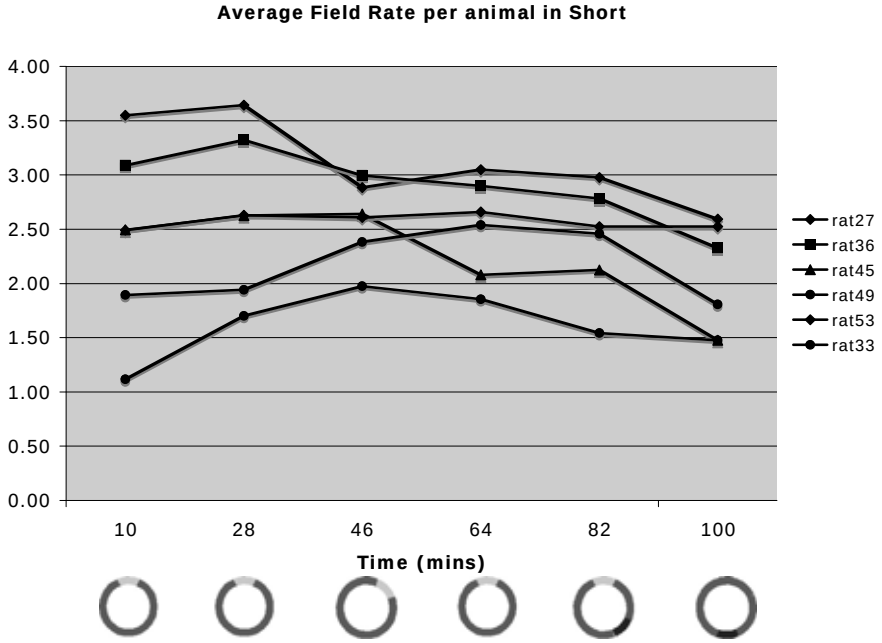
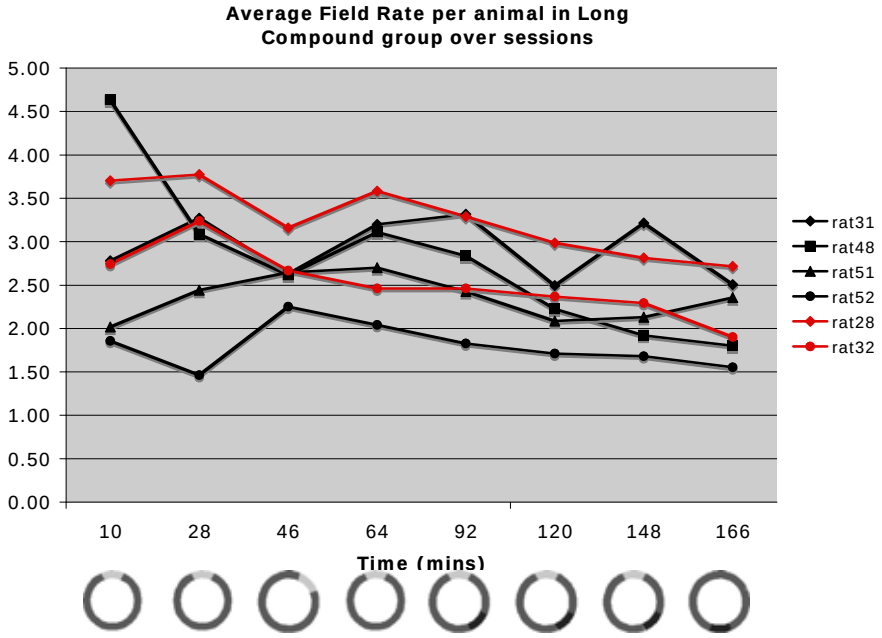

Average Coherence per animal in the
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
10
28
46
64
82
Time (mins)
rat37
rat39
rat42
rat43

Figure 17: Average field rate for individual animals per session for place cells from the short compound group (A), the long compound group (B) and control group (C) plotted against cumulative recording time. Black lines indicate data from animals whose place fields tended to rotate with the novel black card while red lines indicate data from animals whose place fields did not tend to rotate with the black card. No obvious relationship existed between field rate and the stimulus control exerted by the black cue card. Data from the short and long compound rats suggest that field rate decreases gradually over successive recording sessions regardless of manipulations made to the cue cards. In contrast, data from control rats suggest that the field rate tends to decrease earlier and more sharply than in either of the blocking groups. The sharpest decreases in field rate tended to occur at time points coinciding with card removal. While coherence decreased with cue removal for rats in the blocking groups, field rate decreased for rats in the control group. This may be due to the earlier occurrence of cue alterations than in the blocking protocols or an increased sensitivity to cue removal when both cues are present during initial exposure.

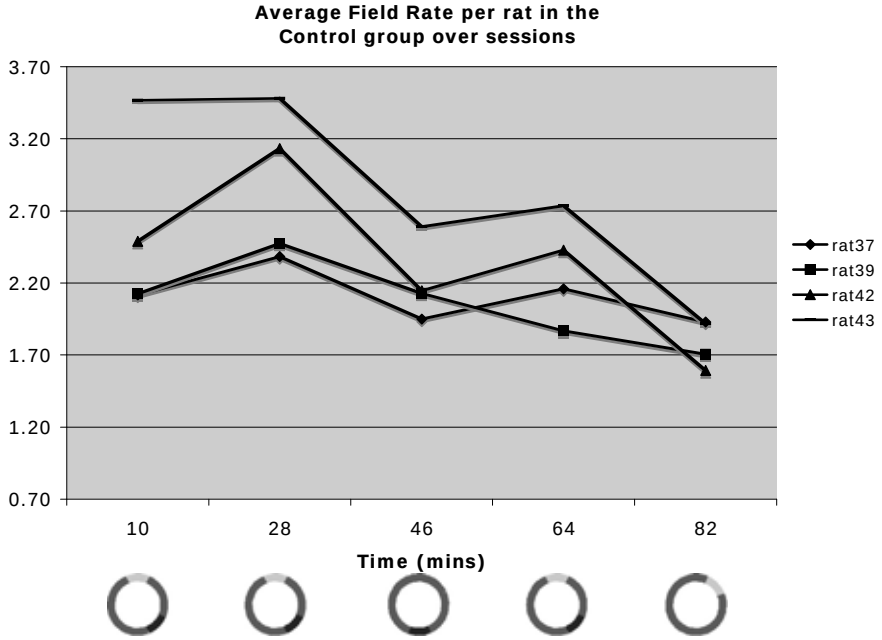
A)


Average Field Rate per animal in Short
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
10
28
46
64
82
100
Time (mins)
rat27
rat36
rat45
rat49
rat53
rat33

B)


Average Field Rate per animal in Long
Compound group over sessions
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
10
28
46
64
92
120
148
166
Time (mins)
rat31
rat48
rat51
rat52
rat28
rat32

C)


Average Field Rate per rat in the
Control group over sessions
3.70
3.20
2.70
2.20
1.70
1.20
0.70
10
28
46
64
82
Time (mins)
rat37
rat39
rat42
rat43

successive sessions are plotted for all three groups over cumulative recording time in figures 18 - 21 respectively.

Coherence:

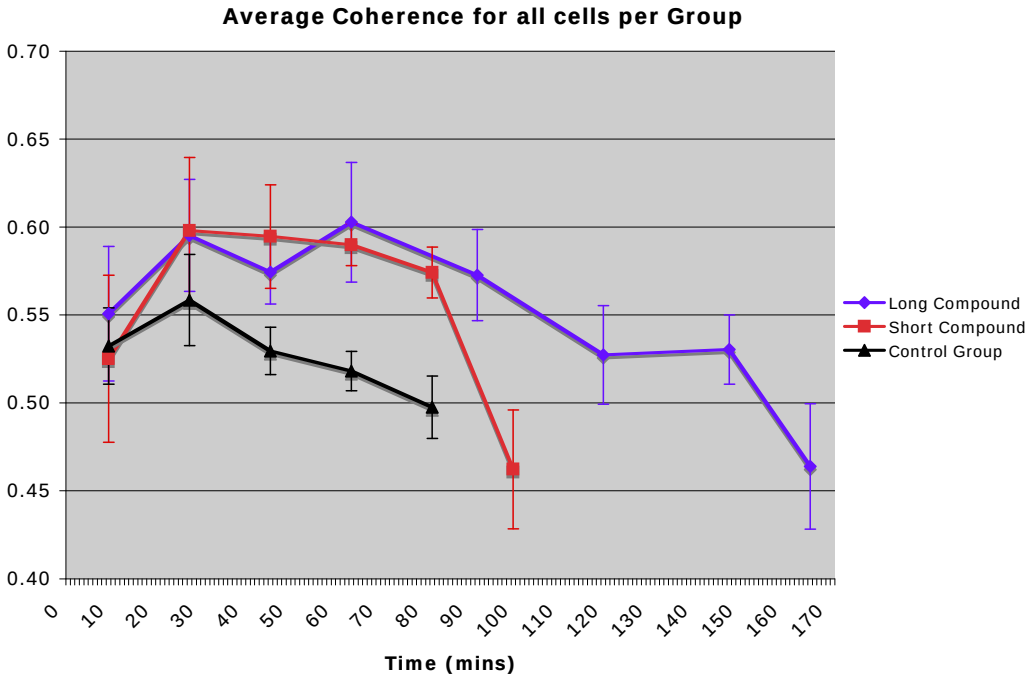
As suggested by figure 18, the pattern of coherence levels for both blocking groups were very similar during the first 5 sessions. When the data from both groups were pooled for these sessions, a significant main effect of coherence was found ($F_{4, 44} = 3.37$, $P > 0.05$). Pair-wise post hoc analyses suggest that the average coherence level in session 1 ($\bar{X} = 0.54 \pm 0.03$) was significantly lower than sessions 2 ($\bar{X} = 0.60 \pm 0.02$), 3 ($\bar{X} = 0.59 \pm 0.02$) and 4 ($\bar{X} = 0.60 \pm 0.02$) (see table 2). This implies that the coherence increased significantly between sessions 1 and 2 and remained at a stable peak value until session 4. This finding is corroborated by paired t-tests that compare the average coherence values for all cells per group between sessions 1 and 2. Significant increases in coherence were found in fields from short compound rats ($\bar{X}_{sess1} = 0.55 \pm 0.02$, $\bar{X}_{sess2} = 0.62 \pm 0.02$; $t_{92} = - 4.77$; $P < 0.001$) and long compound rats ($X_{sess1} = 0.53 \pm 0.02$, $\bar{X}_{sess2} = 0.62 \pm 0.02$; $t_{100} = - 4.01$; $P < 0.001$). This finding is in agreement with several studies of place cell activity in novel environments (Frank et al, 2004; Leutgeb, et al 2004; Mehta, Quirk and Wilson, 2000; Tanila et al., 1997; Wilson and McNaughton, 1993). The significant increase in place cell coherence in the second session for the short and long compound groups may reflect underlying changes in synaptic plasticity. The time course required for this change is in agreement with Frank et al (2004) in that hippocampal neurons required 5-6 minutes of experience to form a stable spatial representation.

When separate repeated measures analysis of variance were carried out for the blocking groups, significant changes in coherence were detected across recording epochs for the short compound group ($F_{5, 25} = 5.99$, $P > 0.001$) and the long compound group ($F_{7, 35} = 8.43$, $P > 0.001$). Coherence levels dropped significantly for short compound rats following white card removal in session 6 (see figure 19). Post hoc pair-wise comparisons suggest that this sharp decline in coherence in session 6 ($\bar{X} = 0.46 \pm 0.04$) was significantly different from all sessions but session 1($\bar{X} = 0.53 \pm 0.05$) (table 3).

This drop in coherence between sessions 5 and 6 was not found in post hoc comparisons for long compound rats suggesting that the decrease in coherence was due to the removal **Figure 18:** A) Place field coherence averaged across rats in each group over cumulative recording time: Blue line = short compound; Red line = long compound group; and black line = control group. The data from both groups were pooled for the first 5 sessions and revealed a significant main effect of coherence. Pair-wise post hoc analyses suggest that the average coherence level in session 1 was significantly lower than sessions 2, 3 and 4 implying an increase in coherence between sessions 1 and 2 that remained at a stable peak until session 4. When separate analyses were carried out for the short and long compound groups, significant changes in coherence were detected across recording epochs in both cases. Coherence levels dropped significantly for short compound rats following white card removal in session 6. Post hoc pair-wise comparisons suggest that this sharp decline in coherence was significantly different from all sessions but session 1. This coherence drop was not found in post hoc comparisons for long compound rats during similar time points during repeated compound exposures to both cue cards. This suggests that the decrease in coherence was due to the removal of the white card. The results of the repeated measures post hoc comparisons indicate that coherence levels in long compound rats began to decline after session 5, as sessions and 4 were significantly different from sessions 6 and 7. This decline was more gradual in comparison to the short compound group suggesting an influence of temporal degradation on coherence. After the white card removal in session 8, coherence levels for long compound rats were significantly lower than all other sessions and were at a similar level to session 6 for the short compound group. Noticeably absent, repeated measures analysis of variance found no significant main effect of coherence for the control group. Although coherence levels between sessions 1 and 2 appear to follow the same increasing trend as the blocking groups, this was not significant. This difference may be due to a smaller sample size or due to the presence of an extra cue card at the time of initial exposure; B) Average coherence difference score for all cells in each group between the successive sessions in figure A plotted against cumulative recording time. The large differences between sessions 1 and 2 reflect the general increase in coherence in the first 2 sessions. The

differences between sessions 5 and 6 for the long compound rats reflect the decrease in coherence following cue removal

A)



B)

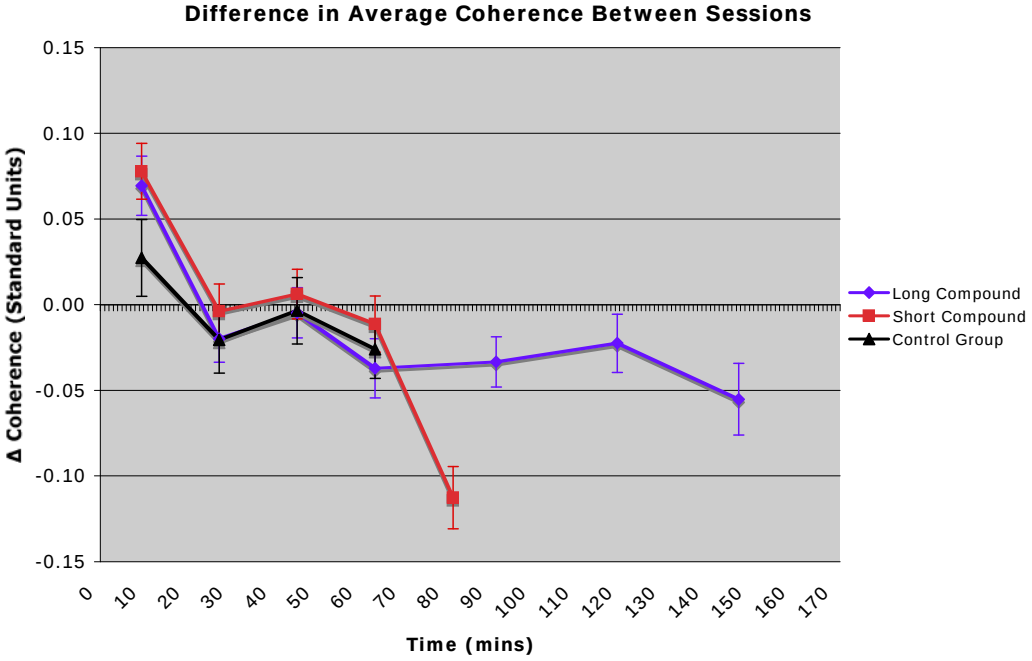
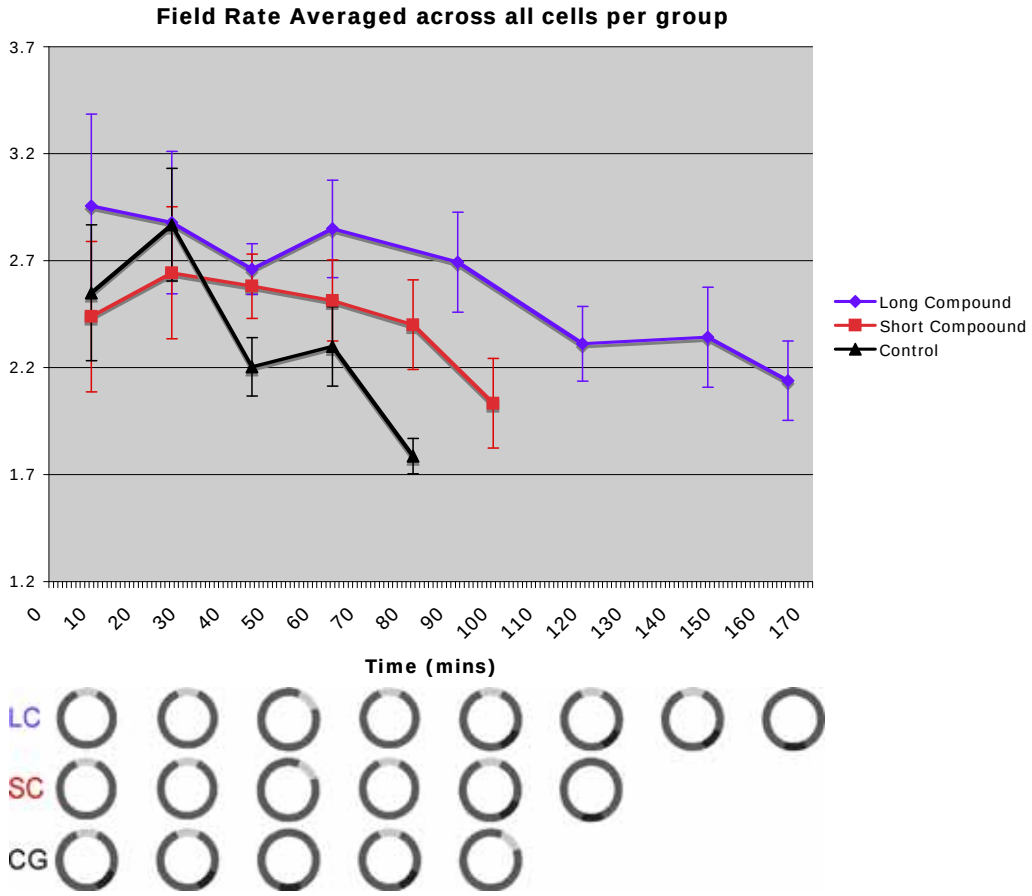


Figure 19: A) Field rate averaged across rats in each group over cumulative recording time: Blue line = short compound; Red line = long compound group; and black line = control group. Significant field rate changes were found across sessions for all three groups. The field rates were generally similar for both blocking groups over cumulative recording time. The firing rate in both cases began to decrease after session 5. While post hoc comparisons indicate that the decreased rate from short compound rats in session 6 was significantly different from sessions 3, 4 and 5, a similar non-significant decrease occurred in the long compound rats during session 6. Therefore, it is unlikely that the drop in field rate during session 6 for short compound rats was due to cue removal. The field rate for the long compound rats was stable between sessions 6 and 7 and decreased again during session 8. The field rate in session 8 was significantly different from session 1 and marginally significant from sessions 2 and 4. This result suggests that while the effects of cue removal cannot be ruled out, the field rate from rats in the blocking groups tended to decrease over cumulative recording time. A more complicated pattern of rate change emerged in the control group. Significant decreases in field rate tended to coincide with cue removal sessions. The field rate dropped significantly following the removal of the black cue between sessions 2 and 3 and only slightly recovered when the cue was replaced in session 4. The field rate dropped again in session 5 after the removal of the black cue card. Post hoc comparisons revealed that session 2 was significantly different than sessions 3 and 4 and that session 5 was significantly lower than all previous sessions; B) Average field rate difference score for all cells in each group between the successive sessions in figure A plotted against cumulative recording time. While the field rate remains relatively similar between successive sessions for the blocking rats, the field rate changes more abruptly for the control group rats during cue removal sessions.

A)


Field Rate Averaged across all cells per group
3.7
3.2
2.7
2.2
1.7
1.2
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
Time (mins)
Long Compound
Short Compoound
Control
LC
SC
CG

B)

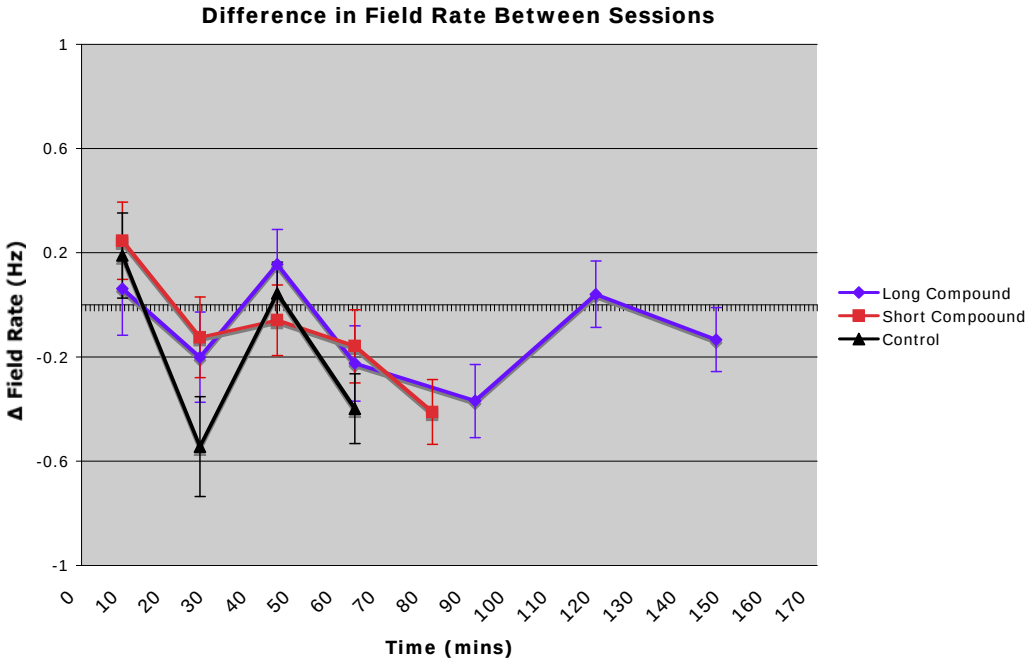
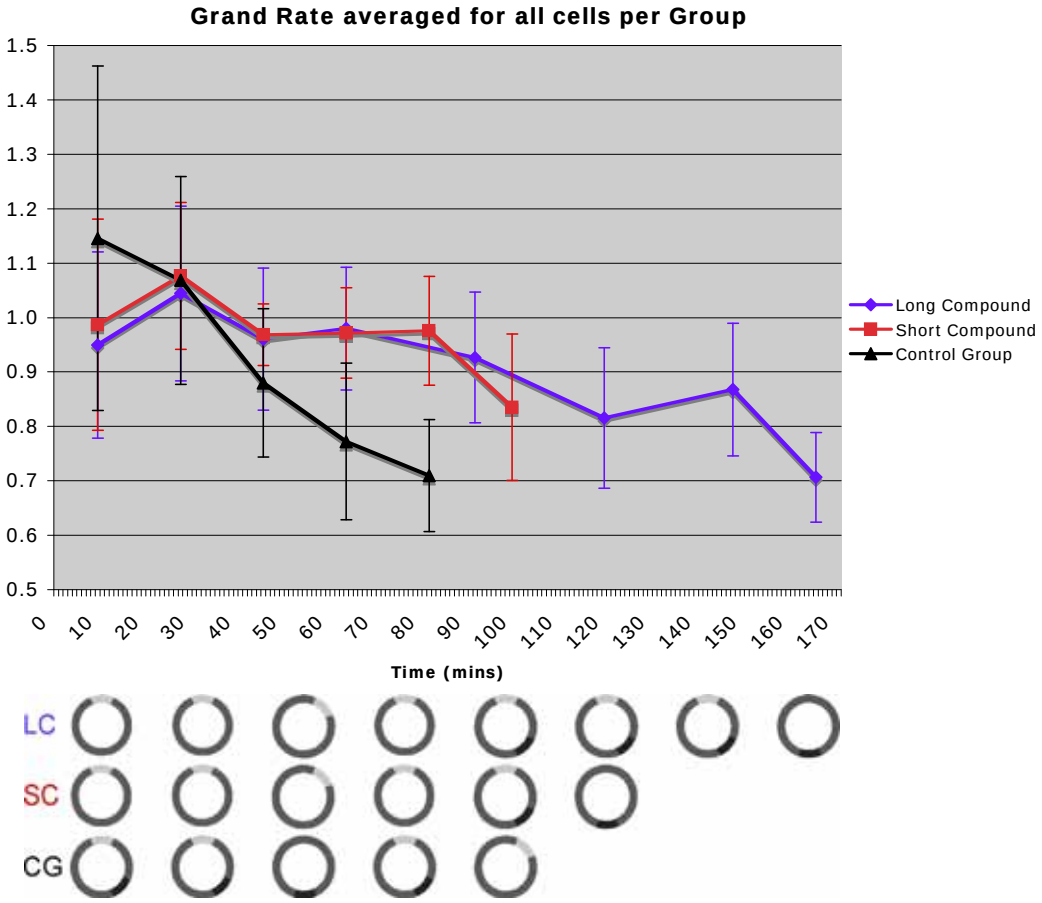

Difference in Field Rate Between Sessions
1
0.6
0.2
-0.2
-0.6
-1
Δ Field Rate (Hz)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
Time (mins)
Long Compound
Short Compoound
Control

Figure 20: A) Firing field grand rate averaged across rats in each group over time: Blue line = short compound; Red line = long compound group; and black line = control group. Significant changes for grand rate were only found in the long compound group. Post hoc comparisons suggest that grand rate and field size had deteriorated significantly during the later recording sessions. The grand rate in session 8 was significantly different from its peak during session 2. These results again suggest a depreciation of field properties over the course of cumulative recording time. While not significant, the grand rate for the control group drops more rapidly than the blocking groups. This may be due to card removal after session 2; B) Average grand rate difference score for all cells in each group between the successive sessions in figure A plotted against cumulative recording time. No obvious differences were detected between successive sessions.

A)


Grand Rate averaged for all cells per Group
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170
Time (mins)
Long Compound
Short Compound
Control Group
LC
SC
CG

B)

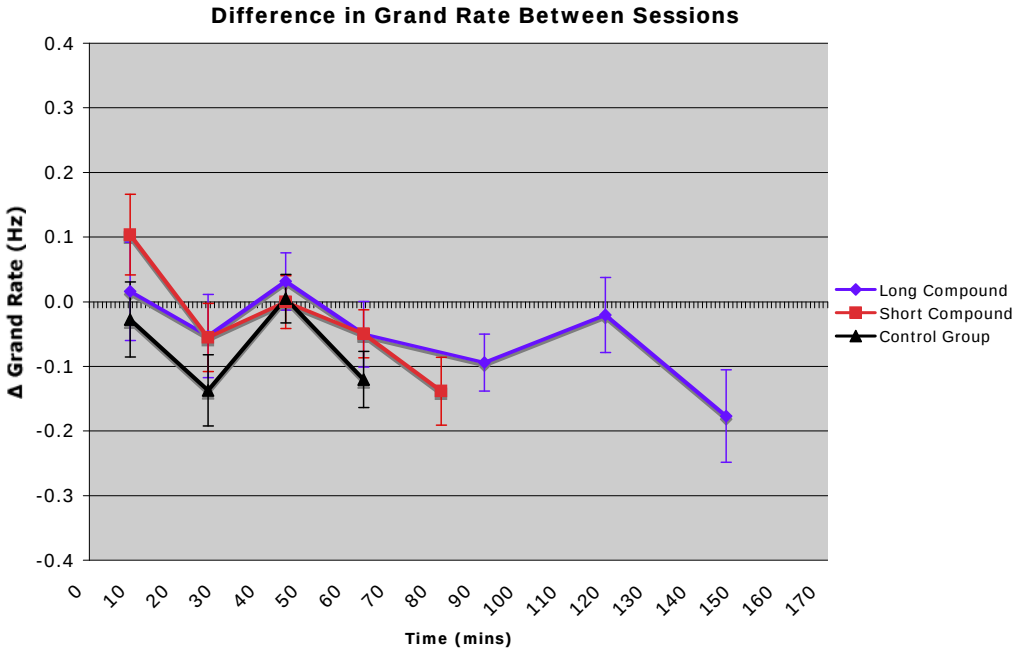
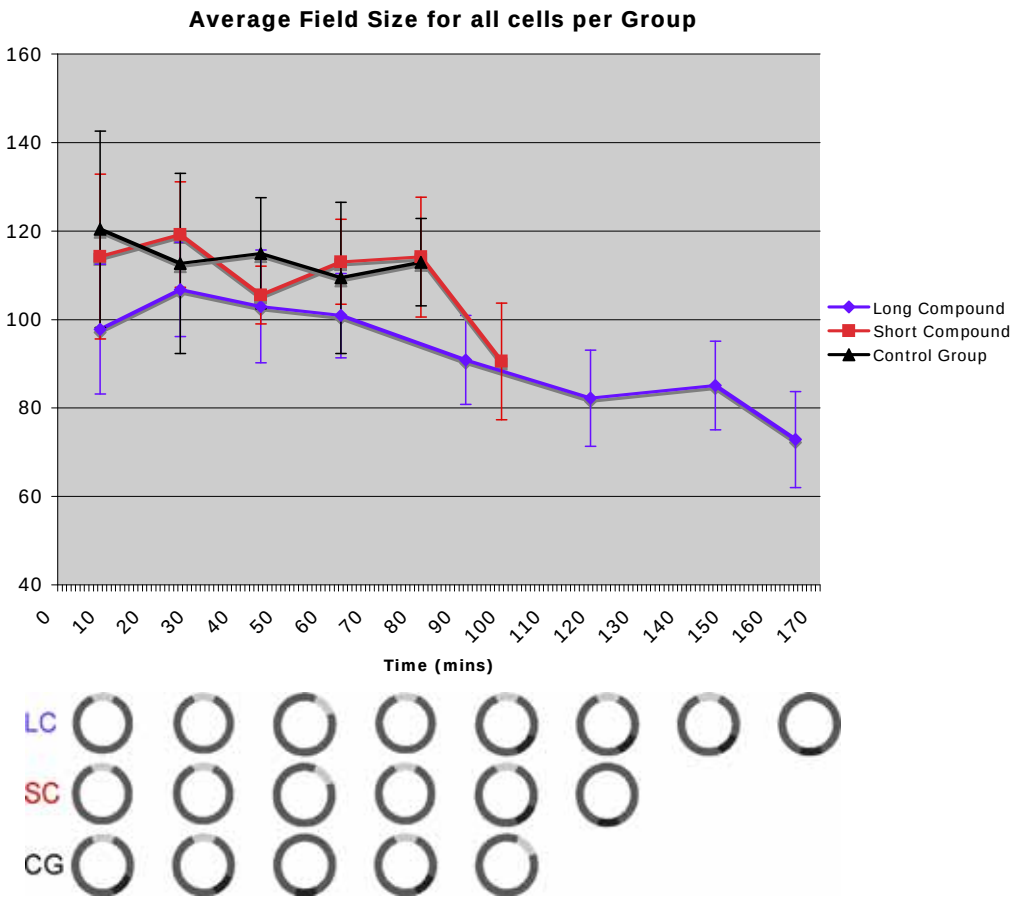
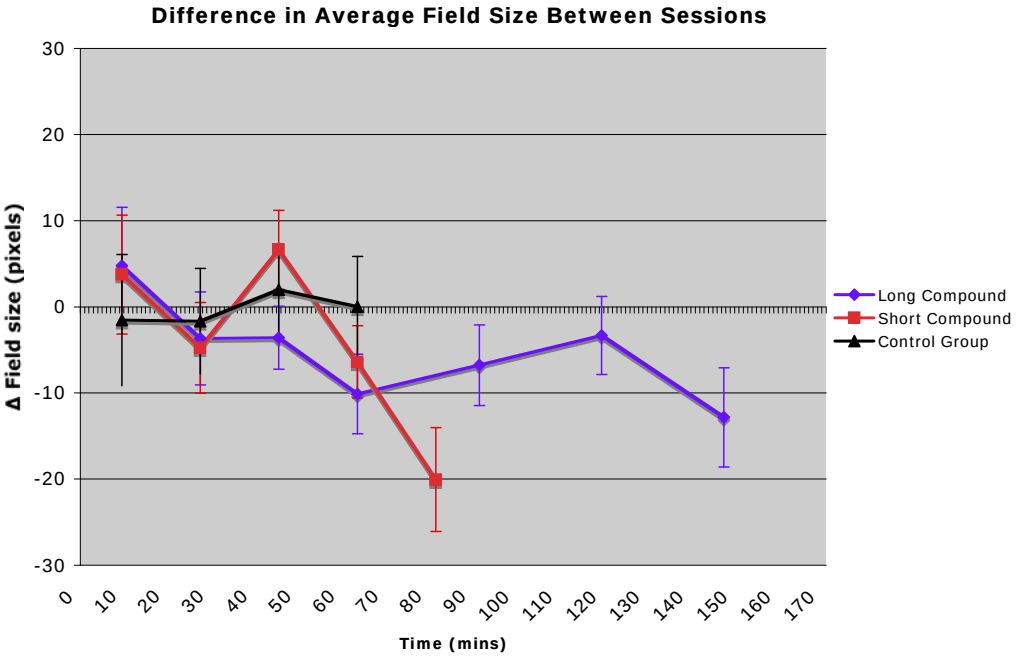

Difference in Grand Rate Between Sessions
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
Δ Grand Rate (Hz)
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170
Time (mins)
Long Compound
Short Compound
Control Group

Figure 21: A) Firing field size averaged across rats in each group over time: Blue line = short compound; Red line = long compound group; and black line = control group. Significant changes for field size were only found in the long compound group. Post hoc comparisons suggest field size had deteriorated significantly during the later recording sessions. The field size in session 8 was significantly different from sessions 2, 3 and 4. These results again suggest a depreciation of field properties over the course of cumulative recording time; B) Average field size difference score for all cells in each group between the successive sessions in figure A plotted against cumulative recording time. The field size difference following card removal between sessions 5 and 6 is much greater than the other sessions.

A)


Average Field Size for all cells per Group
160
140
120
100
80
60
40
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
Time (mins)
Long Compound
Short Compound
Control Group
LC
SC
CG

B)


Difference in Average Field Size Between Sessions
30
20
10
0
-10
-20
-30
Δ Field size (pixels)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
Time (mins)
Long Compound
Short Compound
Control Group

of the white card. The results of a one-way analysis of variance between the coherence values of the cells in each blocking group indicates there was no significant difference between the groups in session 5 ($F_{1, 220} = 0.04$, $P > 0.05$), suggesting that the quality of the fields were similar following the introduction of the novel cue. As the repetition of a compound stimulus session for the long compound group in Session 6 makes it an effective control for the effects of card removal in the short compound group protocol, an additional One-way analysis of variance was made to compare coherence levels for the cells in both groups during session 6. The results of this analysis indicate that the coherence was significantly decreased in the short Compound Group ($F_{1, 220} = 7.88$, $P < 0.01$). The group differences in this case suggest that the drop in coherence was most likely due to the removal of the white cue card and rotation of the novel black card in session 6.The results of the repeated measures post hoc comparisons indicate that coherence levels in long compound rats began to decline after session 5, as sessions 2 ($\bar{X} = 0.60 \pm 0.03$) and 4 ($\bar{X} = 0.60 \pm 0.03$) were significantly different from sessions 6 ($\bar{X} = 0.53 \pm 0.03$) and 7($\bar{X} = 0.53 \pm 0.02$) (see table 4). This decline was more gradual and not as sharp as that of the short compound group. This suggests that there may have been an influence of temporal degradation on coherence. After the white card removal in session 8, coherence levels for long compound rats were significantly lower than all other sessions and were at a similar level to session 6 for the short compound group.

Noticeably absent, repeated measures analysis of variance found no significance main effect of coherence for the control group. Although coherence levels between sessions 1 ($\bar{X} = 0.53 \pm 0.02$) and 2 ($\bar{X} = 0.56 \pm 0.03$) appear to follow the same increasing trend as the blocking groups, this was not significant. This difference may be due to a smaller sample size or due to the presence of an extra cue card.

Field rate:

Significant field rate changes were found across sessions for the short compound group ($F_{5, 25} = 3.51$, $P > 0.05$), long compound group ($F_{7, 35} = 3.30$, $P > 0.01$) and control group ($F_{4, 12} = 9.04$, $P > 0.01$). The field rates were generally similar for both blocking groups over cumulative recording time. The firing rate in both cases began to decrease

after session 5 (see figure 19). While post hoc comparisons indicate that the decreased rate from short compound rats in session 6 ($\bar{X} = 2.03 \pm 0.21$) was significantly different from sessions 3 ($\bar{X} = 2.58 \pm 0.15$), 4 ($\bar{X} = 2.51 \pm 0.19$) and 5 ($\bar{X} = 2.40 \pm 0.21$), a similar but non-significant decrease occurred in the long compound rats during session 6 (see table 5 and 6 respectively). Therefore, it is unlikely that the drop in field rate during session 6 for the short compound rats was due to cue removal. The field rate for the long compound rats was stable between sessions 6 and 7 and decreased again during session 8. The field rate in session 8 ($\bar{X} = 2.14 \pm 0.19$) was significantly different from session 1 ($\bar{X} = 3.0 \pm 0.43$) and marginally significant from sessions 2 ($\bar{X} = 2.88 \pm 0.33$) and 4 ($\bar{X} = 2.85 \pm 0.23$). This result suggests that while effects of cue removal cannot be ruled out, the field rate from rats in the blocking groups tended to decrease over cumulative recording time.

A more complicated pattern of rate changes emerged in the control group. Significant decreases in field rate tended to coincide with cue removal sessions (see figure 19). The field rate dropped significantly following the removal of the black cue between sessions 2 and 3 and only slightly recovered when the cue was replaced in session 4. The field rate dropped again in session 5 after the removal of the black cue card. Post hoc comparisons reveal that session 2 ($\bar{X} = 2.87 \pm 0.26$) is significantly different than sessions 3 ($\bar{X} = 2.20 \pm 0.14$) and 4 ($\bar{X} = 2.30 \pm 0.19$) and that session 5 ($\bar{X} = 1.79 \pm 0.08$) is significantly lower than all previous sessions (see table 7). Significant decreases in cell firing rate following the removal of nearby cues has been found previously by Hetherington and Shapiro (1997) and O'Keefe and Conway (1978). Other studies have also found that alterations in the topological relationship among a configuration of stimuli by interchanging or removing a sufficient number of spatial stimuli generally cause firing fields to degrade (Muller and Kubie, 1987, O'Keefe, 1979; O'Keefe and Nadel, 1978).

Grand rate and field size:

Significant changes for grand rate ($F_{7, 35} = 2.39$, $P > 0.05$) and field size ($F_{7, 35} = 3.44$, $P > 0.01$) were only found in the long compound group. Figures 21 and 22 as well as post hoc comparisons suggest that grand rate and field size had deteriorated

significantly during the later recording sessions (see tables 8 and 9 respectively). The grand rate in session 8 ($\bar{X} = 0.71 \pm 0.08$) was significantly different from its peak during session 2 ($\bar{X} = 1.04 \pm 0.16$) and the field size in session 8 ($\bar{X} = 72.9 \pm 10.8$) was significantly different from sessions 2 ($\bar{X} = 106.8 \pm 10.7$), 3 ($\bar{X} = 102.9 \pm 12.8$) and 4 ($\bar{X} = 100.9 \pm 9.55$). These results again suggest a depreciation of field properties over the course of cumulative recording time.

The lack of a coherence effect in control rats and the significant drop in coherence from rats in the blocking groups following card removal suggests that there was something inherently different in the card removal situation presented to the rats in each protocol. The lack of a coherence effect and the significant decrease in firing rate in control rats following cue removal may be due to the fact that the cues were altered three sessions earlier than in the short compound protocol. The control rats had only had two 10 minute exposures to the arena and both cue cards before the white card was removed. As temporal degradation in firing rate is evident in both short compound and long compound groups following session 4, temporal degradation cannot easily be ruled out as a cause for decreased firing rate for control rats. However, the differing patterns for these 2 neural signals in each protocol are striking. It may be that if the rats in the control group had more exposure to the cylinder that there would have been a change in coherence following cue removal and less of a change in firing rate. In addition, it is uncertain how a significant decrease in coherence might correlate with behavioral performance in a spatial task, or how increased rearing in reaction to a cue removal might effect field coherence.

Finally, field property analysis was also performed for each group and for key session comparisons using the same cell populations as in the above rotation analyses. A Bonferroni correction factor was applied to each successive paired t-test for each group. Paired t-tests revealed the same general pattern for cells that met criteria for rotation analysis as for those that met the basic criteria for field property analysis with the exception of significant differences between sessions 7 and 8 for long compound animals. While a general decrease in field properties was evident, these were not significant. This is likely due to the fact that, due to the decline in field properties over cumulative recording time, the firing fields were approaching the cut off level for inclusion in the

rotation analysis. Excluding units that had fallen below the cut off point likely biased the sample if favor of non-significant differences. Summary tables for the average of each field property per group can be found according to session comparison in Appendix C.

3.9 Firing field similarity during wedge analysis session comparisons

For each place cell the peak correlation coefficient reported by the wedge analysis between each of the three session comparison sessions, field stability and white and black card stimulus control, was normalized by using the Fisher transformation function:

$$z' = 1/2 \ln (1 + x / 1 - x)$$

The normalized cell values were than compared across the three wedge analyses for each group through a one-way analysis of variance. No significant differences were found for the control group ($F_{2, 105} = 0.87$, $p = 0.42$), short compound group ($F_{2,156} = 1.55$, $p = 0.21$) and long compound group ($F_{2,164} = 0.44$, $p = 0.64$). These results are a further indication of field stability across sessions. Even though several field properties decreased significantly, the peak correlation values between sessions for place fields in the rotation analyses did not change. Their spatial firing patterns remained intact.

Discussion

In a blocking experiment, a conditioned response is first established for a conditional stimulus, A, by pairing it with an unconditional stimulus that elicits an unconditioned response. In time, presenting A reliably evokes a conditioned response that closely resembles the unconditioned response. An additional stimulus, B, can be added to A to form a compound stimulus. After the compound stimulus is paired many times with the unconditional stimulus, the more familiar component A is removed and the ability of B to evoke a conditional stimulus is tested. It is commonly found that B evokes only a weak conditional response or none at all: prior conditioning to A is said to "block" conditioning to B. Blocking occurs in the non-spatial domain when the subject's location is irrelevant (i.e., Kamin 1968, 1969) as well as well as in the spatial domain when the subject must use available stimulus information to navigate to a goal (Biegler and Morris, 1999; Chamizo, Sterio and Mackintosh, 1985; Rodrigo, Chamizo, McLaren and Mackintosh, 1997; Hamilton and Sutherland, 1999). Informally, blocking is thought to occur because the novel stimulus does not add any new predictive value with regard tot eh occurrence of the unconditional stimulus.

The work presented here was designed to test if a process analogous to blocking occurs in the hippocampal spatial representation when a novel stimulus is added to a familiar environment. Place cells were recorded with a rat in a gray cylinder with a single white card on its wall. A novel black card was then added to form a compound stimulus. In a final probe session the white card was removed and the black card was rotated. If control by the black card is blocked by the original stimulus configuration, firing fields will not follow the angular location of the novel cue whereas without blocking, firing field rotation will approximate black card rotation. The basic result is that the newer black card exerts near ideal angular control over firing fields for most (10/12) rats. Field organization is detectably reduced when only the black card is present, but place cell activity is otherwise unchanged.

Thus, in contrast to behavioral experiments, blocking does not appear to occur for stimulus control over the hippocampal spatial representation; a novel stimulus which

conveys no new information about contingencies can nevertheless rapidly become the predominant polarizing stimulus in the environment. The black card is a nearly ideal substitute for the white card since the angular positions of firing fields are the same relative to both stimuli.

How can this discrepancy between be reconciled? The key is the realization that despite the introduction of a new stimulus, place cell experiments and (non-spatial or spatial) conditioning experiments differ in two crucial ways. First, no overt unconditioned or conditioned response to the familiar or compound novel stimulus is required of the animals in the case of the place cell experiment. Second, while food is delivered in the place cell experiment, its arrival has no contingent relationship to any of the sensory stimulus configurations. This gap between the place cell and behavioral blocking results can be appreciated in a more formal way. Many behavioral conditioning phenomena can be explained with the Rescorla-Wagner equation (Rescorla and Wagner, 1972):

$$(1) \qquad \frac{dV}{dt} = \alpha(\lambda - V)$$

where dV/dt is the change or increment in associative strength of a conditioned stimulus on any given trial in which the stimulus is paired with a response. The term V is the combined "associative strength" between all of the stimuli present and the unconditioned response, λ is the limit of associative strength, and α is a constant that represents the degree to which the CS is able to enter into an associative relationship with a US. The term λ reflects the maximum amount of associative strength that a given US can support. It is impossible to put the place cell results into the Rescorla-Wagner framework; none of the fundamental concepts of the Rescorla-Wagner model carry over into the type of experience-dependent neural change detected using a blocking-like design.

As an aside, it is important to note that the lack of congruence between incorporation of new stimuli into the place cell representation and behavioral conditioning is not a logical consequence of differences in contingencies. Thus, blocking might have been seen for place cells but in that case a new set of mechanisms would have to been proposed; the fact that blocking is not the rule for place cells only makes the need for new mechanisms more obvious.

While associative learning theory inadequately describes the results of the experiment, it is remarkable that the lack of blocking for the hippocampal spatial representation was one of the critical differences predicted between taxon learning and locale learning in the original statement of the cognitive mapping theory. The cognitive mapping hypothesis suggests that the detection and subsequent investigation of a novel spatial stimulus will result in its rapid incorporation into the pre-existing cognitive spatial representation. Further complimenting the theory, the acquisition of stimulus control in the current experiment occurred without reward in an incidental learning context. The spatial learning in this case was therefore not simply an associative 'stimulus-response' process, but was curiosity driven and presumably took place through the action of 'misplace detectors' (O'Keefe and Nadel, 1978).

For the first time in a spatial study using electrophysiological methods, the study shows evidence of the incorporation of a novel stimulus into a pre-existing spatial representation in the hippocampus. Moreover, the time scale required for this incorporation has also been shown to be quite rapid, occurring in a single exposure. While the results raise several interesting points regarding the nature of spatial learning and hippocampal function, they also raise several questions that I will now attempt to address: 1) Can associative learning theory account for our findings?; 2) How do the results fit cognitive mapping theory?; 3) Can map updating be detected by recording place cells?; 4) Is the process of map updating similar to reconsolidation?; and finally, 5) How can our results be reconciled with behavioral studies that show blocking in the spatial domain?

4.1 Can associative learning theory account for our findings?

The concept of the conditioned stimulus (CS) is easily transferable from associative learning theory to the current experimental design. The conditioned stimuli in our case would be the white and black cue cards. One can infer that learning has taken place in reference to these cues from the degree to which they attain stimulus control over firing field position. However, there was no particular action or behavior that was required of, or elicited by the animal. Any learning that occurred was incidental and occurred as a result of curiosity and exploration. At least in the classical sense, there was

no unconditioned stimulus (US). This does not preclude a discussion of associative learning theory in relation to our experiment. A pre-established link between spatial operant conditioning tasks and place cell activity allows us to make inferences about the process of cue learning (Huxter, Thorpe, Martin and Harley, 2001; Lenck-Santini, Save and Poucet, 2001; Lenck-Santini, Muller, Save and Poucet, 2002; O'Keefe and Speakman, 1987; Speakman and O'Keefe, 1990).

The absence of blocking in the short compound rats and the presence of 2 blocked rats in the long compound group suggests that longer exposure times to both the novel and familiar cue cards may have made blocking possible. This result was best predicted by the associative learning model proposed by Mackintosh (1975). Unlike other associative learning models, such as the Rescorla-Wagner (1972) model, the salience of the CS (α), i.e., how noticeable the stimulus is as determined by its loudness or brightness etc., is not thought to be a constant value. The Mackintosh model proposes that there can be changes in the processing of a conditioned stimulus as a result of experience. In other words, the model focuses on the amount of attention that is awarded a stimulus in relation to its predictive value. Therefore the salience value (α) is variable. On each trial, the associative strength of a conditioned stimulus changes according to the rule:

$$\Delta V_A = \alpha(\lambda - V_A)$$

where V_A refers to the associative strength between stimulus A and the US and ΔV_A indicates the increment of change in associative strength for a given trial. The value of λ is set by the magnitude of the US and reflects the maximum strength that the CS-US association can attain. The value of α, as stated previously, reflects the amount of attention that the stimulus receives. The greater its value, the faster the conditioning will be. The value of α will approach 1 when a stimulus is a good predictor of the US relative to other stimuli that may be available, and when the stimulus is a relatively poor predictor of the US then α will approach 0.

Blocking occurs, according to Mackintosh, because when both stimuli are presented in compound following training to the original stimulus, the original stimulus has become the best predictor of the US. Attention to the novel element will initially be high and in the first compound session will gain some associative strength. However, the

novel stimulus at this point is still a relatively poor predictor of the US. Its associative strength thus rapidly declines during compound training, resulting in little conditioning. Therefore, the Mackintosh model would predict that blocking would not occur on the first compound trial. In other words, the Mackintosh model suggests that blocking is not a consequence of competition between stimuli for reinforcement, but is a consequence of the animal's inattention to the added element. Once the animal has learned that the novel stimulus does not predict anything new in the current learning situation, attention will be shifted toward the original stimulus (Mackintosh, 1975). In this sense, not only does the Mackintosh model predict that increased compound exposure may make blocking more likely, but it also makes allowance for some associative learning to occur following a single exposure to the novel stimulus. Both of these results have occurred in our study.

In the original Pavlovian blocking experiment, Kamin (1968, 1969) entertained the possibility that the transitional trial proves the superimposed stimulus to be redundant as a proposed 'gating mechanism' acts to keep the new stimulus from being perceived on subsequent trials. Kamin discovered that this mechanism could not be overcome even by tripling the amount of compound exposure to both stimuli. Further exposure to the novel stimulus did not make it any less redundant. In other words, the animals may notice the light on the transitional trial but in the following trial will be shown to have no new predictive value. The rat would therefore continue to ignore the novel stimulus on subsequent trials.

As suggested by Mackintosh (1975), the occurrence of blocking with one compound trial is of pivotal importance in evaluating alternative conceptions of classical conditioning. Unfortunately, the evidence for learning during this compound trial appears to be inconsistent. Mackintosh and his colleagues did not see blocking following one compound trial (Dickinson, Nicholas and Mackintosh, 1983; Mackintosh, 1975; Mackintosh, Dickinson and Cotton, 1980), except when the elements of the compound CS were presented sequentially and separated by a trace interval (Dickinson et al., 1983). Revusky (1971) and Gillian and Domjan (1977) did find evidence of blocking in a conditioned taste aversion task with a single compound trial. However, in this case the elements of the compound were also presented sequentially with a short time interval between them. The only published demonstrations of blocking with one compound trial

using simultaneously presented compound stimuli in a conditioned suppression procedure are those of Balaz, Kasprow, and Miller (1982) and Azorlosca and Cicala (1986). The studies which failed to find blocking following one compound trial may be due to the lack of conditioning to the test CS in control groups (Azorlosca and Cicala, 1986). Blocking with one compound training trial appears to remain a tenuous issue that has never been fully resolved. The differences in the findings of these different studies likely lay in the subtle variations of their training procedures.

How much learning occurs during the first compound trial in a fear conditioning task has been studied extensively without a clear answer. The findings of these studies have helped to improve theories of associative learning in the context of fear learning that in some ways may be relevant to the present study. However, associative learning theory to ultimately falls short in explaining my results. The length of exposure to the stimuli and US (i.e., noise or light and shock) occur in a timescale of seconds as opposed to several minutes. In addition, the shock US activates extra-hippocampal structures such as the amygdala (Fanselow, 1998). The cognitive mapping hypothesis suggests that this is expected and learning that is hippocampal dependent is not subject to the rules of associative learning (O'Keefe and Nadel, 1978). Finally, the notion of associability with regard to spatial stimuli (λ) is rather crude. While associability may be applicable to single stimuli in fear conditioning, it is much more complex to use it with respect to the myriad of cues that make up the spatial realm. The geometry or boundary shape of a given environment, stable background cues, the nature of local and distal cues and their perceived stability must all be taken into account. No other spatial studies have suggested a limit for the amount of time or number of exposures necessary that would make the collection of stimuli that make up an environment permanently fixed. The results of the present study, as well as the results of several other studies (Anderson and Jeffery, 2003; Leutgeb et al., 2005; Lever et al., 2002; Muller and Kubie, 1987; Muller, Poucet and Rivard, 2001; Knierim, 2002; Shapiro et al., 1997; Skaggs and McNaughton, 1998; Tanila, Shapiro and Eichenbaum, 1997) have demonstrated the flexibility of hippocampal spatial representations. In any case, the results show that in the first couple of hours of exposure to an environment, novel spatial stimuli can still be associated with the more familiar stimuli of which that environment is composed.

4.2 How do the results fit cognitive mapping theory?

According to the cognitive map theory (O'Keeke and Nadel, 1978), hippocampal dependent spatial learning in the 'locale system' is rapid, unconditional and motivated by curiosity. Locale learning is therefore antithetical to associative learning as it is incremental, conditional on the degree of achieved error reduction and motivated by reward or punishment. According to the cognitive map theory, these features are associated with the acquisition of spatial knowledge in the non-hippocampal dependent 'taxon system'. The formation and stabilization of the cognitive representation for a novel environment within a session and the ability to rapidly update this representation suggests that rats in our study tended to employ the locale system. However, while the incidental nature of the cue learning may in some ways strengthen our supposition that the learning was curiosity driven, the lack of a spatial task makes it impossible to prove what spatial strategies the rats may have employed. In addition, it is unclear how the absence of spatial demands may have lent itself to the absence of blocking.

Kamin (1968, 1969) and Mackitosh's (1975) notion of a gating mechanism that focuses attention on stimuli based on their predictive value was mirrored in a recent proposal by Morris and Frey (1997). The behavioral indication of blocking in the spatial domain (Biegler and Morris, 1999) suggested that rats are sensitive to mismatches between their perception and stored representations of arrays of spatial landmarks, and that their reaction to this mismatch is typically increased exploration. This is also predicted by O'Keefe and Nadel (1978). However, a reaction of increased exploration may not guarantee learning. Biegler and Morris (1997) suggested that incorporation of a spatial landmark into the map of space would only occur to the extent that it predicts a goal location. That exploration of the novel landmark in their study decreased over trials indicated that some information about the cue was rapidly encoded. Whether or not its location was also encoded is uncertain. In any case, the novel landmark in their study was not used to guide search behavior. Morris and Frey (1997) have proposed that the hippocampal based 'automatic recording system' has access to the animal's currently activated spatial map and that it triggers exploratory behavior, or directed attention, when there's a mismatch between the current perception of the environment and expectations

of the environment. If the newly acquired information is needed, for finding a goal location for example, the 'effortful' components of the long-term memory system are engaged and the animal's spatial representation of the environment is updated. When it is not needed to find a goal, there will be no reinforcement signal of a goal directed character and, thus, the error correcting learning rule used for long-term memory need not be engaged. This raises the question as to whether or not an increased spatial demand may increase the likelihood of blocking. However, this would likely depend on the spatial strategies used to solve the spatial task or somehow ensuring that a locale oriented strategy was used (O'Keefe and Nadel, 1978).

As Restle (1957) pointed out, the probability of a particular hypothesis being 'chosen' depends on the demands presented by an experiment. The use of the locale system may be more prevalent when extra-maze cues are available in conjunction with a large number of choices; the taxon system may be preferred when a strong, salient cue might overshadow other stimuli and bias the rat toward guidance learning or a sparse number of cues and few behavioral options favors orientation hypotheses. In an environment that does not provide enough structure to favor any particular strategy, the animal may randomly switch strategies from one trial to the next. There are many factors that might influence the selection of a hypothesis, such as rearing conditions, 'personality', previous experiences, inter-trial intervals and so on (O'Keefe and Nadel, 1978; Restle 1957). However, there is an element of 'free will' here as the subjects in some experimental designs, including the present study, is free to choose any spatial strategy. When both classes of solutions are available and the spatial problem can be solved either way, the decision of which system to activate must first be made (Shelton and Gabrieli, 2004). It is unclear how organisms make this decision but there is evidence that these decisions do take place and that individuals can alternate between spatial strategies. Bohbot et al. (2004) reported that when human subjects were asked to solve a computerized radial arm maze task, about half of the subjects spontaneously chose a locale oriented strategy while the other half spontaneously chose a taxon oriented strategy. Furthermore, data collected using fMRI suggested that there was a significantly greater BOLD signal in the right hippocampus for those subjects that used a locale oriented strategy while there was an increased BOLD signal for the caudate nucleus for

those subjects that chose a taxon oriented strategy. There was also a difference in the time course for the manner in which the signals increased in both brain regions. The increased BOLD signal was seen in the hippocampus for locale strategy users when they initially started the task. Meanwhile, the increased signal in the caudate nucleus was more incremental. It therefore appears that, with practice, activity in the caudate nucleus gradually increased in subjects that chose a taxon oriented strategy that was sustained until the end of the experiment. This finding appears to match well to the differing time courses suggested by O'Keefe and Nadel (1978) in locale and taxon learning. In addition to these findings, Bohbot et al (2007) found that participants that used locale strategies in solving a given spatial task had significantly more gray matter in the hippocampus and less gray matter in the caudate nucleus compared with those that used a taxon oriented strategy. Furthermore, the gray matter in the hippocampus was negatively correlated to the gray matter in the caudate nucleus, suggesting a competitive interaction between these two brain areas.

Using rats as subjects, Packard and McGaugh also found the same time course and functional dissociation between the hippocampus and the caudate nucleus (1996). On day 8 and day 16 of training, rats were placed on an arm in a cross maze opposite to the arm they had normally been started on for a probe trial. Rats that were given saline injections to the hippocampus and caudate nucleus displayed place learning on the day 8 probe trial and response learning on the day 16 probe trial, indicating that with extended training there is a shift in the learning mechanisms controlling behavior. Rats receiving lidocaine injections into the hippocampus showed no preference for place learning or response learning during a probe trial on day 8 but did display a preference for response learning during a probe trial on day 16. This suggests that the hippocampus is necessary for place learning during the initial stages of training. Rats that received injections of lidocaine into the caudate nucleus before probe trials showed a preference for place learning. Inactivation of the caudate nucleus therefore prevented response learning.. This study thus provides further evidence that the hippocampus and caudate nucleus mediate the expression of place learning and response learning, respectively, and that hippcampal dependent place learning is acquired at a faster rate than caudate nucleus dependent response learning.

While it is impossible to know if the 2 blocked animals were using a taxon oriented strategy, one can imagine that the increased exposure to the cylinder could have gradually led to a shift toward taxon oriented strategies (Hartley et al, 2003; Packard and McGaugh, 1996). Several different experiments could be performed to test for this in future. While an experiment involving the simultaneous performance of spatial tasks and place cell recording would be more difficult it would also be ideal. Three different experiments could answer the question of how spatial strategy might relate to the whether or not a novel spatial stimulus is incorporated into the cognitive map:

1) A place preference task (Rossier et al., 2000) such as that used in Lenck-Santini, Muller, Save and Poucet (2002) in which the locale or taxon strategies were encouraged depending on the proximity of the goal to the cue card, or if the goal area was marked. Upon reaching criterion, an additional cue card could be added to the environment. After additional training with the new cue card, the initial card or goal marker could be removed. Would the employed spatial strategy influence whether or not the firing fields rotated in register with the novel card? Would a taxon strategy make blocking more likely?

2) If one wished to replicate the present study with a spatial task that occurred on the same time scale, the best option would be the avoidance task in the rotating arena (Cimadevilla, Fenton and Bures, 2001). Perhaps this is the only task where spatial learning could happen in five 10 minute sessions. However, cue card control over the location of avoidance has yet to be determined.

3) One could repeat the study either with or without a spatial demand placed on the animals and record hippocampal place cells following the temporary inactivation of the striatum (see Packard and McGaugh, 1996) in the trials before compound exposure. Based on the number of animals that tracked the novel cue, one could then look at the incidence of each spatial strategy. If the striatum is required for taxon strategies, then temporary inactivation should encourage the rats to use a locale strategy. This should in turn make it more likely that the rats would track the novel card. Nevertheless, blocking

was not a common occurrence in our hands. Even without temporary inactivation of the striatum, it appears the rats may employ a locale strategy in updating their representation of space.

4.3 Can map updating be detected by recording place cells?

Modifications of field properties such as firing rate are clearly associated with spatial alterations, but whether these changes are a cause or an effect of the updating process remains unclear. Leutgeb at al. (2005) have proposed that this change in rate may be a key feature in the formation of separate population codes for both location and cue configuration, thus allowing hippocampal cell assemblies to simultaneously convey information about where an animal is located as well as what is currently experienced in that location. However, a clear neural signal, as detected through changes in the firing pattern of place cells in their firing field, that could be attributed to the introduction of the novel card was not found in the present experiment.

While no changes in field properties in response to a novel spatial stimulus were detected, changes in other sessions were found. As in several other studies, we also found an initially rapid, then gradual modification of firing fields in response to novel environments (Wilson & McNaughton, 1993; Mehta et al., 2000; Frank, Stanley, & Brown, 2004; Leutgeb, Leutgeb, Treves, Moser, & Moser, 2004). Across all three groups, large increases in coherence were found between the first and second sessions. This result may suggest changes in synaptic plasticity between the first and second session. Moreover, this result may also suggest that the representation of this novel environment was stable by the second session.

Different signals were by the control group and the blocking groups following cue removal. Similar to Leutgeb at al. (2005), while other field properties remained constant a significant reduction in cell firing rate occurred when the black or white cue was removed. This may be similar to Leutgeb et al.'s 'rate remapping', but the interpretation of rate changes for the blocking groups in our experiment is complicated by the decline in firing rate over cumulative recording time. Future studies should contain an additional control group with a similar amount of cumulative recording time and the recording environment held constant. If field rates were constant in an unaltered environment, this

would indicate that the changes in firing rate in our experiment were due to card manipulations. Hetherington and Shapiro (1997) suggested that changes in field rate associated with cue removal might reflect learning or, to be more precise, unlearning. If this is true, then it may suggest that rate changes in hippocampal cells may provide a mechanism that allows for the flexible representation of altered environments. This interpretation of rate change raises the question as to whether or not one would expect decreased firing rate in place cells following cue removal if hippocampal plasticity was prevented. For example, would injecting an NMDA antagonist prevent the drop in field rate associated with cue removal?

While the predominant signal change following cue removal in the control group was a decrease in firing rate, cue removal in the blocking groups tended to elicit a decrease in field coherence. No changes were detected in coherence for fields in the control group. Muller and Kubie (1987) found that removing the only available cue card significantly reduced the coherence of place cells and that without this cue card to anchor the system, arbitrary rotations of firing fields could occur. The remaining black card in our experiment was able to serve as an anchoring spatial stimulus for the majority of rats and prevented arbitrary rotations of the firing fields. However the coherence drop suggests the absence of the original cue caused a clear perturbation to the map. It is not yet known how the change in coherence might correlate with behavioral performance if the rats were required to use the novel cue to locate a goal area. A similar drop in coherence was not found in the firing fields from control rats. This may be due to the occurrence of cue removal early on in the control rats' exposure to the novel environment or because both cues were treated as separate elements of a single cue configuration. These results may also indicate a difference in hippocampal processing during both the control and blocking protocols. In each protocol the hippocampal network is presented with either a pattern completion problem or a more complicated process of first incorporating a new spatial stimulus and then completing the pattern.

4.4 Is the process of map updating similar to reconsolidation?

While the results of our study suggest that rats were able to update their maps to include the novel cue, how the hippocampus was able to accomplish this end remains

unclear. Research into the phenomena of consolidation and reconsolidation has touched on the question of how memories are formed and then potentially altered following recall (Debiec, Ledoux and Nader, 2002; Nader, Schafe and Le Doux, 2000). Yet few studies have investigated these processes in relation to allocentric spatial memory (Suzuki et al., 2004; Morris et al., 2006). Spatial memory is rapidly acquired, as are fear conditioning and inhibitory avoidance (Milekic and Alberini, 2002; Nader et al., 2000). Once an animal has been trained to asymptote in a given spatial task or firing field properties for place cells have stabilized, one can assume that no new learning need occur. If the spatial memory is consolidated at this point, then a sudden change in the environment or reward contingency may make recall associated reconsolidation necessary to facilitate its alteration. According to the cognitive map theory, misplace detectors would update the representation of space and cause new memory coding being engaged. In order to keep up with spatial changes, memory encoding will remain engaged at the time of retrieval. Consolidated spatial representations again become labile and, as a consequence, subject to reconsolidation. Morris et al. (2006) performed an experiment where following 6 days of training in a DMP (delayed matching-to-place) in which the rats were trained to find a submerged platform in a water maze. New memory encoding was required each day as the location of the escape platform changed. Following the first trial, the platform would remain stable for the rest of the trials occurring that day. In contrast to another group of rats whose platform location was kept in a constant location, sensitivity to intrahippocampal anisomycin was found for rats performing the DMP task. As anisomycin blocks protein synthesis required for LTP and memory consolidation, this suggests that there was a reconsolidation process that was necessary for performance in the DMP task. The DMP task therefore requires rapid updating, yet this alteration may entail more of "where" the submerged platform is located in space as opposed to the spatial representation of itself. This feature of the DMP task it analogous to the short compound protocol in our experiment. In both cases the map of space must be updated rapidly, in a one minute trial for the rats in the DMP study and in a single 10 minute session for the short compound rats. The short compound rats likely become aware of the novel cue card in the first few seconds of compound exposure while the rats in the DMP task will find the new platform location on each day in under a minute. While this rapid

learning in the DMP task has been determined to be hippocampal dependent and encoding of the novel platform location of the submerged platform in NMDA receptor dependent (Bast, da Silva and Morris, 2005), neither of these factors have been determined for cue card learning.

While these experiments attempt to demonstrate spatial reconsolidation, much more work has found evidence of the modification of cognitive spatial representations in the hippocampus in the form of remapping (Muller and Kubie, 1987; Muller, Poucet and Rivard, 2001), partial remapping (Anderson and Jeffery, 2003; Knierim, 2002; Lever et al., 2002; Shapiro et al., 1997; Skaggs and McNaughton, 1998; Tanila, Shapiro and Eichenbaum, 1997) or rate remapping (Leutgeb et al., 2005). The notion of map modification has been relatively prevalent since Kentros et al. (1998) showed that pre-established maps, or the ensembles of cells and their firing locations, were unchanged by NMDA blockers. The formation and short-term stability of firing fields in a novel environment was also found to be NMDA independent. However, it was the long-term stabilization of this map that was found to be NMDA dependent. Does this mean that the place cells that comprise a map of space cannot undergo reconsolidation? Are changes in cell firing a type of reconsolidation? It is at this point that the notions of consolidation and the modification of memories become rather vexed.

4.5 How can our results be reconciled with behavioral studies that show blocking?

The general absence of blocking in our study appears to be at odds with other studies that have found behavioral evidence of blocking in the spatial domain (Biegler and Morris, 1999; Chamizo, Sterio and Mackintosh, 1985; Rodrigo, Chamizo, McLaren and Mackintosh, 1997; Hamilton and Sutherland, 1999). These studies imply that even if map updating took place, the subjects did not use the novel spatial cues to find a goal area. Apart from the absence of a spatial task in out study, the key difference in the methodology of our experiment is the nature of our spatial cues. The cues presented to the rats in our experiment were black and white cards placed on the wall of the cylinder while the studies that have found spatial blocking tended to use 3 dimensional landmarks within or beyond the confines of the experimental apparatus (i.e., the landmark arrays from Biegler and Morris, 1999). Recent studies have suggested a dissociation between

contributions toward spatial learning by the local boundary that frames a given environment and spatial landmarks (Doeller and Burgess, 2007; Doeller, King and Burgess, 2007). Central to this proposed dissociation is the general finding that the hippocampus preferentially processes location in relation to local environment boundaries. The firing of hippocampal place cells has been found to reflect distance and direction in relation to local boundaries (Hartley et al., 2000; O'Keefe and Burgess, 1996), but not to intra-maze landmarks (Cressant, Muller and Poucet, 1997). Human hippocampal activation has also been shown to correspond to learning locations relative to local boundaries while the striatum is preferentially activated during local landmark learning (Doeller, King and Burgess, 2007).

Doeller and Burgess (2007) found that learning object-locations relative to intra-maze landmarks appears to obey the rules laid out by associative reinforcement theories in that the landmarks could be overshadowed by the boundary. In addition, landmarks could be blocked by learning about a goal location with respect to landmarks or the boundary. Doeller and Burgess also suggest spatial learning relative to environmental boundaries is incidental, occurring independently of behavioral error or the presence of other predictive cues. Boundary learning did not show evidence of overshadowing or blocking. Thus, spatial learning in relation to the boundary seems inconsistent with associative reinforcement based on a single prediction-error term, potentially requiring separate error signals or landmarks and for the environmental boundary.

The results of the Doeller and Burgess (2007) study therefore reveal key differences with regard to how the brain processes landmarks and cue cards. If the cue cards in our experiment were considered by the animals to be part of the boundary, then this may explain the general absence of blocking in our experimental design. If intra-maze landmarks are processed by extra-hippocampal structures like the striatum, then one might expect that spatial learning with reference to these cues to obey the laws of associative learning (O'Keefe and Nadel, 1978). Learning a goal location in reference to an array of landmarks may therefore block the learning of the same goal location in relation to novel landmarks (i.e., Biegler and Morris, 1999).

4.6 Conclusions concerning blocking in the hippocampal spatial representation

Our study suggests that the hippocampus has the ability to rapidly construct a map of a novel environment and then update that map to incorporate spatial stimuli that are added to the environment. While spatial blocking did occur using our experimental design, it was unlikely. Blocking of stimulus control over the position of firing fields is not a biological imperative.

It is unlikely there could have been a dissociation between firing field location and spatial choice behavior (see Lenck-Santini, Muller, Save, Poucet, 2002 for an example of this) but future studies should attempt to replicate the findings of the current study at the behavioral level. The importance of this experiment becomes underscored by the fact that the only evidence for blocking in the spatial domain has come from behavioral studies (Biegler and Morris, 1999; Chamizo, Sterio and Mackintosh, 1985; Doeller and Burgess, 2007; Rodrigo, Chamizo, McLaren and Mackintosh, 1997; Hamilton and Sutherland, 1999). Would a spatial behavioral choice also rotate in register with a novel cue card? An additional issue raised by Doeller and Burgess (2007) is whether or not there is a distinction between the manner in which the brain processes cues that may be considered to be part of the boundary and intra-maze landmarks. One could modify the design of our experiment to test whether or not spatial choice behavior in relation to a cue card could block learning based on the position of a 3 dimensional intra-maze landmark, or vice versa. If the acquisition of stimulus control by cue cards in relation to a goal area is similar to boundary learning in the Doeller and Burgess (2007) experiment, one might predict cue card learning would block or overshadow novel intra-maze landmarks. One would also expect that learning a goal location in relation to an intra-maze landmark would not block or overshadow a novel cue card.

The experimental design laid out in this study may therefore represent a powerful method of studying the acquisition of spatial learning involving different types of cues. It provides a simple test for the hippocampal network by demanding the creation of a novel representation of space, novelty detection, and incorporation of the novel cue into a pre-existing representation of space. Finally, while associative learning theory provides an almost ubiquitous model for learning, it captures the acquisition of some types of learning

better than others. The acquisition of stimulus control by cue cards over hippocampal firing fields does not obey the rules of associative learning.

Appendix A

Variance per Method of Rotation Analysis

Variance in Results reported by both field rotation analysis programs – The degree of maximum correlation between compared recording sessions tended to have less variance when the positional firing pattern was rotated in 7.5° wedges as opposed to 1° steps.

	WROT		STAND		BROT	
	1° Rotation	7.5° Wedge	1° Rotation	7.5° Wedge	1° Rotation	7.5° Wedge
R27	239.24	16.07	2.25	12.60	646.40	180.63
R33	156.89	270.00	204.48	69.64	490.98	352.57
R36	216.67	151.44	1149.46	33.75	595.94	744.23
R45	280.57	168.75	248.76	182.40	1031.54	526.28
R49	1788.29	613.39	848.00	225.00	1416.81	120.54
R53	134.33	243.75	56.00	240.00	261.33	168.75
R37	733.88	230.63	154.44	239.06	1001.62	275.89
R39	1173.30	360.06	286.38	268.38	550.67	553.39
R42	172.75	84.38	58.14	444.64	1571.33	862.50
R43	200.57	76.88	98.25	126.56	43.30	16.88
R28	2984.27	3939.11	1964.94	2025.92	93.5	113.13
R31	4101.14	95.42	525.07	2313.13	483.50	282.81
R32	183.25	207.81	293.84	128.57	56.29	163.39
R48	4046.20	2233.13	93.67	56.25	3241.67	2587.50
R51	676.47	172.84	55.67	230.05	428.87	132.95
R52	671.47	1381.70	282.48	163.39	3779.37	3751.88
Mean	1109.96	640.33	395.11	422.46	980.62	724.73
STDERR	+/- 346.02	+/- 263.71	+/- 130.43	+/- 173.08	+/- 272.08	+/- 253.44

Appendix B

Field Rotation per Individual Rat, Group and Method of Rotation Analysis

Results reported by both field rotation analysis programs – The first program rotated the entire positional firing pattern for one session in 1° steps. After each step, the pixel-by-pixel correlation between the stationary and rotated firing patterns was computed. After 360 steps, the maximum correlation was found. The angle of maximum correlation was taken as the magnitude by which the field rotated. In the second program, the maximum correlation for the two sessions was calculated for forty-eight 7.5° wedges. As with the first program, the angular position of the maximum correlation was taken as the magnitude of field rotation.

	1° Rotation	7.5° Wedge	1° Rotation	7.5° Wedge	1° Rotation	7.5° Wedge
Short Compound	Stand/Wrot	Stand/Wrot	Stand/Stand	Stand/Stand	Stand/Brot	Stand/Brot
R27	26.71°	33.21°	0.25°	-1.88°	35.20°	29.25°
R33	23.67°	37.50°	6.14°	5.36°	19.88°	25.31°
R36	38.00°	39.23°	-6.20°	1.50°	41.64°	45.00°
R45	38.00°	37.50°	-6.59°	-5.63°	40.58°	38.13°
R49	52.43°	47.14°	-11.00°	15.00°	40.14°	36.43°
R53	55.67°	50.00°	3.00°	2.50°	15.67°	30.00°
	n=51		n=54		n=54	
Means	39.08°	40.76°	-2.40°	2.81°	32.19°	34.02°
STDERR	+/- 5.31°	+/-2.63°	+/- 2.68°	+/- 2.89°	+/- 4.68°	+/- 2.93°
Long Compound	Stand/Wrot	Stand/Wrot	Stand/Stand	Stand/Stand	Stand/Brot	Stand/Brot
R28	63.47°	36.00°	14.00°	17.9°	8.2°	9.75°
R31	-3.00°	25.31°	2.20°	-17.3°	28.67°	33.33°
R32	30.67°	35.83°	11.13°	7.5°	-0.43°	5.36°
R48	70.20°	39.00°	-6.50°	-3.0°	73.50°	67.50°
R51	48.45°	41.59°	0.00°	2.9°	37.55°	38.86°
R52	31.55°	38.86°	-8.86°	-12.9°	26.17°	33.75°
	n=59		n=60		n=47	
Means	40.22°	36.10°	1.99°	-0.80°	28.94°	31.43°
STDERR	+/- 10.87°	+/- 2.33°	+/- 3.75°	+/- 5.33°	+/- 10.58°	+/- 9.16°
Control Group	Stand/Wrot	Stand/Wrot	Stand/Stand	Stand/Stand	Stand/Brot	Stand/Brot
R37	48.10°	45.75°	-10.78°	-7.50°	42.57°	39.64°
R39	38.29°	34.82°	1.17°	-1.67°	35.67°	40.18°
R42	23.67°	32.50°	-3.14°	5.36°	19.00°	37.50°
R43	49.17°	43.75°	5.25°	9.38°	48.60°	48.00°
	5.91°	3.26°	3.43°	3.74°	6.39°	2.30°
	n=39		n=39		n=31	
means	39.80°	39.21°	-1.88°	1.39°	36.46°	41.33°
STDERR	+/- 9.15°	+/- 8.52°	+/- 3.20°	+/- 3.28°	+/- 8.71°	+/- 8.95°
Pooled Average	39.69°	38.63°	-0.62°	1.10°	30.37°	30.89°
	$n_{Total} = 149$		$n_{Total} = 152$		$n_{Total} = 132$	

Appendix C

Average Field Properties for Place Cells used in Rotation analyses Between Key Session Comparisons

Average firing pattern parameters for place cells (n=51) in the second standard session (Session 2) and white card rotation session (Session 3) – Short Compound Group (N=6)

	Session 2	Session 3	Paired *t*	P (*two-tailed*) α/1
Grand Rate	1.11 ± 0.13	1.03 ± 0.09	1.20	0.24
Field Rate	3.22 ± 0.25	3.0 ± 0.20	1.33	0.19
Size	102.7 ± 8.96	102.9 ± 8.21	- 0.03	0.98
Coherence	0.67 ± 0.02	0.67 ± 0.02	- 0.03	0.98
Information	1.82 ± 0 .08	1.83 ± 0.10	- 0.16	0.88

Average firing pattern parameters for place cells (n=59) in the second standard session (Session 2) and white card rotation session (Session 3) – Long Compound Group (N=6)

	Session 2	Session 3	Paired *t*	P (*two-tailed*) α/1
Grand Rate	0.99 ± 0.09	1.05 ± 0.11	- 0.76	0.22
Field Rate	3.23 ± 0.24	3.03 ± 0.20	1.25	0.11
Size	99.5 ± 0.18	98.8 ± 0.17	0.13	0.90
Coherence	0.68 ± 0.02	0.65 ± 0.02	1.56	0.12
Information	2.02 ± 0 .09	1.97 ± 0.08	0.95	0.35

Average firing pattern parameters for place cells (n=39) in the third standard session (Session 4) and white card rotation session (Session 5) –Control Group (N=4)

	Session 2	Session 3	Paired *t*	P (*two-tailed*) α/1
Grand Rate	0.87 ± 0.10	0.79 ± 0.10	2.00	0.05
Field Rate	2.53 ± 0.22	1.99 ± 0.18	4.58	4.8×10^{-5} *
Size	105.6 ± 11.2	115.5 ± 11.9	- 1.86	0.07
Coherence	0.58 ± 0.03	0.55 ± 0.03	0.78	0.22

Information	1.75 ± 0 .11	1.61 ± 0.10	2.68	0.01 *

Average firing pattern parameters for place cells (n=54) in the second standard session (Session 2) and third standard session (Session 4) – Short Compound Group (N=6)

	Session 2	Session 4	Paired *t*	P (*two-tailed*) α/2
Grand Rate	1.03 ± 0.12	0.95 ± 0.08	1.03	0.30
Field Rate	3.06 ± 0.24	2.75 ± 0.19	1.99	0.05
Size	95.1 ± 7.77	99.8 ± 7.26	-0.87	0.39
Coherence	0.65 ± 0.02	0.65 ± 0.02	0.02	0.98
Information	1.83 ± 0 .07	1.75 ± 0.08	1.56	0.12

Average firing pattern parameters for place cells (n=60) in the second standard session (Session 2) and third standard session (Session 4) – Long Compound Group (N=6)

	Session 2	Session 4	Paired *t*	P (*two-tailed*) α/2
Grand Rate	1.08 ± 0.09	1.11 ± 0.11	- 0.5	0.60
Field Rate	3.39 ± 0.23	3.32 ± 0.22	0.57	0.57
Size	109.2 ± 8.14	104.1 ± 8.56	0.89	0.38
Coherence	0.68 ± 0.02	0.67 ± 0.02	0.98	0.33
Information	1.99 ± 0 .09	2.01 ± 0.09	-0.47	0.64

Average firing pattern parameters for place cells (n=38) in the second standard session (Session 2) and third standard session (Session 4) –Control Group (N=4)

	Session 2	Session 4	Paired *t*	P (*two-tailed*) α/2
Grand Rate	0.88 ± 0.08	0.81 ± 0.09	- 1.5	0.07
Field Rate	2.99 ± 0.29	2.44 ± 0.22	3.02	4.6×10^{-3} *
Size	91.5 ± 8.66	101.2 ± 11.7	- 1.33	0.19

Coherence	0.62 ± 0.02	0.59 ± 0.02	1.43	0.16
Information	1.90 ± 0 .11	1.81 ± 0.11	1.39	0.17

Average firing pattern parameters for place cells (n=54) for the compound session (Session 5) and black card rotation session (Session 6) – Short Compound Group (N=6)

	Session 5	Session 6	Paired *t*	P (*two-tailed*) α/3
Grand Rate	1.01 ± 0.87	0.95 ± 0.08	2.26	0.03
Field Rate	3.03 ± 0.17	2.48 ± 0.14	3.96	2.25×10^{-4} *
Size	107.3 ± 6.62	90.6 ± 6.03	2.69	9.6×10^{-3} *
Coherence	0.65 ± 0.02	0.56 ± 0.02	4.19	1.1×10^{-4} *
Information	1.76 ± 0 .09	1.63 ± 0.08	1.97	0.05

Average firing pattern parameters for place cells (n=48) for the third compound session (Session 7) and black card rotation session (Session 8) – Long Compound Group (N=6)

	Session 7	Session 8	Paired *t*	P (*two-tailed*) α/3
Grand Rate	0.89 ± 0.10	0.73 ± 0.07	1.8	0.07
Field Rate	2.68 ± 0.21	2.49 ± 0.18	0.86	0.39
Size	82.8 ± 7.58	72.3 ± 6.85	1.73	0.09
Coherence	0.58 ± 0.03	0.54 ± 0.02	1.63	0.12
Information	1.99 ± 0 .03	2.03 ± 0.02	1.63	0.11

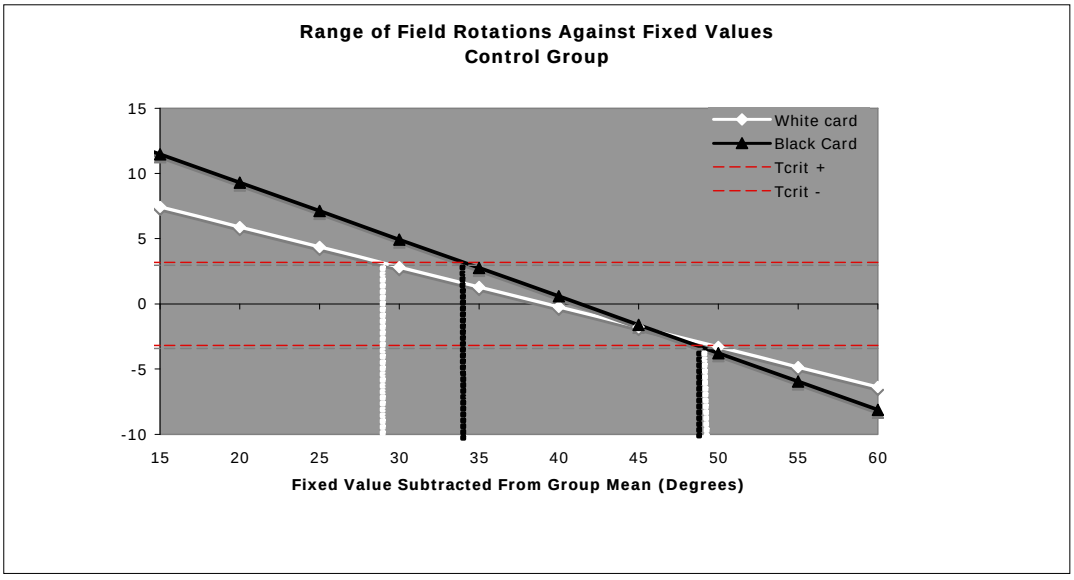
Average firing pattern parameters for place cells (n=31) for the second standard session (Session 2) and black card rotation session (Session 3) –Control Group (N=4)

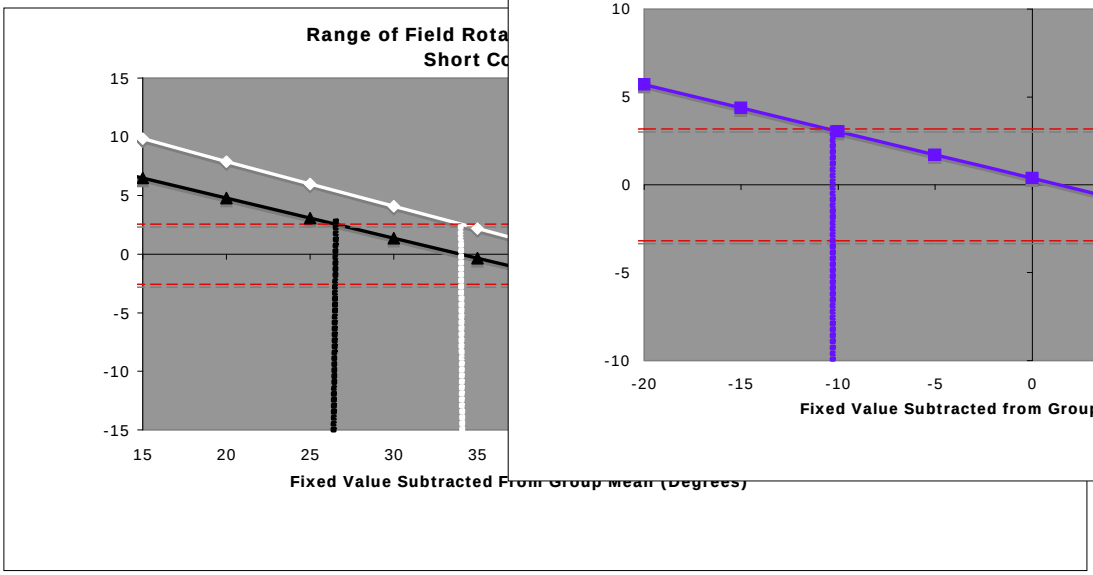
	Session 2	Session 3	Paired *t*	P (*two-tailed*) α/3
Grand Rate	1.0 ± 0.12	0.90 ± 0.12	1.32	0.20
Field Rate	3.03 ± 0.27	2.46 ± 0.21	3.79	6.7×10^{-4} *
Size	101.0 ± 9.62	103.0 ± 9.62	- 0.30	0.77

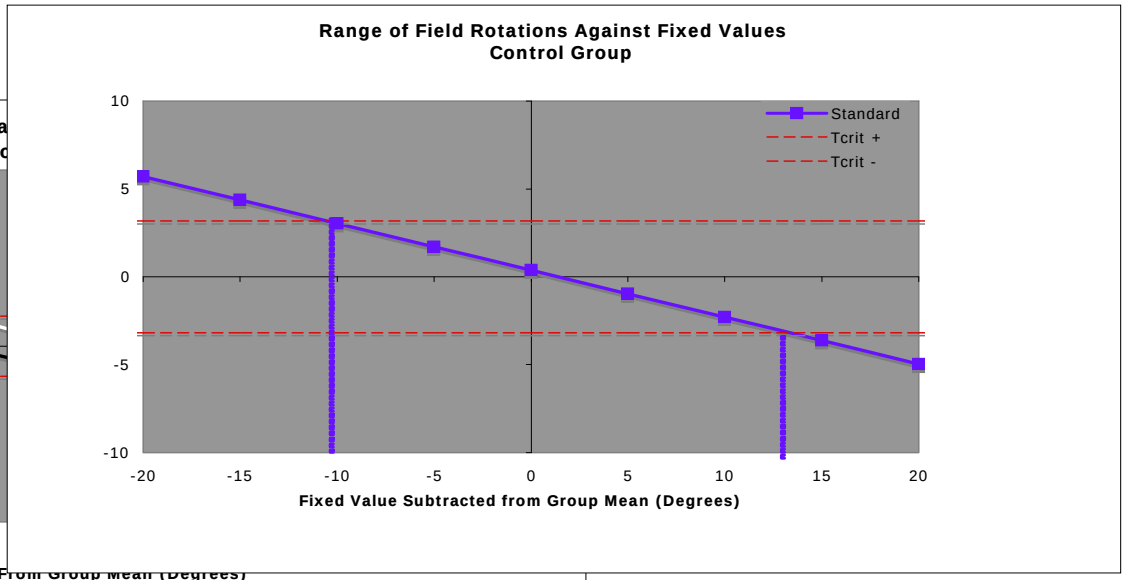
Coherence	0.64 ± 0.02	0.58 ± 0.03	2.66	0.012	*
Information	1.85 ± 0 .10	1.70 ± 0.10	2.41	0.020	

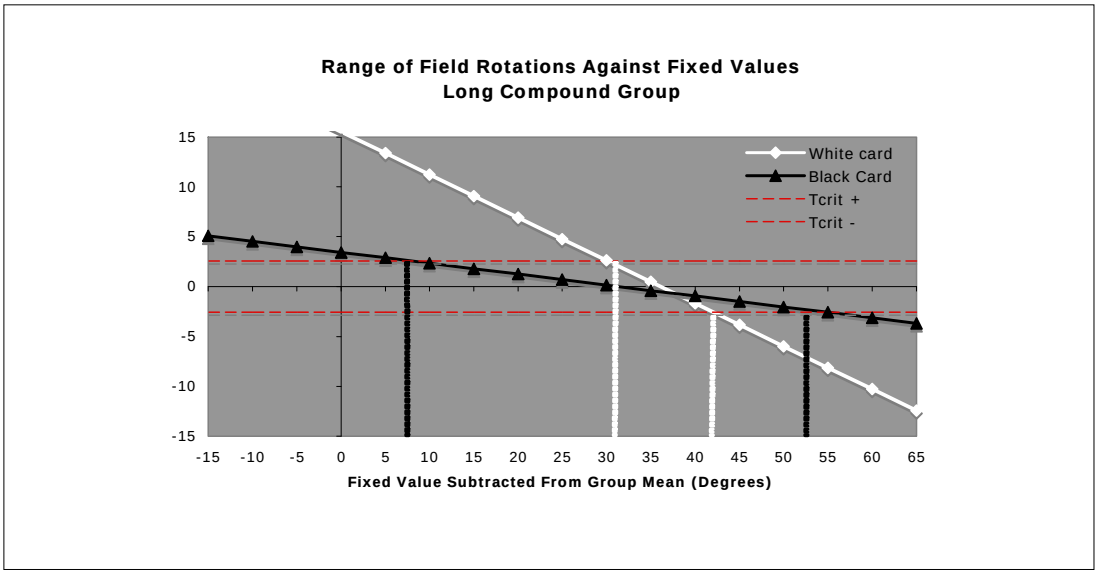
Appendix D

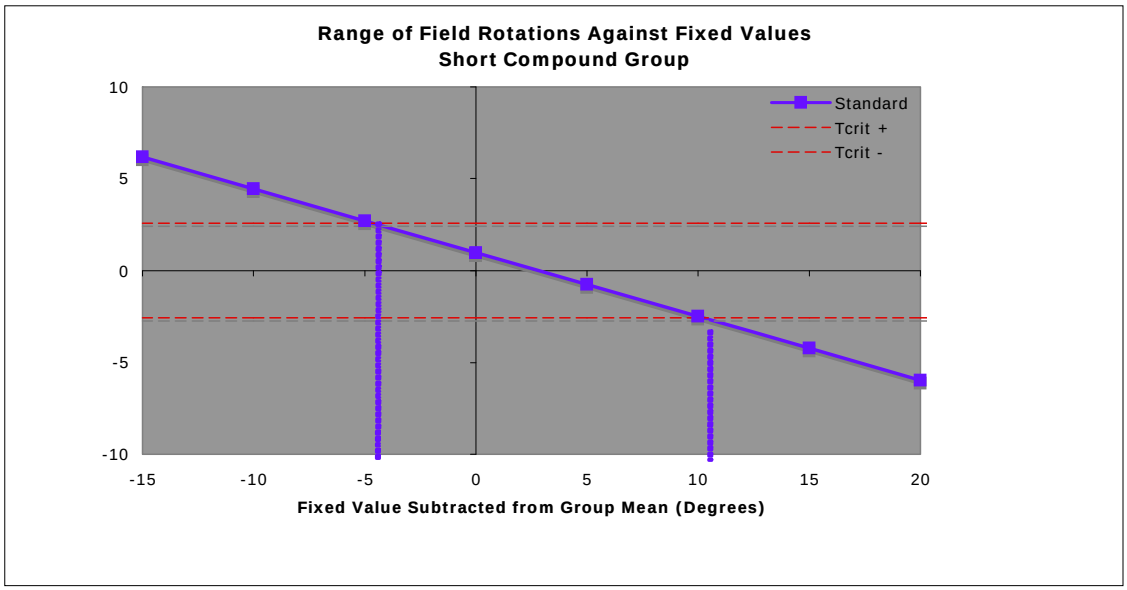
Results of Fixed-value t-tests per group: Determining the angle of peak rotation

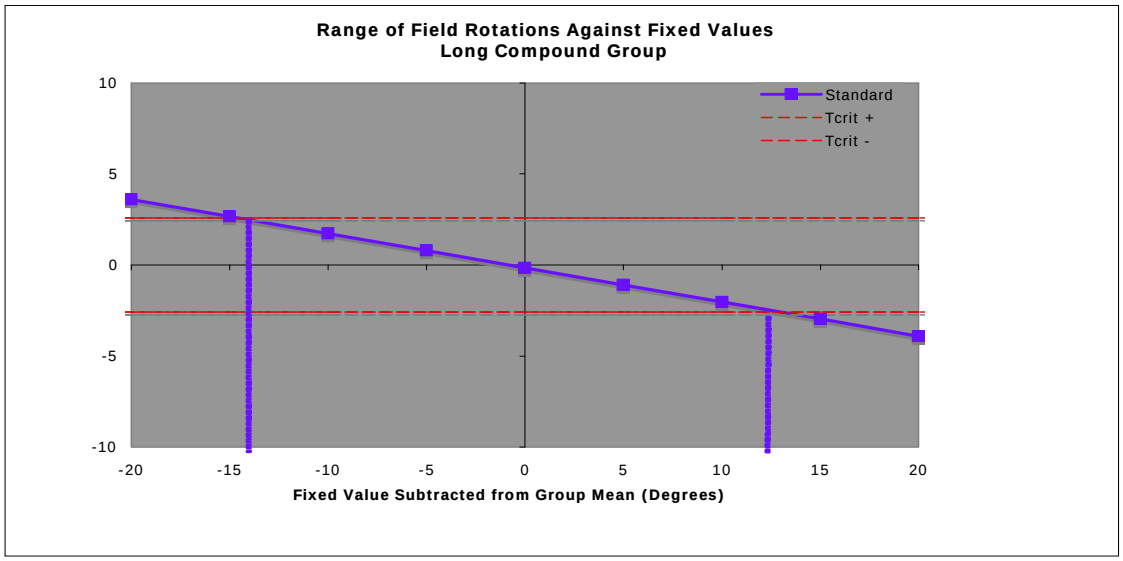



Range of Field Rota
Short Co
15
10
5
0
-5
-10
-15
15
20
25
30
35
Fixed Value Subtracted From Group Mean (Degrees)


Range of Field Rotations Against Fixed Values
Control Group
10
5
0
-5
-10
-20
-15
-10
-5
0
5
10
15
20
Standard
Tcrit +
Tcrit -
Fixed Value Subtracted from Group Mean (Degrees)


Range of Field Rotations Against Fixed Values
Long Compound Group
15
10
5
0
-5
-10
-15
-15
-10
-5
0
5
10
15
20
25
30
35
40
45
50
55
60
65
White card
Black Card
Tcrit +
Tcrit -
Fixed Value Subtracted From Group Mean (Degrees)


Range of Field Rotations Against Fixed Values
Short Compound Group
10
5
0
-5
-10
-15
-10
-5
0
5
10
15
20
Standard
Tcrit +
Tcrit -
Fixed Value Subtracted from Group Mean (Degrees)


Range of Field Rotations Against Fixed Values
Long Compound Group
10
5
0
-5
-10
-20
-15
-10
-5
0
5
10
15
20
Standard
Tcrit +
Tcrit -
Fixed Value Subtracted from Group Mean (Degrees)

Tables

Table 2: The average coherence values for each rat in both blocking groups are pooled both for the first 5 sessions. Repeated measures analysis of variance suggests a significant effect of coherence across sessions. The results of Newman-Keuls pairwise comparisons are shown. Significant differences between pairs of sessions are in bold and italicized. The average coherence was significantly lower in session 1 than sessions 2, 3 and 4.

Time (mins)		10	28	46	64	82
Session		{1}	{2}	{3}	{4}	{5}
Average		0.538	0.597	0.585	0.596	0.573
1	{1}		***0.024***	***0.043***	***0.016***	0.065
2	{2}	***0.024***		0.792	0.988	0.602
3	{3}	***0.043***	0.792		0.528	0.556
4	{4}	***0.016***	0.988	0.528		0.443
5	{5}	0.065	0.602	0.556	0.443	

Table 3: Repeated measures analysis of variance suggests a significant effect of coherence across sessions for rats in the short compound group. The results of Newman-Keuls pairwise comparisons are shown. Significant differences between pairs of sessions are in bold and italicized. The average coherence was significantly lower in session 6 than sessions 2 -5.

Time (mins)		10	28	46	64	82	100
Session		{1}	{2}	{3}	{4}	{5}	{6}
Average		0.525	0.598	0.595	0.590	0.574	0.462
1	{1}		0.165	0.142	0.114	0.128	0.054
2	{2}	0.165		0.914	0.964	0.867	***0.002***
3	{3}	0.142	0.914		0.882	0.788	***0.002***
4	{4}	0.114	0.964	0.882		0.614	***0.002***
5	{5}	0.128	0.867	0.788	0.614		***0.004***
6	{6}	0.054	***0.002***	***0.002***	***0.002***	***0.004***	

Table 4: Repeated measures analysis of variance suggests a significant effect of coherence across sessions for rats in the long compound group. The results of Newman-Keuls pairwise comparisons are shown. Significant differences between pairs of sessions are in bold and italicized. The average coherence was significantly lower in session 8 than sessions 2 -7. Sessions 6 and 7 are significantly lower than sessions 2 and 4.

Time (mins)		10	28	46	64	92	120	148	166
Session		{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
Average		0.551	0.595	0.574	0.603	0.573	0.527	0.530	0.464
1	{1}		0.197	0.537	0.148	0.324	0.538	0.359	***0.002***
2	{2}	0.197		0.346	0.735	0.565	***0.041***	***0.041***	***0.000***
3	{3}	0.537	0.346		0.407	0.942	0.225	0.207	***0.000***
4	{4}	0.148	0.735	0.407		0.526	***0.023***	***0.025***	***0.000***
5	{5}	0.324	0.565	0.942	0.526		0.182	0.146	***0.000***
6	{6}	0.538	***0.041***	0.225	***0.023***	0.182		0.888	***0.007***
7	{7}	0.359	***0.041***	0.207	***0.025***	0.146	0.888		***0.013***
8	{8}	***0.002***	***0.000***	***0.000***	***0.000***	***0.000***	***0.007***	***0.013***	

Table 5: Repeated measures analysis of variance suggests a significant effect of field rate across sessions for rats in the short compound group. The results of Newman-Keuls pairwise comparisons are shown. Significant differences between pairs of sessions are in bold and italicized. The field rate was significantly lower in session 6 than sessions 2 -5.

Time (mins)	10	28	46	64	82	100
Session	{1}	{2}	{3}	{4}	{5}	{6}
Average	2.4385	2.6433	2.5805	2.5135	2.4005	2.0334
1 {1}		0.5984	0.6631	0.6496	0.8176	0.0508
2 {2}	0.5984		0.7037	0.7089	0.5786	***0.0111***
3 {3}	0.6631	0.7037		0.6846	0.6903	***0.0196***
4 {4}	0.6496	0.7089	0.6846		0.7697	***0.0327***
5 {5}	0.8176	0.5786	0.6903	0.7697		***0.0335***
6 {6}	0.0508	***0.0111***	***0.0196***	***0.0327***	***0.0335***	

Table 6: Repeated measures analysis of variance suggests a significant effect of field rate across sessions for rats in the long compound group. The results of Newman-Keuls pairwise comparisons are shown. Significant differences between pairs of sessions are in bold and italicized. The average field rate was significantly lower in session 8 than sessions 1, 2 and 4.

Time (mins)	10	28	46	64	92	120	148	166
Session	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
Average	2.9557	2.8781	2.6608	2.8491	2.6927	2.3111	2.3418	2.1382
1 {1}		0.7441	0.7222	0.8939	0.6827	0.1196	0.1233	***0.0275***
2 {2}	0.7441		0.7937	0.9031	0.7139	0.1824	0.1775	***0.0487***
3 {3}	0.7222	0.7937		0.7064	0.8933	0.3110	0.1848	0.1386
4 {4}	0.8939	0.9031	0.7064		0.5115	0.1749	0.1571	***0.0498***
5 {5}	0.6827	0.7139	0.8933	0.5115		0.3816	0.3088	0.1531
6 {6}	0.1196	0.1824	0.3110	0.1749	0.3816		0.8970	0.4685
7 {7}	0.1233	0.1775	0.1848	0.1571	0.3088	0.8970		0.6664
8 {8}	***0.0275***	***0.0487***	0.1386	***0.0498***	0.1531	0.4685	0.6664	

Table 7: Repeated measures analysis of variance suggests a significant effect of field rate across sessions for rats in the control group. The results of Newman-Keuls pairwise comparisons are shown. Significant differences between pairs of sessions are in bold and italicized. The average field rate was significantly lower in session 5 than all previous sessions. The average field rate in session 2 was significantly higher than sessions 3 and 4.

Time (mins)	10	28	46	64	82
Session	{1}	{2}	{3}	{4}	{5}
Average	2.5496	2.8681	2.2038	2.2984	1.7868
1 {1}		0.1187	0.2034	0.2098	***0.0080***
2 {2}	0.1187		***0.0197***	***0.0275***	***0.0009***
3 {3}	0.2034	***0.0197***		0.6267	***0.0482***
4 {4}	0.2098	***0.0275***	0.6267		***0.0474***
5 {5}	***0.0080***	***0.0009***	***0.0482***	***0.0474***	

Table 8: Repeated measures analysis of variance suggests a significant effect of grand rate across sessions for rats in the long compound group. The results of Newman-Keuls pairwise comparisons are shown. Significant differences between pairs of sessions are in bold and italicized. The average field rate was significantly lower in session 8 than the peak value during session 2.

Time (mins)	10	28	46	64	92	120	148	166
Session	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
Average	0.950	1.044	0.961	0.980	0.927	0.815	0.867	0.707
1 {1}		0.766	0.911	0.948	0.816	0.521	0.680	0.115
2 {2}	0.766		0.669	0.513	0.748	0.250	0.470	***0.028***
3 {3}	0.911	0.669		0.844	0.936	0.575	0.776	0.123
4 {4}	0.948	0.513	0.844		0.947	0.547	0.777	0.103
5 {5}	0.816	0.748	0.936	0.947		0.494	0.548	0.128
6 {6}	0.521	0.250	0.575	0.547	0.494		0.595	0.273
7 {7}	0.680	0.470	0.776	0.777	0.548	0.595		0.238
8 {8}	0.115	***0.028***	0.123	0.103	0.128	0.273	0.238	

Table 9: Repeated measures analysis of variance suggests a significant effect of field size across sessions for rats in the long compound group. The results of Newman-Keuls pairwise comparisons are shown. Significant differences between pairs of sessions are in bold and italicized. The average field size was significantly smaller in session 8 than sessions 2 -4.

Time (mins)	10	28	46	64	92	120	148	166
Session	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
Average	97.822	106.769	102.935	100.877	90.850	82.226	85.079	72.858
1 {1}		0.749	0.836	0.734	0.440	0.316	0.338	0.060
2 {2}	0.749		0.670	0.788	0.399	0.116	0.174	***0.012***
3 {3}	0.836	0.670		0.819	0.536	0.214	0.287	***0.028***
4 {4}	0.734	0.788	0.819		0.507	0.248	0.305	***0.037***
5 {5}	0.440	0.399	0.536	0.507		0.603	0.522	0.202
6 {6}	0.316	0.116	0.214	0.248	0.603		0.751	0.301
7 {7}	0.338	0.174	0.287	0.305	0.522	0.751		0.368
8 {8}	0.060	***0.012***	***0.028***	***0.037***	0.202	0.301	0.368	

References

Anderson, M.I., & Jeffery K.J. (2003). Heterogeneous modulation of place cell firing by changes in context. *J Neurosci. 23(26)*: 8827-8835.

Amaral, D.G., Witter, M.P. (1995). The hippocampal formation In: *The Rat Brain*. Paxinos G (Ed.), Academic Press 2nd edition, 443-493.

Azorlosca, J.L., & Cicala, G. (1986). Blocking of conditioned suppression with 1 or 10 compound trials. *Animal Learning and Behavior 14(2)*: 163-167.

Balaz, M., Kasprow, W.J., & Miller R.R. (1982). Blocking with a single compound trial. *Animal Learning and Behavior 10*: 271-276.

Bast, T., da Silva, B.M., & Morris R.G. (2005). Distinct contributions of hippocampal NMDA and AMPA receptors to encoding and retrieval of one-trial place memory. *J Neurosci. 25(25)*: 5845-5856.

Biegler, R., & Morris, R.G. (1999). Blocking in the spatial domain with arrays of discrete landmarks. *J Exp Psychol Anim Behav Process 25(3)*: 334-351.

Bliss, T., & Lomo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J Physiol. 232(2)*: 331-356.

Bohbot, V.D., Lerch, J., Thorndycraft, B., Iaria, G., & Zijdenbos, A.P. (2007). Gray matter differences correlate with spontaneous strategies in a human virtual navigation task. *J Neurosci*. 27(38): 10078-10083.

Bohbot, V.D, Iaria, G., & Petrides M. (2004). Hippocampal function and spatial memory: evidence from functional neuroimaging in healthy participants and performance of patients with medial temporal lobe resections. *Neuropsychology. 18(3)*: 418-425.

Bostock, E., Muller, R.U., & Kubie J.L. (1991). Experience-dependent modifications of hippocampal place cell firing. *Hippocampus 1(2)*: 193-205.

Chamizo, V.D., Sterio, D. & Mackintosh, N.J. (1985). Blocking and overshadowing between intra-maze and extra-maze cues: a test of the independence of locale and guidance learning. *Quarterly Journal of Experimental Psychology, 37B*, 235-253.

Cheng, K. (1986). A purely geometric module in the rat's spatial representation. *Cognition. 23(2)*: 149-178.

Cheng, K., & Gallistel, C. R. (1984). Testing the geometric power of an animal's spatial representation. In Roitblatt, H. L., Bever, T. G., & Terrace, H. S. (Eds.), *Animal cognition* (pp. 409–423). Hillsdale, NJ: Erlbaum.

Cimadevilla, J.M., Fenton, A.A., & Bures, J. (2001). New spatial cognition tests for mice: passive place avoidance on stable and active place avoidance on rotating arenas. *Brain Res Bull 54(5)*: 559-63.

Cressant, A., Muller, R.U., & Poucet, B. (1997). Failure of centrally placed objects to control the firing fields of hippocampal place cells. *J Neurosci. 17(7)*: 2531-2542.

Cressant, A., Muller, R.U., & Poucet, B. (1999). Further study of the control of place cell firing by intra-apparatus objects. *Hippocampus 9(4)*: 423-431.

Debiec, J., & LeDoux, J.E., & Nader, K. (2002). Cellular and systems reconsolidation in the hippocampus. *Neuron 36(3)*: 527-38.

Dickinson, A., Nicholas, D.J., & Mackintosh, N.J. (1983). A re-examination of one-trial blocking in conditioned suppression. *The Quarterly Journal of Experimental Psychology 35 (1)*: 67–79.

Doeller, C.F., & Burgess, N. (20007). Distinct error-correcting and incidental learning of location relative to landmarks and boundaries. *PNAS,* submitted.

Doeller, C.F., King, J.A., & Burgess, N. (20007). *PNAS,* submitted.

Estes, W. K. & Skinner, B. F. (1941). Some quantitative properties of anxiety. *Journal of Experimental Psychology* 29: 390-400.

Fenton, A., Csizmadia, G., & Muller, R.U. (2000). Conjoint control of hippocampal place cell firing by two visual stimuli. I. The effects of moving the stimuli on firing field positions. *J Gen Physiol. 116(2)*:191-209.

Fox, S.E., & Ranck, J.B. (1975). Localization and anatomical identification of theta and complex spike cells in dorsal hippocampal formation of rats. *Exp Neurol. 49(1 Pt 1)*: 299-313.

Fox, S.E., Ranck, J.B. (1981). Electrophysiological characteristics of hippocampal complex-spike cells and theta cells. *Exp Brain Res. 41(3-4)*: 399-410.

Frank, L.M., Stanley, G.B., & Brown, E.N. (2004). Hippocampal plasticity across multiple days of exposure to novel environments. *J Neurosci. 1;24(35)*: 7681-7689.

Gallistel, R. (1990). *The organization of learning.* MIT Press: Cambridge, USA.

Gillian, D.J., & Domjan, M. (1977). Taste aversion conditioning with expected an unexpected drug treatment. *Journal of Experimental Behavior: Animal Behavior Processes 3*: 297-309.

Gray, J. (2000). *The neuropsychology of anxiety: An enquiry into the functions of the septo-hippocampal system*. 2nd ed. Oxford: Oxford University Press.

Hartley, T., Burgess, N., Lever, C., Cacucci, F., O'Keefe, J. (2000). Modeling place fields in terms of the cortical inputs to the hippocampus. *Hippocampus 10(4)*: 369-379.

Hartley, T., Maguire, E.A., Spiers, H.J., & Burgess N. (2003). The well-worn route and the path less traveled: distinct neural bases of route following and wayfinding in humans. *Neuron 37(5)*: 877-888.

Hamilton, D.A., Sutherland, R.J., Hamilton, D.A., & Sutherland, R.J. (1999). Blocking in human place learning: evidence from virtual navigation. *Psychobiology 27*: 453–461.

Hermer, L., & Spelke E.S. (1994). A geometric process for spatial reorientation in young children. *Nature. 370(6484)*: 57-59.

Herrera, D.G., & Robertson, H.A. (1996). Activation of c-fos in the brain. *Prog Neurobiol. 50(2-3)*: 83-107.

Hess, U.S., Lynch, G., & Gall, C.M. (1995). Regional patterns of c-fos mRNA expression in rat hippocampus following exploration of a novel environment versus performance of a well-learned discrimination. *J Neurosci.15(12):* 7796-7809.

Hetherington, P.A., & Shapiro, M.L. (1997). Hippocampal place fields are altered by the removal of single visual cues in a distance-dependent manner. Behav Neurosci. *111(1)*: 20-34.

Huxter, J.R., Thorpe, C.M., Martin., G.M., & Harley, C.W. (2001). Spatial problem solving and hippocampal place cell firing in rats: control by an internal sense of direction carried across environments. *Brain Res 123(1)*: 37-48.

Jarrard, L.E. (1983). Selective hippocampal lesions and behavior: effects of kainic acid lesions on performance of place and cue tasks. Behav Neurosci. 97(6): 873-89.

Jeffery, K.J., Donnet, J.G., Burgess, N., & O'Keefe, J.M. (1997). Directional control of hippocampal place fields. *Exp Brain Res. 117(1)*: 131-142.

Jeffery, K.J., & O'Keefe, J.M. (1999). Learned interaction of visual and idiothetic cues in the control of place field orientation. *Exp Brain Res. 127(2)*: 151-161.

Jenkins, T.A., Amin, E., Pearce, J.M., Brown, M.W., & Aggleton, J.P. (2004). Novel spatial arrangements of familiar visual stimuli promote activity in the rat hippocampal formation but not the parahippocampal cortices: a c-fos expression study. *Neuroscience 124(1)*: 43-52.

Kamin, L.J. 1968. 'Attention-like' processes in classical conditioning. In M.R. Jones (Ed.). *Miami symposium on the prediction of behaviour: Aversive stimulation*. Univ. of Miami Press, 9-33

Kamin, LJ. (1969). Predictability, surprise, attention, and conditioning. In Campbell, B.A., Church RM (Eds.), *Punishment and Aversive Behavior* (pp. 242-259). New York: Appleton-Century-Crofts.

Kentros, C., Hargreaves, E., Hawkins, R.D., Kandel ER, Shapiro M, & Muller, R.V. (1998). Abolition of long-term stability of new hippocampal place cell maps by NMDA receptor blockade. *Science 280(5372*): 2121-2126.

Knierim, J.J. (2002). Dynamic interactions between local surface cues, distal landmarks, and intrinsic circuitry in hippocampal place cells. *J Neurosci. 22(14)*: 6254-6264.

Knierim, J.J., Kudrimoti, H.S., & McNaughton, B.L. (1995). Place cells, head direction cells, and the learning of landmark stability. *J Neurosci. 15 (3 Pt 1)*:1648-1659.

Lenck-Santini, P.P., Muller, R.U., Save, E., & Poucet, B. (2002). Relationships between place cell firing fields and navigational decisions by rats. *J Neurosci. 22(20)*: 9035-9047.

Lenck-Santini, P.P., Rivard, B., Muller, R.U., & Poucet, B. (2005). Study of CA1 place cell activity and exploratory behavior following spatial and nonspatial changes in the environment. *Hippocampus 15(3)*: 356-369.

Lenck-Santini, P.P., Save, E., & Poucet, B. (2001). Evidence for a relationship between place-cell spatial firing and spatial memory performance. *Hippocampus 11(4)*: 377-390.

Leutgeb, S., & Leutgeb, J. (2007). Pattern separation, pattern completion, and new neural codes within a continuous CA3 map. *Learning and Memory 14*: 745-757.

Leutgeb, S., Leutgeb, J.K., Treves, A., Moser, M.B., & Moser E.I.(2004). Distinct ensemble codes in hippocampal areas CA3 and CA1. Science *305(5688):* 1295-1298.

Leutgeb, S., Leutgeb, J., Barnes, C., & Moser, E., McNaughton, B., Moser, M.B. (2005). Independent Codes for Spatial and Episodic Memory in Hippocampal Neuronal Ensembles. *Science 309(5734)*: 619 – 623.

Lever, C., Wills, T., Caccuci, F., Burgess, N., & O'Keefe, J. (2002). Long-term plasticity in hippocampal place-cell representation of environmental geometry. *Nature 416(6876)*: 90-94.

MacDonald, R.J., & White, N.M. (1993). A triple dissociation of memory systems: hippocampus, amygdala, and dorsal striatum. *Behav Neurosci. 107(1)*: 3-22.

Mackintosh, N.J. (1975). Blocking of conditioned suppression: role of the first compound trial. *J Exp Psychol Anim Behav Process 1(4)*: 335-45.

Mackintosh, N.J., Dickinson, A., Cotton, M.M. (1980). Surprise and blocking: Effects of the number of compound trials. *Anim. Learn. Behav. 8*: 387–391

Marr, D. (1971). Simple memory: a theory for archicortex. *Philos Trans R Soc Lond B Biol Sci. 262(841)*: 23-81.

McLelland, J.L., McNaughton, B.L., & O'Reilly, R.C. (1995). Why there are complementary learning systems in the hippocampus and neocortex: insights from the successes and failures of connectionist models of learning and memory. *Psychol Rev. 102(3):* 419-457.

McNaughton, B., Douglas, R., & Goddard, G. (1978). Synaptic enhancement in fascia dentata: Cooperativity among coactive afferents. *Brain Research 157(2)*: 277-293.

McNaughton, B.L., & Morris, R.G. (1987). Hippocampal synaptic enhancement and information storage within a distributed memory system. *Trends in Neurosciences 10 (10):* 408-415.

McNaughton, B. L. & Nadel, L. (1990). Hebb-Marr networks and the neurobiological representation of action in space. In Gluck, D & Rumelhart, D.E. (Eds.), *Neuroscience and Connectionist Theory* (pp. 1-63). Hillsdale NJ: Lawrence Erlbaum Assoc.

Mehta, M.R., Quirk, M.C., & Wilson, M.A. (2000). Experience-dependent asymmetric shape of hippocampal receptive fields. *Neuron 25(3)*: 707-715.

Milekic, M.H., Alberini, C.M. (2002). Temporally graded requirement for protein synthesis following memory reactivation. *Neuron 36(3)*: 521-525.

Morris, R.G., & Frey, U. (1997). Hippocampal synaptic plasticity: role in spatial learning or the automatic recording of attended experience? Philos Trans R Soc Lond B Biol Sci. 1997 352(1360):1489-503.

Morris, R.G., Garrud, P., Rawlins, J.N., & O'Keefe J. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature 297(5868)*: 681-683.

Morris, R.G., Inglis, J., Ainge, J.A., & Olverman, H.J., Tulloch, J., Dudai, Y., Kelly, P.A. (2006). Memory reconsolidation: sensitivity of spatial memory to inhibition of protein synthesis in dorsal hippocampus during encoding and retrieval. *Neuron 50(3)*: 479-489.

Muller, R.U., & Kubie, J.L. (1987). The effects of changes in the environment on the spatial firing of hippocampal complex-spike cells. *J Neurosci. 9(12)*: 4101-4110.

Muller, R.U., & Kubie, J.L. (1989). The firing of hippocapl place cells predicts the future position of freely moving rats. *J Neurosci. 7(7)*: 1951-1968.

Muller, R.U., Kubie, J.L., & Ranck, J.B. (1987). Spatial Firing Patterns of Hippocampal Complex-Spike Cells in a Fixed Environment . *J Neurosci. 7(7)*: 1935-1950.

Nader, K, Schafe, G.E., & LeDoux, J.E.. (2000). The labile nature of consolidation theory. *Nat Rev Neurosci. (3)*: 216-219.

Nakazawa K., Quirk, M., Chitwood, R., Watanabe M., Yeckel, M., Sun, L., Kato, A., Carr, C., Johnston, D., Wilson, M., & Tonegawa, S. (2002). Requirement for hippocampal CA3 NMDA receptors in associative memory recall. *Science 297(5579)*: 211-218.

O'Keefe, J. (1976). Place units in the hippocampus of the freely moving rat. Exp Neurol 51(1): 78-109.

O'Keefe J. (1979). A review of the hippocampal place cells. *Prog Neurobiol.13(4)*: 419-439.

O'Keefe J., & Dostrovsky, J. (1971) The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely moving rat. *Brain Res 34*:171–175.

O'Keefe J., & Conway D.H. (1978). Hippocampal place units in the freely moving rat: why they fire where they fire. *Exp Brain Res. 31(4)*: 573-590.

O'Keefe J., & Conway D.H. (1980). On the trail of the hippocampal engram. Physiol Psychol 8: 229–238.

O'Keefe, J., & Nadel L. (1978). *The hippocampus as a cognitive map*. Oxford: Clarendon.

O'Keefe, J., & Speakman, A. (1987). Single unit activity in the rat hippocampus during a spatial memory task. *Exp Brain Res. 68(1)*:1-27.

Packard, M.G., & McGaugh, J.L. (1996). Inactivation of hippocampus or caudate nucleus with lidocaine differentially affects expression of place and response learning. *Neurobiology of learning and memory 65*: 65-72.

Quirk, G.J., Muller, R.U., & Kubie, J.L. (1990). The firing of hippocampal place cells in the dark depends on the rat's recent experience. *J Neurosci. 10(6)*: 2008-2017.

Rescorla, R.A., & Wagner, A.R. (1972). A theory of Pavlovian conditioning: Variations in the effectiveness of reinforcement and nonreinforcement. In Black, A.H. & Prokasy, W.F. (Eds.), *Classical conditioning II: Current theory and research* (pp. 64-69). New York: Appleton-Century-Crofts.

Restle, F. (1957). Discrimination of cues in mazes: a resolution of the place-vs.-response question. *Psychol Rev. 64(4)*: 217-228

Revusky, S. (1971). The role of interference in association over a delay. In Konig, W.K., & James, P.H. (Eds.), Animal memory (pp 155-213). New York: Academic Press.

Roberts, A.D., & Pearce, J.M. (1999). Blocking in the Morris swimming pool. *J Exp Psychol Anim Behav Process. 25(2)*: 225-235.

Rodrigo, T., Chamizo, V.D., McLaren, I.P., & Mackintosh, N.J. (1997). Blocking in the spatial domain. *J Exp Psychol Anim Behav Process. 23(1)*: 110-118.

Rolls, E.T. 1989. Functions of neuronal networks in the hippocampus and cerebral cortex in memory. In R. Cotterill (Ed.), *Models of brain function* (pp. 15–33). Cambridge University Press: Cambridge, UK.

Rossier, J., Kaminsky, Y., Schenk, F., & Bures, J. (2000). The place preference task: a new tool for studying the relation between behavior and place cell activity in rats. *Behav Neurosci. 114(2)*: 273-284.

Rotenberg A., Abel, T., Hawkins, R.D, Kandel, E.R., & Muller, R.U. (2000). Parallel instabilities of long-term potentiation, place cells, and learning caused by decreased protein kinase A activity. *J Neurosci. 20(21)*: 8096-8102.

Rotenberg A., Mayford M., Hawkins R.D., Kandel E.R., & Muller, R.U. (1996). Mice expressing activated CaMKII lack low frequency LTP and do not form stable place cells in the CA1 region of the hippocampus. *Cell 87(7)*:1351-1361.

Shapiro, M.L., Tanila, H. & Eichenbaum, H. (1997). Cues that hippocampal place cells encode: dynamic and hierarchical respresentation of local and distal stimuli. *Hippocampus 7*: 624-642.

Shelton, A.L., & Gabrieli, J.D. (2004). Neural correlates of individual differences in spatial learning strategies. *Neuropsychology 18(3*): 442-9.

Scoville, W.B., & Milner, B. (1957). The loss of recent memory after bilateral hippocampal lesions. *Neurol Neurosurg Psychiatry 20*:11–21

Skaggs, W.E., & McNaughton, B.L. (1998). Spatial firing properties of hippocampal CA1 populations in an environment containing two visually identical regions. *J Neurosci. 18(20)*: 8455-8466.

Speakman, A., & O'Keefe, J. (1990). Hippocampal Complex Spike Cells do not Change Their Place Fields if the Goal is Moved Within a Cue Controlled Environment. *Eur J Neurosci 2(6)*: 544-555.

Sutherland, R.J., & Hamilton, D.A. (2004). Rodent spatial navigation: at the crossroads of cognition and movement. *Neurosci Biobehav Rev. 28(7)*: 687-697.

Suzuki, S., Augerinos, G., & Black, A.H. (1980). Stimulus control of spatial behavior on the eight-arm maze in rats. *Learning and Motivation 11*:1-8.

Suzuki, A., Josselyn, S.A., Frankland, P.W., Masushige, S., Silva, A.J., & Kida S. (2004). Memory reconsolidation and extinction have distinct temporal and biochemical signatures. *J Neurosci. 24(20)*: 4787-95.

Tanila, H., Sipilä, P., Shapiro, M., & Eichenbaum, H. (1997). Brain aging: impaired coding of novel environmental cues. *J Neurosci. 17(13)*: 5167-5174

Tanila H., Shapiro, M.L., & Eichenbaum, H. (1997) Discordance of spatial representation in ensembles of hippocampal place cells. *Hippocampus 7*: 613– 623.

Tischmeyer, W., & Grimm, R. (1999). Activation of immediate early genes and memory formation. *Cell Mol Life Sci. 55(4):* 564-574.

Treves, A., & Rolls, E.T. (1991).What determines the capacity of autoassociative memories in the brain? *Network Comput. Neural Syst. 2*: 371–397.

Treves, A., & Rolls, E.T. (1992). Computational constraints suggest the need for two distinct input systems to the hippocampal CA3 network. *Hippocampus 2(2)*: 189-199.

Vann, S.D., Brown, M.W., Erichsen, J.T., & Aggleton, J.P. (2000). Fos imaging reveals differential patterns of hippocampal and parahippocampal subfield activation in rats in response to different spatial memory tests. *J Neurosci. 20(7)*: 2711-2718.

Wan, H., Aggleton, J.P., & Brown, M.W. (1999). Different contributions of the hippocampus and perirhinal cortex to recognition memory. *J Neurosci. 19(3)*: 1142-1148.

Wilson, M.A., & McNaughton, B.L. (1993). Dynamics of the hippocampal ensemble code for space. *Science. 261(5124)*: 1055-1058.

Worden, R. (1992). Navigation by fragment fitting: a theory of hippocampal function. *Hippocampus. 2(2)*: 165-187.

Zhu, X., Brown, M., McCabe, B.J., & Aggleton, JP. (1995). Effects of the novelty or familiarity of visual stimuli on the expression of the immediate early gene c-fos in rat brain. *Neuroscience. 69(3)*: 821-829.