CONTEXTUAL LEARING IN THE LIMBIC MEMORY CIRCUIT

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Context plays a critical role in memory. Information obtained during learning gets bound to the context and the context itself can serve as a potent retrieval cue. It has been well known since the 1970's that the hippocampus (HP) plays a critical role in processing contextual information. Research has shown that learning two lists of items in two different contexts facilitates learning compared to learning both in one context, and damage to the HP abolishes this advantage. Electrophysiology studies have indicated the HP forms contextual representations of an environment quickly and that the representation remains stable thereafter. The HP also encodes non-spatial information such as events, cues and time. The HP and AT are strongly interconnected and it is well known now that damage to the anterior thalamic nuclei (AT) produces severe memory deficits in multiple memory domains similar that following damage to the HP. However, it is not known whether the AT is critically involved in contextual memory processes like the HP. The first chapter evaluated whether the AT is involved in contextual learning of a context dependent odor discrimination task. The results showed that like the HP, damage to the AT abolishes the contextual advantage of learning two sets of odor discrimination problems in separate contexts. The second chapter evaluated how neuronal representations in the HP change when learning two contexts concurrently. While studies have evaluated how neuronal representations change between context exposures, none have evaluated the concurrent development of two contexts. The results showed that several exposures to the same context are required to form a stable HP representation of the two contexts. The final

chapter evaluated what event responses arise when learning the same task as chapter one and how these event representations change with learning. The results show that as learning progressed, event responses decreased in number. This decrease may be due to the responses becoming part of a general trial sequence indicating the stabilization of the representation as learning progressed. The current dissertation provides new evidence for how the memory circuit encodes contextual memory.

BIOGRAPHICAL SKETCH

Matthew received his B.A. degree from the department of psychology at California State University, Northridge in May of 2003. As an undergraduate, he worked at UCLA with Dr. Bernard Balleine where he studied the motivational control of goal-directed behavior which resulted in his first publication in 2007 in the Journal of European Neuroscience. Matthew then received his Master's degree from the psychology department at California State University, Long Beach in May of 2007. His research as a Master's student focused on the cellular response to injury in the hippocampus. For his thesis research he travelled to Maine to conduct field research aimed at comparing the response to hippocampal injury in food-storing and non-storing birds. His thesis work was published in 2009 in Developmental Neuroscience. As a Ph.D. student, his research focused on the limbic memory circuit and the different contributions this circuit makes to contextual learning and memory. His graduate work has led to a first author publication in behavioral neuroscience in 2012, a third author review paper in Frontiers in Human Neuroscience in 2014, and has 2 first author and 1 second author manuscript in preparation for publication. Dr. Law will be joining the lab of Dr. Jonathan Lifshitz at the University of Arizona Medical School in January 2015 to study potential treatments for post concussive cognitive deficits.

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ABBREVIATIONS

Ach – acetylcholine

AT – anterior thalamus

AD – anterior dorsal thalamic nucleus

AM – anterior medial thalamic nucleus

ANOVA – analysis of variance

AP – anterior/posterior coordinate

AV – anterior ventral thalamic nucleus

CA1 – cornu Ammonis region 1

COM – center of mass

fMRI – functional magnetic resonance imaging

GABA - *gamma*-Aminobutyric acid

HD – head direction

HP – hippocampus

ID – identification

IEG – immediate early gene

IF – infield firing rate

ITI – intertrial invterval

LAT – lateral coordinate

LD – lateral dorsal thalamic nucleus

LED – light emitting diode

LMM – linear mixed model

LSD – least significant difference

LTP – long term potentiation

ML – medial lateral coordinate

N/A – not available

OF – outfield firing rate

PETH – perievent time histograms

PTD – pyrithiamine-induced thiamine deficiency

Rdg – retrosplenial dysgranular cortex

RGa – retrosplenial granular A cortex

RGb – retrosplenial granular B cortex

RRI – rate remapping index

RSC – restrosplenial cortex

SEM – standard error of the mean

WKS – Wernicke-Korsakoff syndrome

INTRODCUTION

The hippocampus (HP) has long been thought of as the centerpiece of learning and memory. Following the finding in patient H. M., that ablation of the HP and surrounding region leads to anterograde amnesia (Scoville & Milner, 1957), researchers focused on the HP as the primary structure responsible for learning and memory. Since then, the HP has become the most widely studied structure in the history of neuroscience. Following recovery from the surgery, H. M., and others, were evaluated on a battery of memory tests that, for the first time, revealed that there are anatomically circumscribed cortical areas that subserve memory, that there are different durations for memory (short term versus long term) and there are multiple memory systems (Scoville & Milner, 1957). Since the original finding in H. M., there has been a host of research in multiple animal species as well as in humans on the role of the HP in memory.

Research has confirmed that although the HP is vital for many types of memory, it works in concert with multiple other structures (e.g., Aggleton & Brown, 1999; Eichenbaum, 2000; McGaugh, 2004). The HP is tightly interconnected with the anterior thalamus (AT) and retrosplenial cortex (RSC), both structures that have been implicated in similar memory domains as the HP (e.g., Aggleton & Brown, 1999; Aggleton & Nelson, 2014; Jankowski *et al.*, 2013; Nelson, Hindley, Haddon, Vann, & Aggleton, ; Valenstein *et al.*, 1987). For instance, all three regions have been implicated in episodic memory (e.g., Aggleton & Brown, 1999; Scoville & Milner, 1957; Valenstein *et al.*, 1987) spatial memory and navigation (e.g., Aggleton & Nelson, 2014; Hartley, Lever, Burgess, & O'Keefe, 2013; Miller, Vedder, Law, & Smith) and the HP and RSC have both been found to play a critical role in contextual memory (e.g., Butterly, Petroccione, & Smith, 2012; Keene & Bucci, 2008a, 2008b; Smith & Bulkin, 2014; Smith & Mizumori, 2006a). However, to date there have been no studies that have evaluated the AT and

its potential role in contextual memory. While we know that the HP is involved in contextual memory, the means by which contextual information is encoded has yet to be revealed. Although the RSC has also been implicated in similar memory domains, the focus of the dissertation is the contributions of the HP and AT in context dependent memory processing.

Connectivity

The HP and AT are reciprocally interconnected and are also tightly interconnected with the RSC. The AT is comprised of three main nuclei, the anterior dorsal (AD), anterior ventral (AV) and anterior medial (AM). However, more recently the lateral dorsal nuclei (LD) have also been included as a component of the AT. The HP projects to the AT both directly and indirectly (through the mammillary bodies) via the fornix. These projections arise from the subiculum, not the HP proper and within the subicular complex there are distinct populations of cells that project to the AT and mammillary bodies (Aggleton, Desimone, & Mishkin, 1986; Aggleton *et al.*, 2010; Ishizuka, 2001; Wright, Erichsen, Vann, O'Mara, & Aggleton, 2010). Almost every mammillary body cell projects to the AT, suggesting this is an indirect yet important pathway from the HP to the AT (e.g., Wright *et al.*, 2010; Vann *et al.*, 2007). The AT projects back to the hippocampal formation via the cingulum bundle through the subiculum, except for the AM nucleus which has very limited projections back to the hippocampal formation (Jankowski *et al.*, 2013; Shibata, 1993a, 1993b; van Groen, Kadish, & Wyss, 1999; van Groen & Wyss, 1995; Wyss, Swanson, & Cowan, 1979).

The RSC and AT are reciprocally interconnected in a manner specific to each AT nuclei and serve as an indirect route of connectivity from the AT to the HP. The RSC granular A (Rga) cortex receives projections primarily from AD and LD, with sparse projections to AV (Sripanidkulchai & Wyss, 1986; van Groen & Wyss, 1990a). Rga then has strong projections

back to AV and LD, with sparse projections to AD (Shibata, 1998; Sripanidkulchai & Wyss, 1986; van Groen & Wyss, 1990a; Vann, Saunders, & Aggleton, 2007). The RSC granular B (Rgb) receives projections from AD, AV, and LD and projects back to AV and LD, and sparsely to AD (Shibata, 1998; Sripanidkulchai & Wyss, 1986; van Groen & Wyss, 1990a). The RSC dysgranular cortex (Rdg) receives most of the thalamic projections from AM and LD with minimal innervation from AD and AV and then projects primarily back to AM and LD (Shibata, 1998; Shibata & Kato, 1993; Sripanidkulchai & Wyss, 1986; van Groen, Vogt, & Wyss, 1993). The HP and RSC are also interconnected, but the focus of these experiments is the AT and HP

Episodic Memory

Episodic memory is the ability to remember a past personally experienced event, when, and where it occurred. This was Tulving's original definition of episodic memory (Tulving, 1972). Tulving then changed his definition to include autonoetic consciousness, the ability to project into the past as well as the future, as a necessary component for episodic memory (Tulving, 2002). Episodic memory allows an individual to encode multiple features of an event, with the ability to distinguish and sequence that event upon recall and add some background. The importance of the limbic circuit in memory processing became evident with patient H.M. However, it has since become evident that damage to other regions in this circuit can also lead to amnestic pathology. Wernicke-Korsakoff syndrome (WKS) results from damage to the AT and mammillary bodies due to severe thiamine deficiency (e.g., Mair, Warrington, & Weiskrantz, 1979; Victor, Adams, & Collins, 1971). People suffering from this syndrome suffer from severe amnesia, indicating a role for an intact limbic circuit for proper memory function. This is further evidence that the HP is not the sole structure for encoding and retrieving episodic memories.

Episodic Memory and the HP. Deficits in episodic memory have been associated with damage to the HP or as a result of neurodegenerative diseases such as Alzheimer's (e.g., Scoville & Milner, 1957; Swaab *et al.*, 1998). In addition to the dramatic temporal lobectomy performed in patient H.M., there is similar evidence supporting a role for the HP in episodic memory. For instance, several studies evaluated patients with unilateral and bilateral HP damage in a virtual environment where subjects can navigate through the environment on their own, meet other virtual people, and/or obtain items in the environment. (Burgess, Maguire, Spiers, & O'Keefe, 2001; Spiers, Burgess, Hartley, Vargha-Khadem, & O'Keefe, 2001; Spiers, Burgess, Maguire *et al.*, 2001). The patients with bilateral temporal lobe damage (patient Jon) and those with damage to the left HP were impaired on the contextual memory questions as well as recalling who gave them items compared to control subjects. Rosenbaum et al., (2008) compared HP and neocortical volumes of patients with and without amnesia as well as the ability to recall autobiographical memories. They found that the severity of episodic memory loss correlates with HP volume loss and is not correlated with neocortical volume loss (Rosenbaum *et al.*, 2008). In another case study, patient K.C., who also suffers from amnesia due to damage to the HP and some of the surrounding temporal lobe structures, was also incapable of forming new memories and performed poorly on many tasks that require an intact HP (for review see, Rosenbaum *et al.*, 2005). Interestingly, it was also shown that K.C. had difficulty, compared to those with an intact HP, when asked to devise a fictional narrative of himself in the future (Rosenbaum, Gilboa, Levine, Winocur, & Moscovitch, 2009). These cases and many more highlight the importance of an intact HP for episodic memory function in humans.

Due to the fact that animals do not have the capacity for language, it is quite difficult to assess the ability for episodic memory, especially the aspect of mental time travel in animals as

added by Tulving. However, there is evidence now that animals are in fact capable of episodic memory, or at the least, episodic-like memory. Food storing birds exhibit a seasonal increase in HP neuronal recruitment as well as an increase in HP volume that coincides with food storing behavior in the fall (Law, Gardner, Allen, & Lee, 2010; Smulders, Sasson, & DeVoogd, 1995; Smulders, Shiflett, Sperling, & DeVoogd, 2000). Clayton and Dickinson (1998) trained food caching scrub jays (*Aphelocoma coerulescens*) to store two different items at two different time points. One of the items becomes spoiled at long delays (worms), while the other never spoils (seeds). The food-storing jays would therefore search for worms preferentially at short delays and seeds during long delays in a highly accurate manner. This showed they were capable of knowing what item was stored (worms vs. seeds), when the item was stored (avoided worms at long delays) and where the items were stored (did not make errors searching empty or degraded caches). This was the first demonstration that animals are capable of at least episodic-like memory (Clayton & Dickinson, 1998).

While the first real evidence for episodic memory in animals was done in birds, several experiments have since shown that rats too are capable of episodic memory. Babb and Crystal (2006) performed a similar foraging study to that of Clayton and Dickinson (1998) in rats. The rats were trained to remember a specific event that would clue them in on the location of a particular food item in an 8 arm radial maze. There was an initial session where four of the arms were available, two of which contained a preferred reward (i.e., flavored). The initial session was followed by either a short or long delay. After the short delay, all the arms are open and regular chow pellets were baited in the arms that were initially blocked. After the long session, the flavored pellets were in the initial location and the four initially blocked arms are baited with regular chow pellets. The results showed that rats were significantly more likely to visit the

flavored pellet locations over the chow pellets first after a long interval. They also learned to inhibit their visits to the flavored locations during short intervals

There are several other experiments on rats that suggest they are capable of episodic memory. One study showed that perhaps the cue of *how long ago* is more important than *when* in a foraging experiment designed to analyze different foraging techniques after different delays and times of day (Roberts *et al.*, 2008). Some tasks have been used which rely on a rat's innate preference for novel items (e.g., Kart-Teke, De Souza Silva, Huston, & Dere, 2006). These experiments expose rats to several familiar or novel items that can be in either the same or different location, and provide analysis about the amount of time spent exploring novel items or previously encountered items in different locations. Rats spend more time evaluating novel items, familiar items in novel locations, or older items over recently explored items (e.g., Kart-Teke *et al.*, 2006). The results indicated that they are capable of remembering the what, where and when of an event.

Episodic Memory and the AT. Damage to the AT has also been found to lead to profound amnesia similar to that of damage to the HP. The prime example is those who suffer from WKS (Kopelman, 1995). WKS is a disorder associated with malnutrition but is primarily seen in chronic alcoholism (Harper & Kril, 1990a, 1990b; Kopelman, 1995; Victor *et al.*, 1971). The main contributor is a prolonged thiamine deficiency that results in a bilateral lesion to the AT, several other midline thalamic nuclei and the mammillary bodies with minimal to no damage in the HP (Gold & Squire, 2006; Kopelman, Thomson, Guerrini, & Marshall, 2009; Langlais, Zhang, & Savage, 1996; Sullivan & Pfefferbaum, 2009; Victor *et al.*, 1971). However, the difference between the amnestic syndrome WKS and Wernicke's encephalitis (which does not produce sever amnesia) results from damage to the AT and the extent of the amnesia directly

correlated to the extent of damage to the AT (Gold & Squire, 2006; Harding, Halliday, Caine, & Kril, 2000). Gold and Squire (2006) compared patients with HP damage to those with damage to the AT and found remarkably similar deficits on several declarative memory tasks. Additionally, it has been found that there are deficits in spatial and temporal memory recall, with an even greater deficit when both spatial and temporal aspects of recall are needed for memory performance (Postma et al, 2006).

Although there has not been as much focus on AT damage and episodic memory in rodent models, there is a rodent model of WKS, the pyrithiamine-induced thiamine deficiency model (PTD) (e.g., Langlais & Savage, 1995; Langlais *et al.*, 1996; Mair, Anderson, Langlais, & McEntee, 1985). This model produces pathology which mimics that seen in humans and has been shown to respond to high levels of thiamine supplementation, as is seen in human subjects with WKS (Langlais & Savage, 1995). Similar to HP damage, the PTD rats show impairments in the delayed non-matching-to-position task (Langlais & Savage, 1995; Roland & Savage, 2007) the Morris water maze (e.g., Langlais, Mandel, & Mair, 1992), and the spontaneous alternation task (e.g., Vetreno, Anzalone, & Savage, 2008). Although there have not been any direct animal studies evaluating episodic memory, these deficits observed in the PTD rats closely parallel those seen following hippocampal damage (see below), which further highlights the importance of the AT in proper memory function.

Spatial Cognition

Spatial cognition and the HP. Following the major discovery of place cells in rats (O'Keefe, 1976), one major theory that emerged was that the HP forms a cognitive map (a mental representation of space) which allows for navigation within an environment. This cognitive map would also encode the prominent cues and features of the environment in order to

facilitate navigation. In support of this theory, there were many findings that HP neurons are selective to the geometry of the environment (e.g., Kubie & Ranck, 1983; O'Keefe & Burgess, 1996) and that lesions to the HP produce spatial impairments (e.g., H Eichenbaum, Stewart, & Morris, 1990; Morris, Garrud, Rawlins, & O'Keefe, 1982; Olton, Becker, & Handelman, 1979). Although the arrangement of place cells is orthogonal, when populations of place cells are recorded, the rat's current location can be predicted to within one cm (Wilson & McNaughton, 1993). These same cells can also represent different locations in different environments without losing their preferred locations in previous environments, suggesting that external cues influence the formation of place fields (O'Keefe & Conway, 1978a). In fact, testing in the same environment but changing a single white cue with a black cue leads to place cell remapping, the dramatic change in place cell firing involving new firing rates or for new locations (for review, see Muller, Poucet, Fenton, & Cressant, 1999). Remapping of place cells can happen through the rotation of cues (e.g., Muller & Kubie, 1987a), changes in task demands (e.g., Markus *et al.*, 1995; Smith & Mizumori, 2006b), and changes in mnemonic strategies (Yeshenko, Guazzelli, & Mizumori, 2001). Lesions to the HP result in impaired spatial learning and disrupt place field formation (Eichenbaum *et al.*, 1990; Olton *et al.*, 1979; Sutherland, Kolb, & Wishaw, 1983). The HP is necessary for spatial cognition and damage to this structure produces retrograde amnesia on spatially demanding tasks.

Following HP lesions rats are seriously impaired on radial arm maze tasks (e.g., Bouffard & Jarrard, 1988), the Morris water maze (e.g., Morris, Schenk, Tweedie, & Jarrard, 1990), on the elevated T maze (e.g., Bannerman *et al.*, 1999) as well as other spatially demanding tasks. Inactivation of the HP region prior to acquisition, just after acquisition or two hours after acquisition on the Morris water maze task leads to impairments both on acquisition and

immediately after training, but not if the inactivation was two hours after acquisition (Florian & Roullet, 2004). These results indicate that the HP is critically involved in the consolidation of spatial information. Other studies have found deficits in acquisition and retrieval of spatial memories, further supporting the important role of the HP in spatial cognition (e.g., Farr, Banks, La Scola, Flood, & Morley, 2000; Riedel *et al.*, 1999).

Spatial Cognition: Path Integration. Path integration (or dead reckoning) is the use of internal or idiothetic cues, such as linear and angular self motion, to navigate through an environment. Many of these internal cues are produced by the AT and subiculum, (Head direction cells (HD), Taube, 1995; Taube, Muller, & Ranck, 1990) and the entorhinal cortex (grid cells, Fyhn, Molden, Witter, Moser, & Moser, 2004) to name a few. These different cell types combined have been proposed to drive place cells observed in the HP (McNaughton, Battaglia, Jensen, Moser, & Moser, 2006). Head direction cells have been recorded in the monkey subicular complex (Rolls, 2006) and grid cells have been found in the human entorhinal cortex (Doeller, Barry, & Burgess, 2010) suggesting path integration capabilities in primate species. The importance of head direction cells in path integration has been studied primarily in rodents and it has been shown that the AT also contains head direction cells suggesting a critical role in spatial navigation.

In rodents, it has been well established that idiothetic cues are necessary for path integration and the generation of place fields (e.g., Foster, Castro, & McNaughton, 1989; Markus, Barnes, McNaughton, Gladden, & Skaggs, 1994). If an animal is restrained and is moved through an environment they will not exhibit HP place fields (Foster *et al.*, 1989). Place cells are also responsive to velocity and direction of movement in that they alter the size and firing rate of HP place cells (McNaughton, Barnes, & O'Keefe, 1983). Interestingly, place cells

can be either unidirectional or omnidirectional. Markus et al. (1995) found that place fields were directional when the rat was traversing a radial maze where there are only two directions in which the rat can move, forward and then back. They also found that place fields were omnidirectional when the rat was exploring an open environment in which the place field could be entered from any given angle. This indicates that neurons encode the interactions between locations, their significance and the behaviors required for travel in a given environment (Markus *et al.*, 1995).

Although path integration is a well established process, it requires the use of allocentric cues as well. Gothard et al. (1996) used a linear track with a start box and a fixed reward site at the end of the track. The start box could be moved anywhere along the track as the rat ran to the goal arm. Therefore the return journey varied in distance according to where the box had been moved. Cellular recordings in the HP showed that when the rat returned to the start box at longer distances, the transition between place cell coordinates was smooth. When the return to the box was a shorter distance, the rat used external cues to update the coordinates and the place fields shifted abruptly to the cells that fire near the start box as opposed to those that fire at that specific location on the track. This suggests that the rat was using idiothetic cues to navigate to the goal, but when returning, used external stimuli to update its position (Gothard, Skaggs, Moore, & McNaughton, 1996).

Spatial Cognition: Humans. Following the finding that HM could no longer create new memories, most of the human literature focused on memory mnemonics and episodic memory aspects of the HP. However, following the research on spatial cognition in mammals (specifically rats) more research has focused on spatial cognition in humans. Several studies using fMRI have shown that the HP is vital in place learning tasks (e.g., Bohbot, Iaria, &

Petrides, 2004), a virtual water maze task (e.g., Astur, Taylor, Mamelak, Philpott, & Sutherland, 2002; Cornwell, Johnson, Holroyd, Carver, & Grillon, 2008; Goodrich-Hunsaker, Livingstone, Skelton, & Hopkins), and other spatial tasks (Gillner & Mallot, 1998; Goodrich-Hunsaker & Hopkins, 2010). The initial studies evaluating retrograde amnesia for spatial memory used patients with medial temporal lobe damage. The results indicated that these patients were capable of remembering and describing landmarks, remembering spatial relationships and were capable of sketching maps of known environments (e.g., Rosenbaum *et al.*, 2005; Rosenbaum *et al.*, 2000). However, Maguire et al. (Maguire, Nannery, & Spiers, 2006) performed the same tasks and found no deficits until a patient with discrete HP damage and extensive experience as a London taxi driver performed a virtual reality navigation task. They found that when patient TT used main arterial roads, he was capable of successful navigation. When he had to use side streets and roads less commonly used, the spatial navigation impairment was evident. They concluded that the HP is necessary for the retrieval of spatial memories when navigating through complex large scale spaces, regardless of experience. fMRI studies have also shown that the right HP is involved in spatial navigation of a virtual environment (Maguire *et al.*, 1998) and that damage to the right HP leads to severe deficits in the same task (Spiers, Burgess, Hartley *et al.*, 2001; Spiers, Burgess, Maguire *et al.*, 2001).

Although rare, some cellular recordings have been done in humans, and results have found HP cells that respond to places in the environment, the goal location, locations from certain viewpoints and cells that respond to conjunctions of place, view and goal, in both the temporal and frontal lobes (Ekstrom *et al.*, 2003). They also found that the HP contained cells that fired for location, goal and conjunctively for location and goal, while the parahippocampus contained cells that responded to view, view and location, and view and goal. So as in rodent

and avian studies, the HP plays a major role in spatial navigation, contains cells that respond to specific locations, and have conjunctive cells that code for location and goals or items.

Spatial cognition in the AT. The AT also plays a critical role in spatial cognition. The AT and HP are tightly interconnected and as the HP contains place cells, the AT contains HD cells: cells that discharge whenever the rats head is orientated in a specific direction in a horizontal plane (Taube, 1995). The majority of HD cells are located in the AD nucleus, but are also found in the AV nucleus (and LD, Mizumori & Williams, 1993; Taube, 1995). Similar to place cells, HD cells lock to the features of an environment and remain stable upon re-exposure (e.g., Goodridge & Taube, 1995; Taube & Burton, 1995). Additionally, HD cells will rotate their orientation when a prominent cue in the environment is rotated indicating the use of alocentric information within the environment to anchor the directional representation (e.g., Goodridge & Taube, 1995; Taube & Burton, 1995). Lesions to the AT disrupt the ability of HP place fields to rotate with a shifted visual cue (Calton *et al.*, 2003) and lead to disruptions in a path integration task requiring rats to use ideothetic cues (Frohardt, Bassett, & Taube, 2006). Therefore the AT and HP are considered to work in concert with other structures to successfully navigate through an environment.

The AT is also involved in other spatial memory processes aside from path integration. Lesions to the AT lead to deficits in spatial alternation on a T-maze task (Aggleton, Hunt, Nagle, & Neave, 1996; Aggleton, Neave, Nagle, & Hunt, 1995), the radial arm maze (e.g., Mair, Burk, & Porter, 2003; Mitchell & Dalrymple-Alford, 2006; Mitchell, Dalrymple-Alford, & Christie, 2002; Moran & Dalrymple-Alford, 2003) the Morris water maze (e.g., Warburton & Aggleton, 1999; Wilton, Baird, Muir, Honey, & Aggleton, 2001) and other spatially demanding tasks. These deficits mimic those following lesions to the HP which further emphasizes the role for an

extended memory circuit. Interestingly, one study looked at the role of unilateral lesions to the HP and AT in either the ipsilateral or in contralateral hemispheres and tested them on the three spatial memory tasks mentioned above (Warburton, Baird, Morgan, Muir, & Aggleton, 2001). They found the largest deficits in the group with contralateral lesions to the HP and AT compared to ipsilateral group. They suggested that this is further evidence that spatial memory processes are the result of a conjoint effort of an extended memory system, and not a single structure (Warburton *et al.*, 2001).

Contextual Memory

Context was originally defined as the background information that is present during a learning situation in a given environment. It is well known that the HP encodes this information. However, many findings have found that HP neurons respond to non geometric features (or non-spatial features) of a learning situation such as changes in color associated with an environment (e.g., Anderson & Jeffery, 2003a), task demands (e.g., Markus *et al.*, 1995; Smith & Bulkin, 2014; Smith & Mizumori, 2006b), and strategies for solving a task (Yeshenko, Guazzelli, & Mizumori, 2004; Yeshenko *et al.*, 2001). It is now accepted that the HP is also involved in processing contextual information regardless of its spatial qualities, (for reviews, see Anagnostaras, Gale, & Fanselow, 2001; Maren, Yap, & Goosens, 2001; Myers & Gluck, 1994) however, contributions of the AT to contextual memory have never been evaluated. Smith & Mizumori (2006) have defined context as referring to "a particular situation or set of circumstances that must be differentiated from other situations in order for subjects to retrieve the correct behavioral or mnemonic output". It must be noted that the previously mentioned place fields are considered part of the context during learning, as space is a critical component of any context. This particular relationship has been termed spatial context and is only one element

of the context processed by the HP (e.g., Jeffery, Anderson, Hayman, & Chakraborty, 2004; Mizumori, Ragozzino, Cooper, & Leutgeb, 1999; Smith & Mizumori, 2006a).

Interference is a problem that all memory systems must overcome (McClelland, McNaughton, & O'Reilly, 1995; M. L. Shapiro & Olton, 1994). Interference refers to the inability to retrieve target items from memory due to the presence of competing non-target items. One reason context plays such a critical role in memory is that it allows subjects to segregate information according the relevant context, which reduces interference. For example, human subjects who learn two lists of items in different contexts show better memory retrieval than subjects who learn the lists in the same context (for review, see Smith & Vela, 2001). Although mostly done in rodent models, fear conditioning has also been performed in humans with similar results. For example, neuroimaging studies have shown that cued fear conditioning recruits the amygdala (Buchel, Morris, Dolan, & Friston, 1998; LaBar, Gatenby, Gore, LeDoux, & Phelps, 1998) and contextual fear conditioning recruits the HP (Hasler *et al.*, 2007; Marschner, Kalisch, Vervliet, Vansteenwegen, & Buchel, 2008), similar to results seen in rodents (e.g., McGaugh, 2004). It was also found that the role of the HP decays over time suggesting a time limited role in the consolidation of fear memories, in line with animal studies (Marschner *et al.*, 2008). Context also plays an extremely important role in episodic memory; however, this will be discussed later.

The role of the HP in processing context has been extensively studied in rodent models. Many such experiments focus on electrophysiological recordings while the animal is exposed to a novel context after extensively learning a previous context. Recordings from HP neurons have shown that place cells will remap when an animal experiences a new context (e.g., Frank, Stanley, & Brown, 2004; Smith & Mizumori, 2006b)} and that neuronal firing patterns in the HP

can be controlled by the context itself (Smith, Kolarick, & Mizumori, 2003; Smith & Mizumori, 2006b; Smith, Munoz, & Turner, 2003). Lesions to the HP impairs a rat's performance in context-dependent list learning odor discrimination tasks (Smith, Butterly, Petroccione, & Masterton, 2007), impairs behavioral flexibility between contexts (e.g., Kim & Fanselow, 1992; Penick & Solomon, 1991; Smith, Wakeman, Patel, & Gabriel, 2004), reduces the ability to respond to changes in a familiar environment (Good & Honey, 1991; Save, Buhot, Foreman, & Thinus-Blanc, 1992; Save, Poucet, Foreman, & Buhot, 1992) and impairs contextual fear conditioning (e.g., Maren & Fanselow, 1997). Interestingly, a recent study found that individual neurons within the HP produced different place fields in two different contexts, but as rats learned the relationship between two items and their valence was learned (rewarded or not) in these two contexts, some place cells began to fire conjunctively for both the place and the item represented in that place (Komorowski, Manns, & Eichenbaum, 2009). Not only did these conjunctive cells emerge as the animal gained experience, but they also correlated with the rats performance on the task such that on error trials, the item-position cells acted solely as position cells. This research indicates that when an animal enters a novel environment, HP neurons first create a spatial map of that environment and as they become more familiar with the environment, they begin to process the contextual information to form a more distinct and coherent memory which incorporates information for the location, items or both (Komorowski *et al.*, 2009).

Interactions of the HP and AT with the RSC

The RSC (as previously mentioned) also shares strong interconnections with the AT and HP and has been implicated in many of the same memory processes. Damage to the RSC leads to anterograde and retrograde amnesia (Valenstein *et al.*, 1987) that is very similar to the amnesia following AT or HP damage in humans. The RSC is involved in spatial memory

processes as well, and damage to this region leads to deficits on the Morris water maze (Harker & Whishaw, 2002; Pothuizen, Davies, Albasser, Aggleton, & Vann, 2009; Vann & Aggleton, 2002), radial arm maze (Keene & Bucci, 2009; Vann & Aggleton, 2002), and t-maze alternation tasks (Pothuizen, Davies, Aggleton, & Vann, 2010), and deficits similar to those following HP or AT damage. The RSC is also involved in contextual learning as is the HP (Butterly *et al.*, 2012; Keene & Bucci, 2008a, 2008b; Smith & Bulkin, 2014; D. M. Smith & Mizumori, 2006a), however, the contributions from the AT to context-based memories has yet to be examined. Therefore, these three structures along with several others have been thought of as an extended memory system that works together rather than ascribing single functions to any one structure (Aggleton & Brown, 1999; Aggleton & Nelson, 2014; Aggleton *et al.*, 2010; Miller *et al.*, 2014).

Further evidence that these systems work in concert comes from immediate early gene studies (IEG) creating lesions to the different regions. A lesion to the HP of rats produces a decrease in IEG expression (hypoactivity) in the AT (Jenkins, Amin, Brown, & Aggleton, 2006) and RSC during a spatial foraging task known to elicit IEG expression. Likewise, a lesion to the AT also leads to hypoactivity in the HP and RSC as well as other interconnected structures (Jenkins, Dias, Amin, & Aggleton, 2002; Jenkins, Dias, Amin, Brown, & Aggleton, 2002; Jenkins, Vann, Amin, & Aggleton, 2004). Additionally, lesions to the AT lead to a loss of long term depression in the RSC, an important component of neuroplasticity (Garden *et al.*, 2009). These results indicate that a lesion to one of these structures not only disrupts the function of that brain region, but also produces detrimental and long lasting disruptions to interconnected regions. Therefore it is easy to conclude that these primary memory structures all work in concert and modulate each other in order to support healthy memory function.

Current Studies

The aim of my current studies is to further evaluate the role of the limbic memory circuit in contextual learning. The first chapter looks to evaluate the role of the AT in contextual learning. Although the HP has been shown to be critically involved in the formation of contextual representations and the HP and AT are involved in many of the same memory tasks, no studies have ever evaluated whether the AT participates in contextual learning. The second chapter aims to analyze how two contextual representations develop concurrently. Previous studies have only focused on the development of a single representation or the change in HP representations from a well known context to a novel context. The final chapter (which is part of another study, see appendix) aims to analyze event responses in a HP dependent odor discrimination task and evaluate how they change with learning. I was the primary investigator of the first three chapters and a major contributor to the experiment in the appendix. Additionally, the experiment in the appendix is part of the experiment in chapter 3 and therefore was included in this dissertation.

CHAPTER 1

THE ANTERIOR THALAMUS IS CRITICAL FOR OVERCOMING INTERFERENCE IN A CONTEXT-DEPENDENT ODOR DISCRIMINATION TASK

ABSTRACT

The anterior thalamus (AT) is anatomically interconnected with the hippocampus and other structures known to be involved in memory, and the AT is involved in many of the same learning and memory functions as the hippocampus. For example, like the hippocampus, the AT is involved in spatial cognition and episodic memory. The hippocampus also has a well-documented role in contextual memory processes, but it is not known whether the AT is similarly involved in contextual memory. In the present study, we assessed the role of the AT in contextual memory processes by temporarily inactivating the AT and training rats on a recently developed context-based olfactory list learning task, which was designed to assess the use of contextual information to resolve interference. Rats were trained on one list of odor discrimination problems, followed by training on a second list in either the same context or a different context. In order to induce interference, some of the odors appeared on both lists with their predictive value reversed. Control rats that learned the two lists in different contexts performed significantly better than rats that learned the two lists in the same context. However, AT lesions completely abolished this contextual learning advantage, a result that is very similar to the effects of hippocampal inactivation. These findings demonstrate that the AT, like the hippocampus, is involved in contextual memory and suggest that the hippocampus and AT are part of a functional circuit involved in contextual memory.

INTRODUCTION

The anterior thalamus (AT) plays a critical role in learning and memory and shares many functions with the hippocampus. For example, the AT has a well-known role in spatial cognition, as indicated by the presence of head direction cells, which fire whenever the subject faces a particular direction regardless of position in the environment (Taube, 1995), and numerous findings of spatial memory deficits following AT lesions (e.g., Aggleton *et al.*, 1996; Byatt & Darymple-Alford, 1996; Sziklas & Petrides, 1999; Warburton & Aggleton, 1999; Warburton, Baird, & Aggleton, 1997). Also, like the hippocampus, the AT has been implicated in episodic memory. AT damage is associated with severe amnesia in Wernike-Korsakoff syndrome (e.g., Harding *et al.*, 2000; Kopelman, 1995; Mair *et al.*, 1979) and other forms of amnesia (e.g., Cipolotti *et al.*, 2008; Graff-Radford, Tranel, Van Hoesen, & Brandt, 1990). Recent studies of animal models of memory, such as the odor sequence memory task, have also shown an involvement of the AT (Wolff, Gibb, & Dalrymple-Alford, 2006).

The AT includes the anterior dorsal, anterior ventral and anterior medial nuclei. The lateral dorsal nucleus has similar functions (e.g., head direction neurons, Mizumori & Williams, 1993) and is sometimes considered part of the anterior nuclear group (Saunders, Mishkin, & Aggleton, 2005; van Groen, Kadish, & Wyss, 2002; Wright *et al.*, 2010). The AT is a major sub-cortical projection target of the hippocampal system, specifically the subiculum, with direct fornix projections and indirect projections via the mammillary bodies (Aggleton *et al.*, 2010; Ishizuka, 2001; Meibach & Siegel, 1975; Nadel, Willner, & Kurz, 1985; Saunders *et al.*, 2005; Shibata, 1992). The AT projects back to the hippocampal system directly and indirectly via the retrosplenial cortex (e.g., Shibata, 1993a; van Groen *et al.*, 1993; van Groen & Wyss, 1990a, 1990b, 1995), which is also involved in spatial cognition and memory (Cho & Sharp, 2001;

Valenstein *et al.*, 1987). These anatomical and behavioral observations have lead several authors to propose that these structures are part of a functional circuit that supports learning and memory processes (for review, see Aggleton & Brown, 1999; Gabriel, 1993; Mizumori, Cooper, Leutgeb, & Pratt, 2000).

In addition to its role in spatial cognition and episodic memory, the hippocampus has a well-documented role in contextual memory (for review, see Bouton, 1993; Hirsh, 1974; Smith, 2008). For example, hippocampal neurons exhibit context-specific firing patterns (Anderson & Jeffery, 2003b; Nadel *et al.*, 1985; Smith & Mizumori, 2006b) and lesions impair conditioned responses to contextual cues (Kim & Fanselow, 1992; Phillips & LeDoux, 1992). Another component of this circuit, the retrosplenial cortex, has also been implicated in contextual memory (Keene & Bucci, 2008a). Although no studies have directly tested the involvement of the AT in contextual learning, indirect evidence suggests an AT role in context. Studies of instrumental discrimination learning have identified patterns of neuronal activity in the AT and the retrosplenial cortex that are specific to a particular context and depend on how well the subjects have learned the task, suggesting that the firing patterns may reflect the association of the learned behavior with the learning context (Freeman, Cuppernell, Flannery, & Gabriel, 1996). Consistent with this idea, fornix lesions, which disconnect the hippocampus from the AT, disrupt these firing patterns and impair the ability to learn different discriminations in separate contexts (Smith *et al.*, 2004). Additionally, studies of immediate early gene expression indicate that AT neurons respond when subjects are exposed to a novel environment or re-exposed to a context where they had received a shock (Jenkins, Dias, Amin, Brown *et al.*, 2002; Yasoshima, Scott, & Yamamoto, 2007).

In the present study, we assessed the role of the AT in contextual memory processes by temporarily inactivating the AT and training rats on a recently developed context-based list learning task (Butterly *et al.*, 2012), which was adapted from classic studies of human memory showing that learning two lists of items in separate contexts produces less interference and better recall than learning the two lists in the same context (Bilodeau & Schlosberg, 1951). In our task, rats are trained on one list of odor discrimination problems, followed by training on a second list in either the same context or a different context. In order to induce interference, the two lists contain overlapping items with reversed predictive values. In our previous study (Butterly *et al.*, 2012), we showed that, as with human subjects, control rats that learned the two lists in separate contexts performed better than rats that learned the two lists in the same context. However, temporary lesions of the hippocampus completely and selectively abolished this contextual learning advantage. Instead, rats with hippocampal lesions performed as if they had learned the two lists in the same context, indicating that the hippocampus is needed when subjects must use contextual information to overcome interference. In the present study, we examine the effects of temporary AT lesions on this same task. A finding of a lesion induced deficit would be the first demonstration of an AT role in contextual learning and memory processes.

METHODS

Subjects, Surgical Procedures, and Microinfusions

The subjects were 36 adult male Long Evans rats (Charles River Laboratories, Wilmington, MA) weighing 300-350 g at the time of surgery. Guide cannula (Plastics One, Roanoke, VA) were stereotaxically positioned just above the target location so that the infusion cannula, which protruded 1.0 mm beyond the tip of the guide, would be positioned bilaterally in the AT (2.3mm posterior from bregma, 1.5mm lateral to bregma, and 3.8mm ventral to the

cortical surface, Paxinos & Watson, 1998). The rats were given an antibiotic (5 mg/kg Baytril) and an analgesic (5 mg/kg ketoprofen). All procedures complied with guidelines established by the Cornell University Animal Care and Use Committee. After one week for recovery from surgery, the rats were placed on a restricted feeding regimen (80–85% of free feeding weight) and they began training.

Temporary lesions were induced with the $GABA_A$ agonist muscimol. Thirty minutes prior to relevant training sessions, muscimol or saline was infused bilaterally. The cannulae were left in place for one minute after the infusions. A commonly used muscimol concentration (1 µg/µl, Butterly *et al.*, 2012) produced general impairments in behavioral responding, exploration, etc., even when minimal volume (0.2-0.3 µl) was injected into the anterior thalamus. We titrated the dose in pilot animals by decreasing the concentration until the maximum concentration was found that reliably allowed rats to perform the task with no observable suppression of behavioral responding and that dose (0.2–0.3 µl of a solution containing 0.118 µg/µl of muscimol, infused over the course of 1min) was used for all subjects.

In the present study, we specifically targeted the anterior dorsal, anterior ventral nuclei and lateral dorsal nuclei because these nuclei are an integral part of the hippocampal-retroslpenial-anterior thalamic circuit of interest here (Aggleton *et al.*, 2010; Saunders *et al.*, 2005). The anterior medial nucleus is also interconnected with the anterior cingulate and prelimbic cortices (Shibata, 1993a, 1993b; Shibata & Kato, 1993; van Groen *et al.*, 1999), the latter of which plays a different, non-contextual role in this task (D. Smith, unpublished data). For this reason, the anterior medial nucleus was not specifically targeted and inactivation was likely minimal.

Apparatus and General Training Procedures

The training made use of a well-known digging task used to study olfactory memory (H. Eichenbaum, 1998), in which rats are trained to dig in cups of odorized bedding material to retrieve buried food rewards (45 mg sucrose pellets, Bioserve, Frenchtown, NJ). All of the rats were first trained on one list of odor discrimination problems. They were then given either muscimol or saline infusions and were trained on a second list of odors either in the same context or a different context. Thus, the experimental manipulations took place during training on the second list in a 2 X 2 design with lesion condition (saline or muscimol) and context condition (same or different) as factors.

The details of the apparatus, stimuli and training procedures have been published previously (Butterly *et al.*, 2012). Briefly, the two contexts differed along the following dimensions: color of the chamber (white or black), color of the curtains surrounding the training area (black or white), substrate in the chamber (uncovered Plexiglass floor or a black rubber mat), the 65 dB continuous background masking noise (white noise or pink noise) and the ambient odor left by wiping out the chamber with baby wipes prior to each training session (unscented or scented, Rite Aid, Inc). Additionally, the rats were transported in covered cages to the experimental area by different methods in the two contexts (via a cart or carried by hand).

The rats were trained in Plexiglas chambers (45 cm wide X 60 cm long X 40 cm deep) equipped with a removable divider, which separated the odor presentation area from an area where the rats waited during the intertrial interval. Odor cues were presented in ceramic dessert cups (8.25 cm in diameter, 4.5 cm deep) which fit into circular cutouts cemented to the floor of the chamber to discourage the rats from moving the cups or tipping them over. Thirty-two pure odorants served as cues. The amount of each odorant was calculated so that they produced an equivalent vapor phase partial pressure when mixed with 50 ml of mineral oil (Cleland, Morse,

Yue, & Linster, 2002) and 10 ml of the resulting odorant solution was then mixed with 2 L of corncob bedding material and stored in airtight containers.

Prior to training, the rats were given two 15-min sessions of acclimation to each of the two contexts. The rats were then shaped to dig in cups of bedding for a sucrose reward. After the rats had learned to reliably retrieve the rewards, they began training on the first list of odor discrimination problems. Each list contained eight odor pairs (16 different odors). Within each pair, one odor was rewarded and the other was not. The predictive value of the odors (rewarded or non-rewarded) was counterbalanced across subjects and their locations (left or right side of the chamber) were randomized. The daily training sessions consisted of 64 trials (eight trials with each odor pair, presented in an unpredictable sequence).

At the start of each trial, the experimenter placed the two cups containing the odorized bedding into the chamber and removed the divider so that the rat could approach the cups and dig until he retrieved the reward. A digging response was recorded if the rat displaced any of the bedding, except for incidental displacement (e.g., stepping into the cup while walking over it). After consuming the reward, the rat was returned to the waiting area for an intertrial interval of ~15 s while the experimenter prepared the cups for the next trial. The rats were given daily training sessions on List 1 until they reached a behavioral criterion of 90% correct choices on two consecutive sessions.

After reaching the criterion on List 1, rats were assigned to one of four groups for training on List 2. Rats were given training on List 2 in either the same context or a different context and they received AT infusions of either muscimol or saline (yielding 4 groups: saline – different context, muscimol – different context, saline – same context and muscimol – same context). Infusions were given 30 min prior to each of the first 3 training sessions on List 2. The

rats were then given 2 additional training sessions with no further infusions. To create maximal interference between items on the two lists, half the odors on List 1were included on List 2, but their predictive value was reversed. Half of these repeated odors had been rewarded (+) previously and the other half were not previously rewarded (-). For example, if the first two odor pairs on List 1 were A+/B- and C+/D-, then the first two odor pairs on List 2 would be X+/A- and D+/Y-. This ensured that the rats could not adopt a simple strategy of avoiding more familiar odors or approaching novel odors.

The percentage of trials with a correct choice for each training session served as our dependent measure of performance. Analyses involving within subjects variables were submitted to a linear mixed model analysis with Bonferroni correction for multiple pairwise comparisons following significant main effects (SPSS, IBM, Armonk, NY). In order to account for individual rat differences, rat ID was added as a random effect and was included in the overall error term. Analyses not involving within subjects variables were submitted to standard ANOVA. All post hoc tests and planned comparisons were Bonferroni corrected.

Pellet Detection

Previous studies have indicated that rats cannot smell the buried rewards (Butterly *et al.*, 2012). Nevertheless, most of the rats (n = 22) were tested to ensure that they could not directly detect the pellets. After the completion of training, the rats were given a session consisting of 64 trials (eight trials with each rewarded odor from List 2). On each trial, the rats were presented with two cups containing the same odor. However, only one of the cups was baited. If the rats could directly detect the pellets, they would be expected to perform better than chance (50%). The rats chose the baited cup 48.56 ± 1.83% (Mean ± SEM) of the time, which did not differ significantly from chance performance ($t_{(21)} = -0.79$, $p = 0.44$).

Histology

After completion of training, the rats were deeply anesthetized and transcardially perfused with 4% paraformaldehyde and their brains were removed, sectioned at 40 µm, mounted on slides and stained with cresyl violet. The stained sections were used to verify that the placement of the cannulae was within the AT. In addition to the nissl stained tissue, five rats were given infusions of fluorescent muscimol (0.2–0.3 µl of a solution containing 1.0 µg of fluorescent muscimol in 1.0 µL of saline with 0.2 µL of DMSO to aid dissolution, infused at a rate of 0.1 µL/min, Molecular Probes, Carlsbad, CA) 30 min prior to perfusion in order to estimate the spread of the drug (Allen *et al.*, 2008). For these brains, additional sections were collected and counterstained with Fluorogel with Tris buffer (Electron Microscopy Sciences, Hatfield, PA). Placement of the infusion cannulae were verified in the nissl stained tissue, and subjects with misplaced cannulae were excluded from the main analysis of the effects of lesions and were instead included in a 'missed infusion' group that served as an additional control condition, as described below. Cannula locations and an estimate of the muscimol spread based on fluorescent muscimol are illustrated in Figure 1. These estimates suggested that the muscimol infusions were primarily confined to the AT.

RESULTS

List 1 Performance

All of the rats were trained until they reached the behavioral criterion on List 1 before they began training on List 2. The rats took an average of 4.44 ± 0.12 (Mean ± SEM) training sessions to reach the criterion. The average performance on the final day of training was 96.27 ±

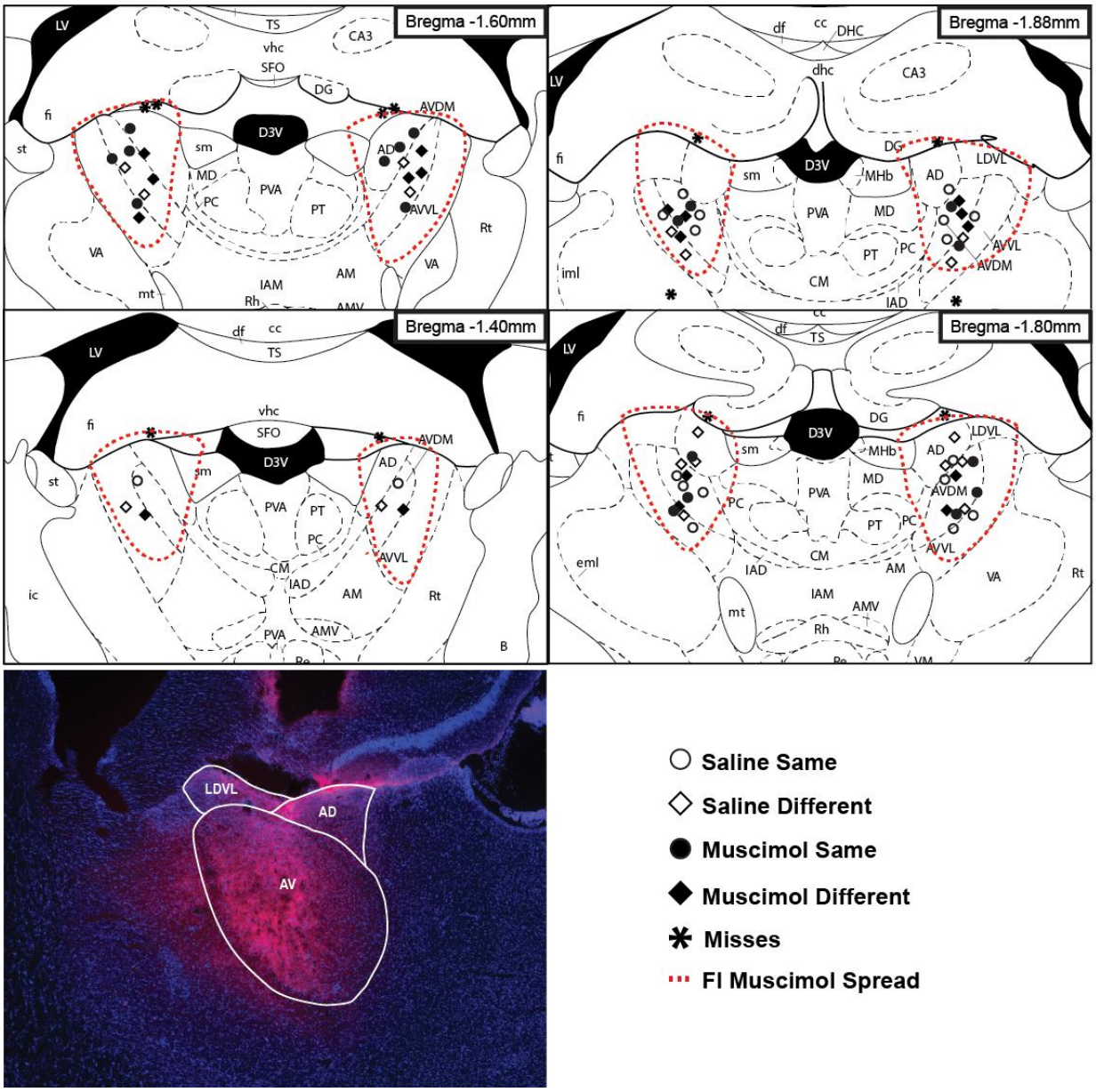

Figure 1. Infusion sites for subjects in the four experimental groups and the location of the misplaced infusions are shown on figures adapted from Paxinos and Watson (1998). Coordinates, in mm from bregma, are indicated for each panel and estimated spread of the infusions based on fluorescent muscimol infusions is indicated by the red dashed line. An example of fluorescent muscimol spread is also shown.

0.42% correct and there were no performance differences between groups on the final day of List 1 (F[3,32] = 0.977, p = 0.416).

Effects of Temporary Anterior Thalamic Lesions

To assess the effects of the context manipulation and the temporary AT lesions, the average percentage of trials with a correct response for each session of List 2 was submitted to a linear mixed model (LMM) analysis with context condition (same or different) and lesion condition (saline or muscimol) as between subjects factors and training session (5 sessions of List 2) as a within subjects factor (Figure 2). The LMM revealed a significant main effect of context (F[1,32] = 11.05, p < 0.01) and a main effect of lesion condition (F[1,32] = 10.65, p < 0.01). The analysis also revealed a significant interaction of lesion and training session (F[4,128] = 7.98, p < 0.001). This was likely attributable to the experimental design, which involved saline or muscimol infusions during the first three sessions but not during the last two sessions. Therefore, we decomposed this interaction by performing two additional LMM analyses, one to compare the performance of control and lesion subjects across the first three (infusion) sessions of List 2 and a second to compare performance during the final two (no infusion) sessions. The analysis of the infusion sessions again revealed a main effect of the context condition (F[1,32] = 7.46, p < 0.01) and a main effect of the lesion condition (F[1,32] = 16.64, p < 0.001). The interaction of the context and lesions factors did not achieve significance (F[1,32] = 3.16, p = 0.085). Although rats given muscimol lesions were significantly impaired during the infusion sessions, their performance caught up to that of the control rats when infusions were no longer given (effect of lesion condition during the final two sessions: F[1,32] = 0.14, p = 0.713).

Our previous study showed that control rats performed significantly better when they learned the two lists in different contexts, but rats with hippocampal lesions did not benefit from

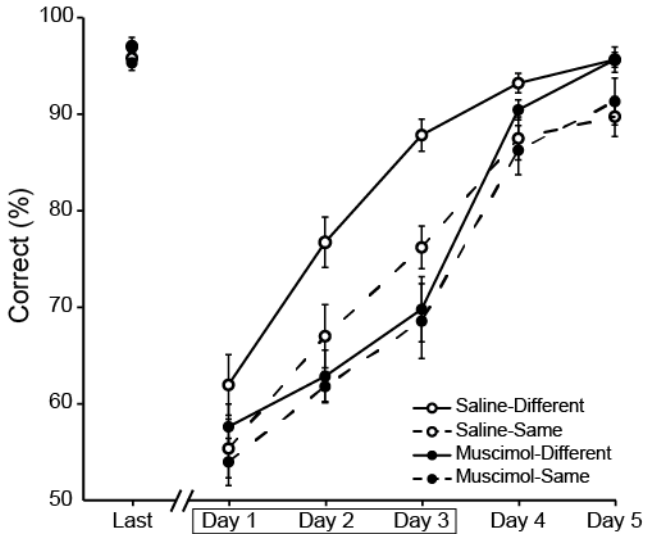

Figure 2. Average percent correct choices are shown for saline control rats (open circles) and muscimol rats (filled circles) and for the different context (solid lines) and same context conditions (dashed lines). Performance data are shown for the final session of List 1 training (Last) and the five training sessions of List 2. Muscimol or saline infusions were given prior to the first three training sessions of List 2, indicated by the box.

learning in different contexts (Butterly *et al.*, 2012). A major goal of the present study was to determine whether AT lesions would have a similar effect. Planned comparisons confirmed that control rats that learned List 2 in a different context performed significantly better than rats that learned the two lists in the same context ($t_{(16)} = 4.42$, Bonferroni corrected $p < 0.05$). However, rats with temporary AT lesions showed no advantage of learning the two lists in separate contexts, compared to rats that learned the lists in the same context ($t_{(16)} = 0.786$, $p = 0.443$).

Several muscimol subjects (N=8) were excluded from the above analyses due to misplacement of the infusion cannulae (Figure 1). However, since the placement of these cannulae was outside of the AT, they provide a good control for the possible effects of muscimol infusions extending beyond the AT. An ANOVA of performance during the infusion sessions revealed no difference between the saline group and the misplaced muscimol infusion group ($F[1,32] = 2.62$, $p = 0.13$, Figure 3), suggesting that the spread of muscimol outside of the AT was not likely responsible for the effects seen in our experimental groups.

Assessment of Interference in Each Condition

The experimental design allowed for a direct comparison of the effects of interference in each condition. If proactive interference occurred, performance should decline when subjects had to learn a second list of conflicting items after learning the first list. If no interference occurred, then performance on List 2 should be just as good as, or better than, performance on List 1. We computed an interference index for each subject by subtracting the average percent correct on List 1 from the average percent correct on List 2. These difference scores reflect the change in performance from List 1 to List 2, with positive numbers indicating facilitation and negative numbers indicating interference (Fig. 4). The scores were submitted to a two way ANOVA with lesion condition (control or muscimol) and context condition (same or different) as between

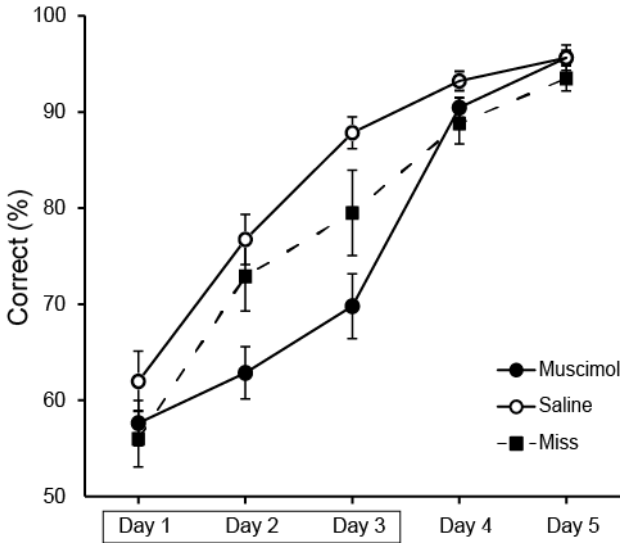

Figure 3. Average percent correct choices are shown for saline control rats (open circles) and muscimol rats (filled circles) and rats with misplaced infusions (black squares). Performance data are shown for the five training sessions of List 2. Muscimol or saline infusions were given prior to the first three training sessions of List 2, indicated by the box.

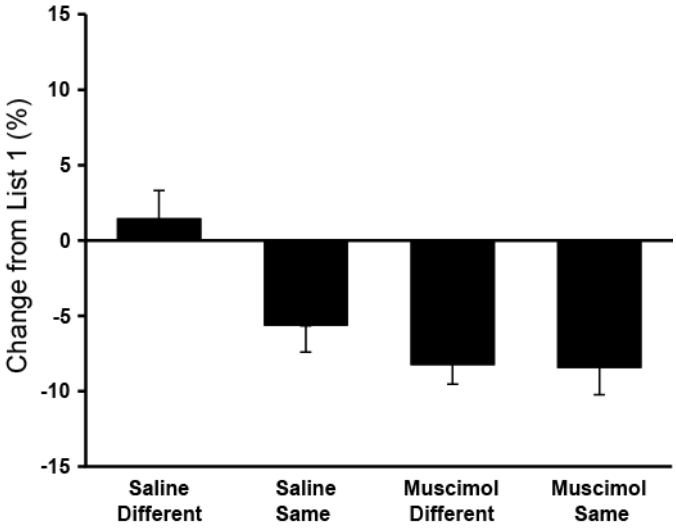

Figure 4. The change in performance from List 1 to List 2, computed as the average percent correct on List 2 minus the average percent correct on List 1, is shown for each of the experimental groups. Facilitation is indicated by better performance on List 2 than on List 1 (positive values) while interference is indicated by worse performance on List 2 (negative values).

subject's factors. This analysis revealed a significant interaction of the lesion and context conditions (F[1,32] = 6.53, $p < .05$). Consistent with the above analysis of the percent correct data, post hoc comparisons showed that control rats experienced significantly less interference when they learned the two lists in different contexts, compared to learning the two lists in the same context ($t_{(15)} = 2.79$, Bonferroni corrected $p < 0.05$) but rats with temporary AT lesions showed equivalent amounts of interference regardless of whether they learned the lists in different contexts or the same context ($t_{(15)} = -1.118$, $p = 0.28$).

Comparison of Anterior Thalamic and Hippocampal Inactivation

Previously we showed that the hippocampus is critical for using contextual information to facilitate learning in this task (Butterly *et al.*, 2012). As described above, there is substantial evidence that the AT and the hippocampus are part of a functional circuit and these two regions may have similar functional roles. Thus, we expected lesions in the two regions to have similar effects. However, inspection of the current data suggested that the AT lesions may have produced a more severe deficit than the dorsal hippocampal lesions of the previous study. To formally examine this possibility, a LMM analysis was used with lesion location (AT or hippocampus, from the previous study: a single injection of 0.5µL of 1µg/µL muscimol infused into the dorsal hippocampus of each hemisphere, 3.6 mm posterior and 2.6 mm lateral to Bregma, 2.2 mm ventral to the cortical surface) as the between subjects factor and training session (the three muscimol infusion sessions of List 2 training) as the within subjects factor. The analysis showed no main effect of lesion location (F[1,32] = 2.69, $p = 0.111$) but did reveal a significant main effect of training session (F[1,32] = 51.38, $p < 0.001$) and a significant lesion location by training session interaction (F[2,64] = 3.97, $p < 0.05$, Fig. 5). Simple effects tests

revealed a performance difference that just failed to reach significance on the second infusion day (Bonferroni corrected $p = 0.06$) and a significant difference on the third day (Bonferroni corrected $p < 0.05$) where the hippocampal lesion groups performed better than the AT lesion group. Comparisons involving data collected during different experiments can be problematic due to the possibility of systematic changes in uncontrolled variables. However, these concerns are somewhat mitigated here since the data were collected in the same laboratory using the same procedures, materials and apparatus.

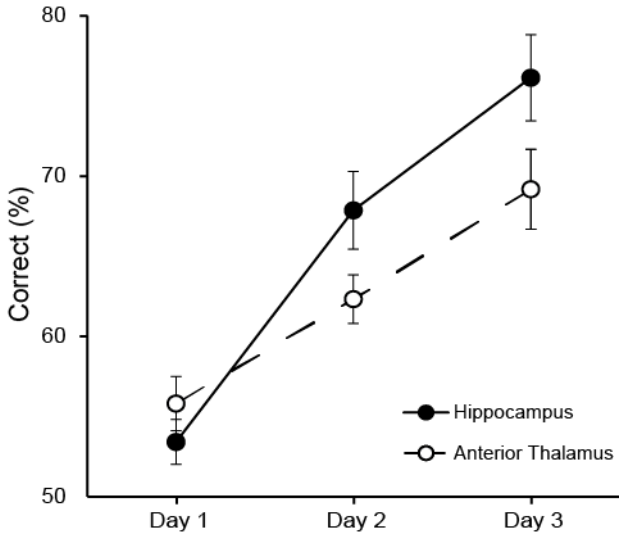

Figure 5. Average percent correct choices are shown for rats given muscimol infusions in the dorsal hippocampus (black circles, Butterly *et al.*, 2012) and for rats given muscimol infusions in the AT (open circles). Performance data are shown for the three training sessions in which muscimol infusions were given on List 2.

DISCUSSION

In line with previous list learning studies in humans (Bilodeau & Schlosberg, 1951) and rodents (Butterly *et al.*, 2012), rats that learned the two lists with overlapping items in different contexts performed better than rats that learned the two lists in the same context. However, AT lesions completely abolished this contextual advantage. The lesions were also associated with increased susceptibility to interference in the different context condition. These results suggest that the AT, like the hippocampus, plays a critical role in the use of contextual information to overcome interference. Previous work has shown that the AT and hippocampus work together in the domains of spatial cognition and episodic memory (for review, see Aggleton & Brown, 1999; Aggleton *et al.*, 2010; Mizumori *et al.*, 2000). However, these results are the first demonstration of a critical role of the AT in contextual learning and memory processes.

The hippocampal role in processing contextual information has been appreciated since the 1970s (Hirsh, 1974) and many studies have shown that the hippocampus is needed for various forms of contextual memory (for reviews, see Bouton, 1993; Hirsh, 1974; Smith, 2008). More recently, we have suggested that hippocampal context coding is important because it provides a means of overcoming interference (Butterly *et al.*, 2012; Smith & Mizumori, 2006b). Specifically, the hippocampus is thought to generate a new representation whenever the subject encounters a novel context and, through learning processes, these context representations become associated with the behaviors and memories that go with that context. Later, when the subject returns to the same context, the hippocampal representation is re-expressed, resulting in the priming of context-appropriate memories and behaviors, thereby minimizing interference caused by intrusions of memories relevant to other contexts. The present results suggest that the

AT also plays a key role in this process and are the first demonstration that the AT is involved in the resolution of interference.

The fact that temporary lesions of the AT and the hippocampus (Butterly *et al.*, 2012) both impair this task is consistent with the literature on spatial cognition and human amnesia, which suggest that these structures are part of a functional circuit that supports various cognitive processes. Context is an important point of convergence for theoretical accounts of spatial cognition and episodic memory, which are often treated as distinct functions. A number of authors have suggested that the spatially localized firing patterns (i.e. place fields) seen in the hippocampus can best be thought of as a neural representation of the spatial context (Anderson & Jeffery, 2003b; Nadel *et al.*, 1985; Smith & Mizumori, 2006a) and episodic memories necessarily include memory for the spatial and temporal context along with the events that occurred. Thus, episodic memory deficits could arise from deficits in context processing (Smith, 2008). Recent research suggests that remembering past episodes and imagining future episodes both involve a process of constructing a representation of the context and the events that occur in that context (Schacter, Addis, & Buckner, 2008). Consistent with this idea, amnesics show a striking deficit in the ability to mentally construct highly contextualized imaginary future episodes such as a trip to the beach or a birthday party (e.g., imagery, sense of presence, etc., as opposed to generalized knowledge such as the beach has sand, water, etc., Hassabis, Kumaran, Vann, & Maguire, 2007). The present results suggest that the AT, like the hippocampus and retrosplenial cortex, plays a key role in processing the contextual information that is a critical component of spatial cognition and episodic memory.

Empirical evidence suggests that the AT and hippocampus are components of a functional memory circuit that supports various memory processes. Many studies have shown

that lesions of either structure produce similar impairments (Aggleton, Hunt, & Rawlins, 1986; Aggleton *et al.*, 1995; Anagnostaras *et al.*, 2001; Butterly *et al.*, 2012; Celerier, Ognard, Decorte, & Beracochea, 2000; Fortin, Agster, & Eichenbaum, 2002; Wolff *et al.*, 2006). As mentioned above, neurons in the AT and retrosplenial cortex exhibit context specific response patterns during instrumental discrimination learning and these firing patterns are degraded by fornix lesions (Smith *et al.*, 2004). Other studies have shown that AT neurons fire in synchrony with the hippocampal theta rhythm (Tsanov, Chah, Vann *et al.*, 2011; Tsanov, Chah, Wright *et al.*, 2011; Tsanov, Wright *et al.*, 2011) and direct hippocampal projections to the AT exhibit long term potentiation while indirect projections (via the mammillary bodies) exhibit long term depression (Tsanov, Vann *et al.*, 2011). These interactions are bi-directional. AT neurons are thought to contribute directional information to hippocampal representations (Knierim, Kudrimoti, & McNaughton, 1995; Mizumori, Miya, & Ward, 1994; Muir & Taube, 2002). Lesions of the hippocampus cause hypoactivity in the AT during radial maze performance (T. A. Jenkins *et al.*, 2006) and, conversely, lesions of the AT cause hypoactivity in the dorsal hippocampus during exposure to a novel context (Jenkins, Dias, Amin, Brown *et al.*, 2002) and deficits in many hippocampal dependent tasks (Aggleton *et al.*, 1996; Byatt & Darymple-Alford, 1996; Gibb, Wolff, & Dalrymple-Alford, 2006; van Groen *et al.*, 2002; Warburton *et al.*, 2001; Wolff *et al.*, 2006).

The above discussion emphasizes the shared functional role of the hippocampal-AT circuitry. However, there is also evidence suggesting that these two regions make distinct contributions to memory. For example, the AT is critically involved in instrumental discriminative avoidance learning, including a simple (non-contextual) discrimination task (Gabriel *et al.*, 1983). In contrast, lesions of the fornix or hippocampus have no effect on this

kind of learning (Kang & Gabriel, 1998; Smith *et al.*, 2004). Additional evidence comes from the domain of spatial navigation, where the primary spatial firing correlate of AT neurons is head direction (i.e., elevated firing when the subject is facing the preferred direction, regardless of location within the environment, Mizumori & Williams, 1993; Taube, 1995; Tsanov, Chah, Vann *et al.*, 2011) while the primary spatial correlate of hippocampal neurons is the place field (i.e., elevated firing in a particular location within the environment, O'Keefe & Nadel, 1978). Interestingly, neurons in both regions are sensitive to the context but the way that the neurons respond to context change differs. In the hippocampus, place fields in one context shift to new and unpredictable locations or disappear altogether in a new context (for review, see Colgin, Moser, & Moser, 2008). Indeed, hippocampal place cells may acquire entirely different correlates in a new context (e.g., non-spatial reward or odor responses, H. Eichenbaum, Kuperstein, Fagan, & Nagode, 1987). In contrast, AT head direction neurons may change their preferred direction in a new context, but all the neurons shift as a coherent unit, rather than remapping individually in an unpredictable manner, and they continue to exhibit directional firing rather than acquiring new correlates in the new context (Goodridge & Taube, 1995; Yoder *et al.*, 2011). Thus, hippocampal representations of the spatial context are highly orthogonalized whereas AT representations, while they do differ from one context to another, may not be as distinct.

Attempts to disentangle potentially different contributions of the AT and hippocampus are complicated by the fact that in addition to disrupting any intrinsic function of the region in question, the lesions also probably disrupt the dynamics of the entire circuit resulting in a variety of indirect effects. For example, AT lesions dampen activity in the hippocampus and retrosplenial cortex (Jenkins, Dias, Amin, & Aggleton, 2002; Jenkins, Dias, Amin, Brown *et al.*,

2002). Additionally, although AT lesions do not directly affect ACh transmission in the hippocampus and do not alter baseline levels of ACh, AT lesions do cause a dramatic reduction in behaviorally induced ACh efflux in the hippocampus (Savage, Hall, & Vetreno, 2011). These effects may be partially mitigated by the use of temporary inactivation procedures, as in the present study, but even temporary removal of a key component of the circuit is likely to have widespread effects. Thus, in addition to understanding the functional contributions of each region to contextual memory, it will also be necessary to understand the relevant circuit dynamics. The neural reuse hypothesis suggests that a given region may participate in many different circuits and that the resulting cognitive functions depend more on the currently active circuit than the intrinsic cognitive function of individual regions (Anderson, 2010). This may be the case with the AT and the hippocampus, which may be two nodes that participate in overlapping, but not identical circuits. The AT participates in a circuit that supports discriminative avoidance learning (Gabriel *et al.*, 1983) and another circuit that supports spatial, episodic and contextual memory, whereas the hippocampus only participates in the latter circuit and is not needed for discriminative avoidance learning (Kang & Gabriel, 1998).

Recent studies have shown that another component of this circuitry, the retrosplenial cortex, is also critically involved in contextual learning and memory processes (Smith, Barredo, & Mizumori, 2011). The retrosplenial cortex is the major cortical projection target of the AT and is reciprocally interconnected with the hippocampus (e.g., Shibata, 1993a; van Groen *et al.*, 1993; van Groen & Wyss, 1990a, 1990b, 1995). We have shown that hippocampal neurons exhibit several kinds of highly context specific response patterns, including spatial firing, reward related firing and firing during the intertrial delay period. In contrast, retrosplenial neurons specifically differentiated the particular cues that uniquely identify the context (Smith *et al.*,

2011). Thus, the hippocampus and retrosplenial cortex both play a role in contextual learning and memory processes, but neurophysiological data suggest that they may make somewhat different contributions. It remains for future experiments to determine whether the AT neurons also make a unique contribution to contextual memory.

CHAPTER 2

LEARNING TWO CONTEXTS CONCURRENTLY REQUIRES MULTIPLE EXPOSURES TO FORM STABLE CONTEXT REPRESENTATIONS IN THE HIPPOCAMPUS

ABSTRACT

Cells in the hippocampus (HP) are known to encode the spatial information of an environment. These place cells produce a mental representation of the environment or context by firing whenever the animal is in a specific location (place field). There is a wealth of evidence suggesting that the representation for a context develops rapidly and remains stable in subsequent exposures for days, weeks and months after the initial formation. Interestingly, when a rat is introduced to a different context, a different neuronal representation arises that may or may not include the same cells that participated in the initial representation. A change in the location of a place field is known as remapping. Although there have been multiple experiments describing how these representations form and change (remap), no one has yet to evaluate how the HP develops representations of two environments as they are learned concurrently. Therefore, the goal of the current study was to evaluate how two contextual representations develop simultaneously. Rats foraged for food while alternating between two different contexts while neuronal firing patterns were recorded in the HP. The results showed that the representations of the two contexts are unstable upon re-exposure to the same context on the first day of learning, contrary to previous results that context representations develop rapidly and remain stable. The initial instability of the representation on the first day is due to large scale place field remapping between visits to the same context as if they were two distinct contexts. Therefore I conclude that when the HP has to encode two distinct contexts concurrently, it takes

time to stabilize within-context representations and this process is dependent on memory consolidation to generalize across the two exposures as one context.

INTRODUCTION

The hippocampus (HP) of rats has long been known to contain place cells, cells that exhibit spatially localized firing, and the spatial location in which these cells discharge are referred to as place fields (O'Keefe & Dostrovsky, 1971). It has been proposed that ensembles of these cells give rise to a neural representation of the spatial layout of the environment, or a cognitive map, that provides the animal with a mental representation of the outside world (O'Keefe & Nadel, 1978). The spatial firing of place cells is influenced by many environmental factors such as proximal and distal cues (e.g., Renaudineau, Poucet, & Save, 2007; M. Shapiro, Tanila, & Eichenbaum, 1997), the geometric configuration of an environment (e.g., Jeffery & Anderson, 2003; Wills, Cacucci, Burgess, & O'Keefe, 2005), behavioral demands (Smith & Mizumori, 2006b), as well as ambient background cues such as odor or color (e.g., Anderson & Jeffery, 2003a) . Together, these examples illustrate the range of factors that contribute to the formation of spatial context representations.

Context plays pivotal role in memory and allows for easy memory retrieval without interference (e.g., Smith & Bulkin, 2014; Smith & Mizumori, 2006b). For instance, studies evaluating list learning tasks in humans and rodents have shown that learning two conflicting lists of items in two different contexts leads to enhanced performance compared to learning two lists in the same environment (Butterly *et al.*, 2012; Godden & Baddely, 1975; Law & Smith, 2012). When learning two lists in the same context, the information learned on the first list interferes with learning the second list. Learning the two lists in separate contexts allows for the two lists to be segregated into two distinct memories and therefore reduces interference. As information is learned, it is bound to the context in which learning takes place and the context

itself can serve as a retrieval cue (Smith, 1988). This suggests context plays a critical role in both the formation and recall of memories and can serve to guide behavior.

Many studies have evaluated how changes in the environment influence neuronal representations in the HP (e.g., Anderson & Jeffery, 2003b; Muller & Kubie, 1987b). This change in HP neuronal representations is known as remapping and there are several ways in which remapping occurs. The first type occurs when a cell fires in one context but then does not fire in that same context upon re-exposure (e.g., Muller & Kubie, 1987a). This has been referred to as binary remapping. Another type of remapping (global) occurs when a cell has a preferred firing location in one exposure to a context that then changes its preferred firing location upon subsequent re-exposure (e.g., Muller & Kubie, 1987b). The final type of remapping occurs when a place cell retains its preferred firing location but significantly changes the rate at which that cell fires in the preferred location, and fires more or less frequently. This has been referred to as rate remapping and may provide a means for the preservation of spatial information of a given context while providing a means to distinguish the experience from previous ones (e.g., Leutgeb *et al.*, 2005; Mankin *et al.*).

The clearest example of remapping occurs when rats learn about one environment and then are placed in a new environment (e.g., Frank *et al.*, 2004; Muller & Kubie, 1987b). Place cells that fired in the initial environment globally remap (e.g., fire in a new location) in the new environment in order to establish a unique representation. Changing the context completely is an obvious means to alter HP representations, but more subtle manipulations can also lead to remapping. For example, one study examined hippocampal firing while manipulating the task demands without changing the environment (Smith & Mizumori, 2006b). In that study, they trained rats to always approach the east arm of a plus maze for a reward during the first 15 trials

of a session and then had to approach the west arm for the remaining 15 trials (Smith & Mizumori, 2006b). During learning the HP representation diverged into two distinct behaviorally-defined context representations (go east and go west). This indicates that the behavioral demands of the task defined the context just as it can be defined by the environment. The context is a situation, which can be defined by different kinds of cues, task demands, events, etc.

Previous studies have examined how remapping occurs as the hippocampus shifts from one context representation to another. For instance, several experiments have examined how manipulating two environments can influence remapping in the HP using an apparatus in which the walls can be altered, therefore morphing the apparatus from a cylinder to a square box (or vice versa). The manipulation between the two shapes occurred as a gradual change over several exposures to the apparatus. One study found that as the box was morphed from a square to a more circular structure, the place fields that represented the box spontaneously shifted to the representation of the circular enclosure (Wills *et al.*, 2005). In contrast, other researchers reported that representations shifted gradually from the square box to the cylinder. This may have been due to differences in the way the experiment was carried out such as pre-training in the different morph shapes and the use of a cue card that morphed in size along with the box (Leutgeb *et al.*, 2005). These finding show that the transition from one representation to another can happen in different ways, either through a sudden shift or through a slower, smoother transition.

Although there is a wealth of research analyzing how HP contextual representations change due to environmental manipulations (e.g., Frank *et al.*, 2004; Muller & Kubie, 1987b), behavioral demands (Eschenko & Mizumori, 2007; Ferbinteanu & Shapiro, 2003; Smith &

Mizumori, 2006b), or geometry (Jeffery & Anderson, 2003; Leutgeb *et al.*, 2005; Wills *et al.*, 2005), the focus of those studies had been how the representations change or how the representations develop while learning a single novel context. Thus, the formation of single context representations is well understood, as is the shift between two well consolidated representations. However, animals frequently need to learn about several new contexts simultaneously as environmental contingencies change. To date, no studies have examined how multiple new representations form in the hippocampus. Therefore, the current study is designed to look at how two distinct contextual representations develop together over time. Rats are trained to forage for chocolate sprinkles in two different contexts in an ABAB format for 8 days while recording neuronal firing patterns from the CA1 region of the HP.

METHODS

Subjects and Microdrive Implantation

The subjects were 4 adult male Long Evans rats (Charles River Laboratories, Wilmington, MA) weighing 300–350 g at the time of surgery. Rats were implanted with one of two types of microdrives. Two rats were implanted with microdrives containing 12 independently moveable tetrodes (Harlan, Neuralynx Inc., Bozeman, MT) aimed unilaterally in the CA1 region of the HP (-3.5mm [AP], =2.5mm [LAT] from bregma, (Paxinos & Watson, 1998). The other two rats received implants of a microdrive containing 24 independently moveable tetrodes aimed bilaterally in the CA1 region (-4.0 mm [AP], ± 2.5 mm [ML] from bregma (Paxinos & Watson, 1998)). For both drives, tetrodes consisted of four strands of 12.7 mm platinum/iridium (90/10%) wire (California Fine Wire Company, Grover Beach, CA) that were platinum-plated to reduce impedance to between 120 and 200 kOhms at 1 kHz. At the end of surgery, each tetrode was lowered 1 mm into tissue. Additionally, reference wires were

lowered bilaterally into the corpus callosum and two stainless steel ground screws was implanted in the cerebellum. The rats were given an antibiotic (5 mg/kg Baytril) and an analgesic (5 mg/kg ketoprofen) prior to surgery to reduce pain or infection. All procedures complied with guidelines established by the Cornell University Animal Care and Use Committee. After recovery from surgery, the rats were placed on a restricted feeding regimen (80–85% of free feeding weight) and training began.

Following recovery, rats foraged for chocolate sprinkles in a cylindrical apparatus in order to train them to forage for sprinkles and to lower tetrodes into the CA1 region. This apparatus was distinct from the two contexts used in the experiment. Half of the tetrodes were lowered every other day until a majority of them reached the CA1 region. The lowering of the tetrodes was done gradually, at a rate of approximately 40μm per day over 2-4 weeks. The amplitude and phase of theta waves, the amplitude and sign of sharp-wave events, and the presence of theta modulated complex spiking cells were used to determine when the electrodes were localized within CA1. The experiment began once isolatable single units were obtained with spike waveforms that matched those of pyramidal neurons. Prior to the start of the experiment, the rats were given two 15 min acclimation sessions in each of the contexts so that neophobia would not inhibit exploration on the first day of testing. During data acquisition, tetrodes that did not have cell populations or that had lost a population on days that testing occurred were lowered (~10–30μm) after the recording session in order to maximize the cell population the following day.

General Training Procedures and Neuronal Recording

Once tetrodes were in CA1, rats foraged for chocolate sprinkles in two distinct contexts. Foraging took place in 2 Plexiglas boxes measuring 100cm x 100cm x 50cm deep. The contexts

differed in the following ways: box color (white or black), surface medium (Plexiglas floor or matted floor), surrounding wall color (white in black context and black in white context), background noise (pink or white noise), background odor (mint or vanilla), and position of researcher dispersing the sprinkles (different location for the two contexts). Recordings were obtained during 15 min block intervals in an ABAB format (except for rat #1412 who only ran an ABA format) and counterbalanced across days (Figure 1). There was a 5 min ITI period between each block as well as before and after all the blocks for the final two rats. For the ITI period, rats were placed in a plastic container (an opaque plastic trash can, 30 cm diameter, 65 cm height) lined with bedding.

Data Collection and Neuronal Spike Sorting

Neuronal spike and video data were collected throughout the task with Cheetah Data Acquisition System (Neuralynx, Bozeman, MT). Signals from the electrodes were amplified 2,000-10,000 times, filtered at 600 Hz and 6 kHz, and digitized. All waveforms exceeding a user-defined threshold were stored along with the time of occurrence for offline analysis. Standard spike sorting techniques were used to distinguish multiple unit records into single units (Spikesort 3D, Neuralynx). Waveform features used for sorting included spike amplitude, spike width, waveform principle components, and waveform area. The rat's position was monitored by digitized video (sampled at 30 Hz) of an LED array attached to the rats head.

Classification of Responses

The data was analyzed to determine whether the recorded neurons exhibited spatial firing within one or both of the contexts. These analyses were conducted using programs custom-written in Matlab (The MathWorks, Natick MA). The floors of the recording chambers were divided into ~3.5 x 3.5 cm square pixels, and the firing rate of each neuron was determined by

dividing the total number of spikes in each pixel by the amount of time the rat spent in that pixel. These rate maps were smoothed using an algorithm that replaced the value in each pixel with the average of the values in that pixel plus the adjacent eight pixels. Place fields were defined as any set of 6 or more contiguous pixels where the neuron fired with a rate at least twice the baseline firing rate. Any neuron with a peak firing rate of < 3 Hz or with a place field occupying greater than 50% of the total floor area of the recording chambers was excluded from further analysis to eliminate any putative interneurons.

Data Analysis

Spatial firing rate maps were constructed by calculating firing rates in the pixels, as described above, and the data were smoothed by convolution with a 4 bin Gaussian kernel with unity sum. Spatial bins that contained less than one second of occupancy (following smoothing) were discarded. For display purposes only, firing rate maps were interpolated linearly with 3 points between each sampled data value. To analyze the degree of similarity between place fields, pixel-by-pixel pairwise correlations (Pearson's r) were computed between the firing rate maps generated for each neuron. These were first calculated for each of the observations across blocks of the same context (see Figure 1), and then for each of the observations across blocks involving visits to different contexts. These values were then averaged to produce a single within-context correlation score and a single across-context correlation score for each neuron. To assess the extent to which each neuron exhibited rate remapping across and within contexts, rate remapping indices were calculated according to the methods described by Leutgeb et al. (2005). These indices were computed by taking the difference between the maximum firing rate and the minimum rate during the two blocks and dividing the difference by the summed rates. A score of 0 on this index indicates no change in rate, while 1 indicates maximal rate change.

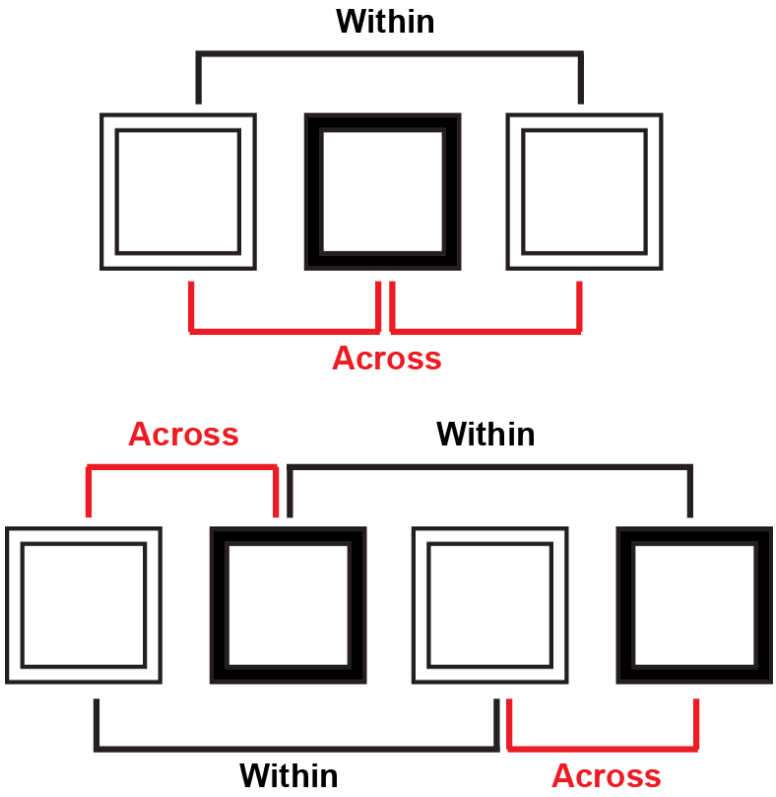

Figure 1. Pictorial representation of the context presentations for each day. For one rat, there were 3 blocks per session. For the other rats, there were 4 blocks per session. The order of the presentation of contexts was counterbalanced.

Spatial correlation scores, rate remapping index and block by block firing rate submitted to repeated measures ANOVAs. All other neuronal data was submitted to one way ANOVAs or chi square analyses for proportion data.

To calculate normalized information scores, an information score was calculated as described in (Markus *et al.*, 1994):

$$I = \sum P_i \frac{R_i}{R} \log_2 \frac{R_i}{R}$$

For spatial information, $P_i$was the probability for occupancy of bin i, R_i was the mean firing rate for bin i, and R was the overall mean firing rate. A distribution of 500 pseudo information values was calculated by randomly offsetting spike times using uniform random values ranging from 5 to 100 seconds. The values used for comparison between sessions were z transformed using this distribution (i.e. the number of standard deviations from the mean of the distribution of pseudo information values). The z transformations were then submitted to ANOVA to evaluate the change in information across days.

Histology

Following the final day of recording, rats were deeply anesthetized and transcardially perfused. The brains were removed, cryoprotected, and sectioned into 40-µm coronal slices. The sections were stained with cresyl violet in order to verify the placement of electrodes in the CA1 region of the HP. Each section of the HP with tetrode tracks was collected for analysis and all of the tetrode wires were identified by following their tracks across sections. Tracks were determined by following small angular deviations from the tetrodes across multiple sections until the track ended. The end location was determined as the placement of a given tetrode.

Recordings from a tetrode were included in the analyses if the deepest position of the tetrode was in or just below the CA1 region.

RESULTS

A total of 656 neurons were recorded during the study. Some tetrodes were lowered on a daily basis to maximize the number of recorded cells, and this led to the potential for movement of tetrodes or brain tissue hours after lowering tetrodes which could lead to the loss or gain of cells. This could lead to spurious remapping results if a cell had a place field in one context but not the subsequent visit to that context due to the losing/gaining the cell. To avoid this problem, I carefully examined the data of every cell using a strict criterion and excluded any cell that exhibited drift during a session, had a response profile that was not stable through the session, was cut off by the preset threshold or was determined to be an interneuron. This resulted in a total of 251 cells that met the criteria and were analyzed in the results. Cells were classified into four categories. There were binary cells that had a place field in one context exposure but not the other (Figure 2A), cells that globally remapped between repeated exposures (Figure 2B), cells that showed rate remapping between same context exposures (Figure 3A) and cells that remained stable between exposures to the same context (Figure 3B). Additionally, I observed cells that showed remapping between exposures to different contexts (adjacent plots, Figure 2 & 3). The pattern of these types of responses over a session and across days was then quantified to evaluate the development of context representations in the two contexts.

Development of Spatial Firing Patterns

The goal of the study was to analyze how two different contextual representations develop over the course of experience. Therefore, I analyzed the average within-context pixel by pixel correlation scores for each neuron, which represented the degree of similarity with respect

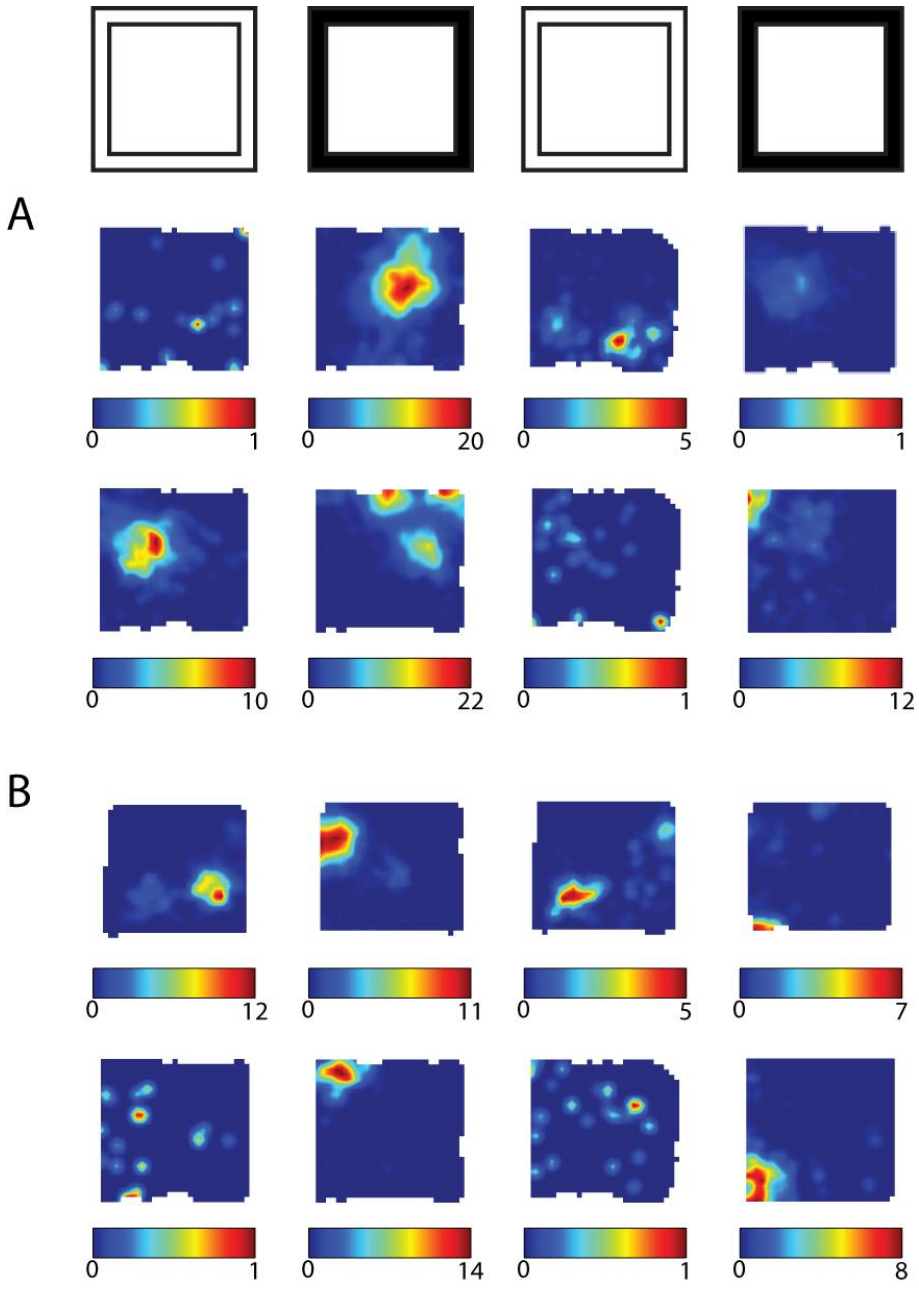

Figure 2. Representative spatial firing rates for the different context exposures (top) for 4 different cells. The scale bar indicates range in firing rates (Hz) for the cell in each context exposure. A) Represents two different cells that exhibited a binary response between exposures to the same context. The first cell shows a cell that doesn't really fire in the first white exposure but has a place field in the second exposure and has a place field in the first black exposure but not the subsequent exposure. The second cell has a place field in the first white exposure but not the subsequent exposure. B) Two cells that exhibited global remapping. The first shows that the cell has a different place field location for each context exposure indicating global remapping in both contexts. The second cell has a place field in the first exposure to the black context that remaps to a new location in the subsequent exposure.

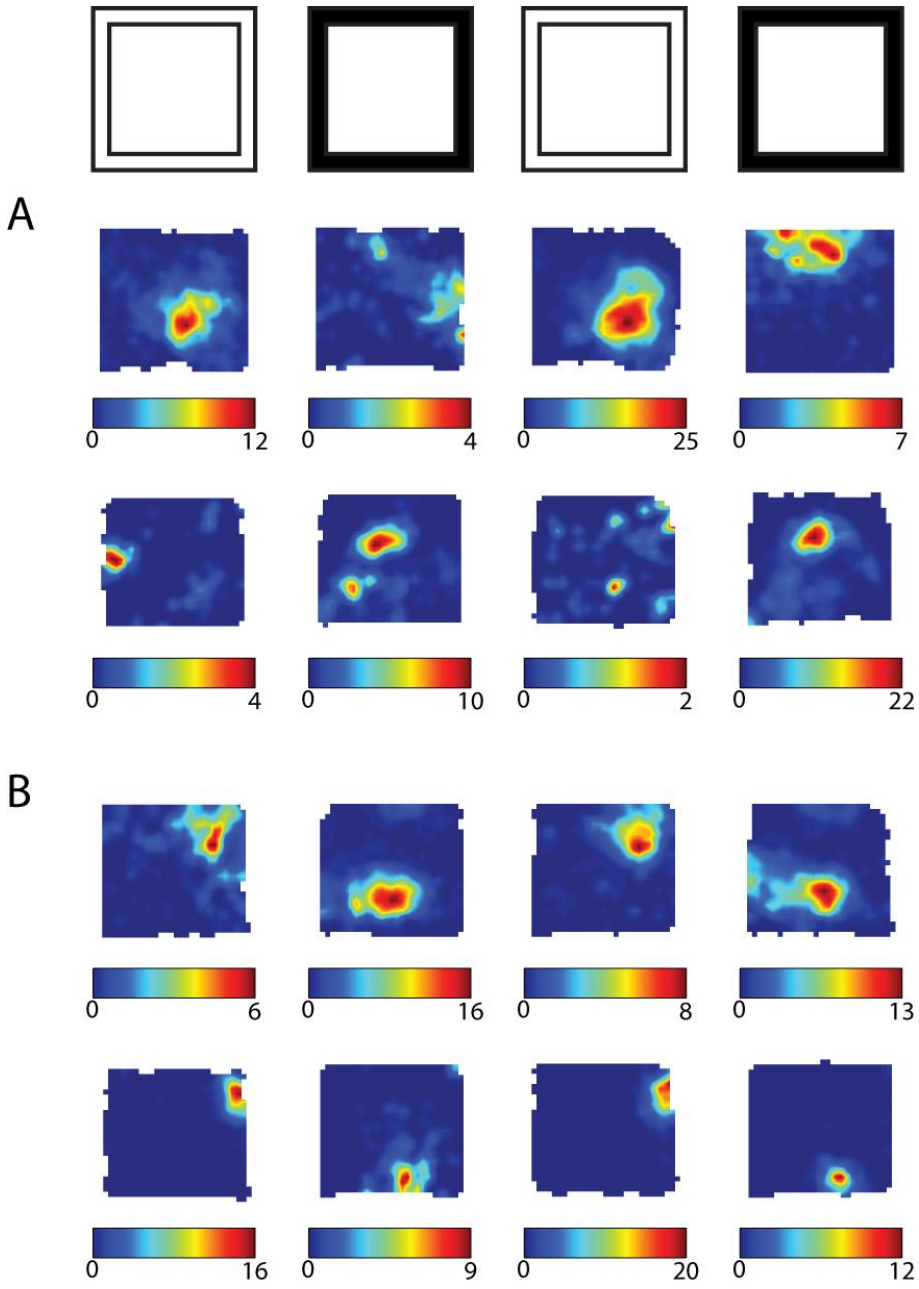

Figure 3. Representative spatial firing rates for the different context exposures (top) for 4 different cells. The scale bar indicates range in firing rates (Hz) for the cell in each context exposure. A) Two cells that exhibited rate remapping. The first cell has a place field in the same location in both white contexts and the second in both black contexts, but in the second exposure the firing rate more than doubled. B) Two examples of cells that maintained a stable place field. In both cases the cells had the same place fields in both the white and black context exposures. Although there were differences in the firing rate, the difference was too small to qualify as rate remapping.

to the location of preferred firing of cells on repeat visits to the same context. I performed the same analysis on the average between-context spatial correlation scores for each neuron, which represented the degree of similarity of the locations of place fields between visits to different contexts. A repeated measures ANOVA was run with spatial correlation type (average within and between contexts) as the within subjects factor and day as the between subjects factor, because different populations of neurons were recorded on each day. Results showed a significant main effect of spatial correlation type ($F[1,240] = 711.524$, $p < 0.001$), a main effect of day ($F[7,240] = 2.810$, $p < 0.01$) and an interaction of spatial correlation type by day ($F[7,240] = 3.239$, $p < 0.01$). Not surprisingly, the within-context neuronal representations were more correlated than the between context correlations, indicating the spatial representations were more similar across repeated visits to the same context than across visits to different contexts (Fig. 4, compare the solid and dashed lines). However, the within-context correlations changed with learning. They started out relatively low on Day 1, increased on Day 2 and significantly increased on Day 3, and remained high thereafter (Tukey LSD, Day 2 $p = 0.33$, Day 3-8 all $p < 0.05$). This indicated that, surprisingly, the two context representations were initially somewhat unstable (for examples see Figure 5) and only became more stable with experience (for examples see Figure 6). This result stands in contrast to previous reports that in new environments place fields become stable over the course of a few minutes (e.g., Frank *et al.*, 2004; Muller & Kubie, 1987a; Thompson & Best, 1990).

Changes in Spatial Firing that Underlie the Development of Different Context Representations.

In the previous section, I showed that firing during repeated visits to the same context were less stable early in learning than after several sessions (Fig. 4). This change in the stability

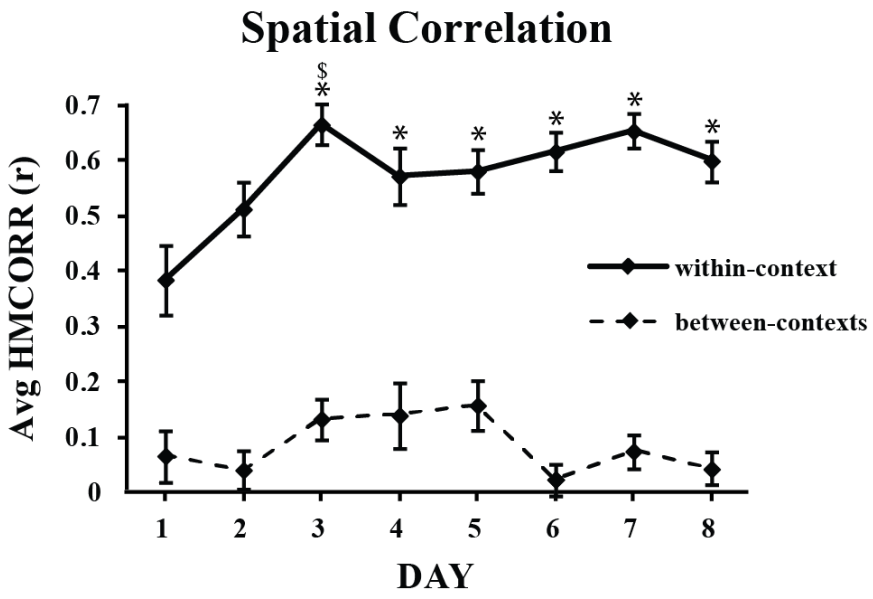

Figure 4. Average spatial correlations scores of neurons are shown for repeated visits to the same context (solid line) and across visits to different context (dashed line). Both of those correlations scores are shown for neurons recorded across the eight recording sessions (Day) taken from each rat. The * indicates time points that are significantly different from Day 1 and $ indicates time points significantly different from Day 2.

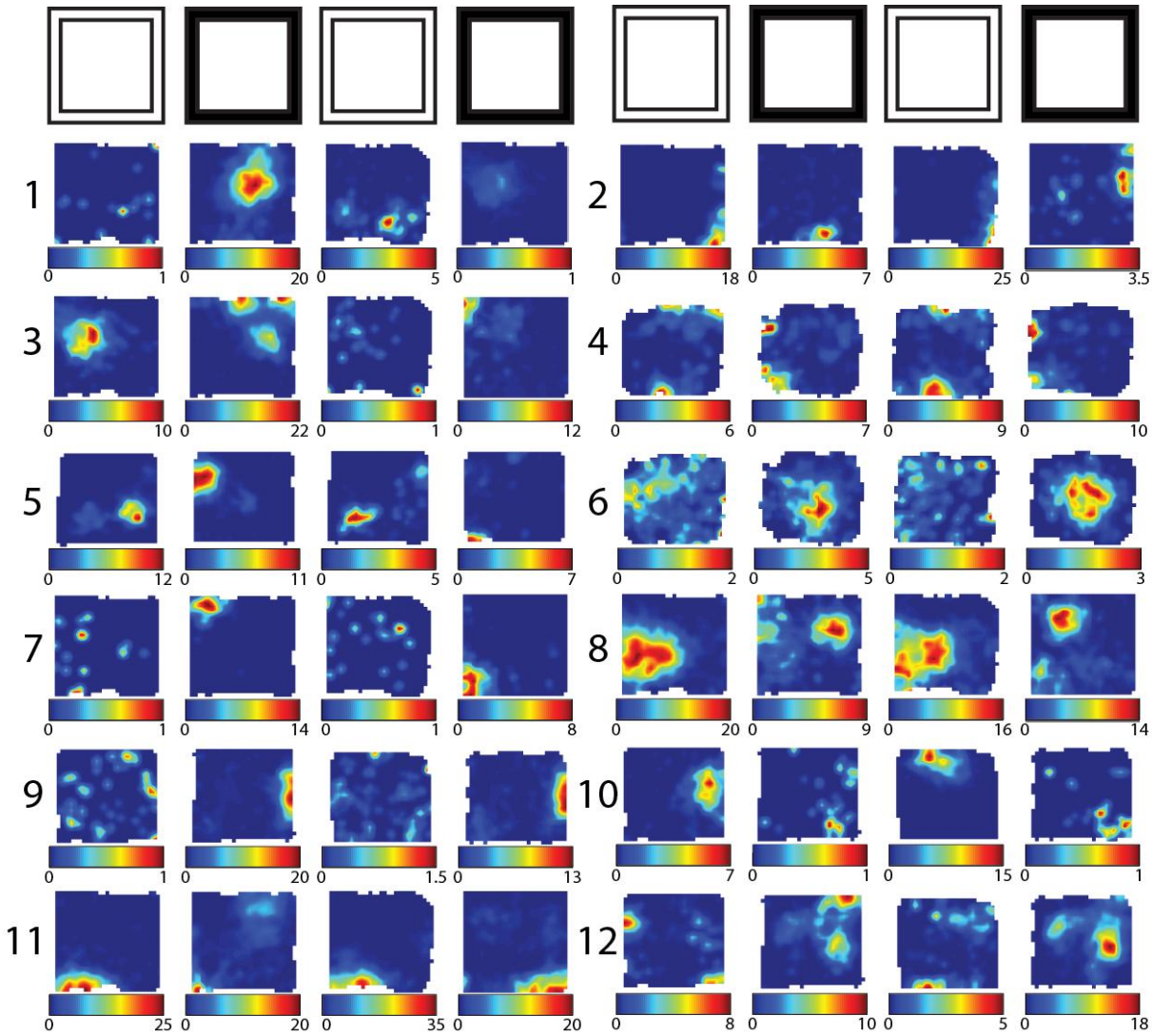

Figure 5. Representative spatial firing rates for the different context exposures (top) for 12 different cells of the first day of training. The scale bar indicates range in firing rates (Hz) for the cell in each context exposure. Many of the cells exhibited remapping across visits to the same context on the first day of training.

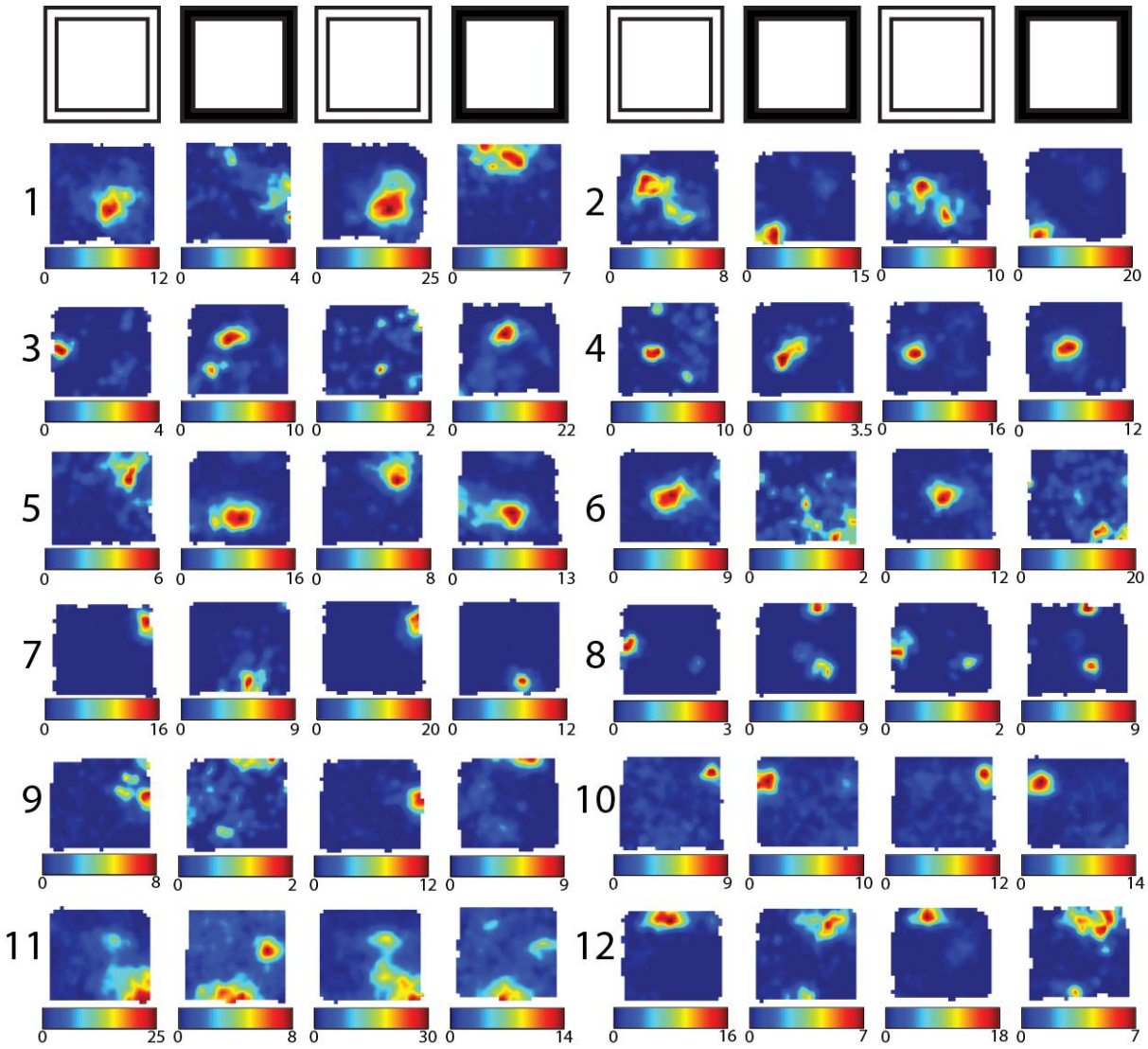

Figure 6. Representative spatial firing rates for the different context exposures (top) for 12 different cells from the final two days of training. The scale bar indicates range in firing rates (Hz) for the cell in each context exposure. Cells exhibited more stability following the initial days of training.

of context representations could have been caused by several factors. For example, the number of neurons with place fields could change with experience or the individual place fields could be unstable, perhaps exhibiting remapping even though the rat was revisiting the same context.

Changes in the Number and Size of Place Fields. One possibility for the lack of a stabilized representation on Day 1 is there were fewer place fields present leading to an incomplete representation. In order to assess this, I included every neuron on each day and determined if they had a place field or did not have a place field. If a cell had a place field in either exposure to context A or context B it was counted as having a place field in that context. This led to a total of two entries per cell with the potential for 2 place field entries for any cell, one for each context. A Chi-square analysis revealed that the percentage of place fields did increase over days ($\chi^2(7, N = 498) = 14.692$, $p < 0.05$) with Day 1 having the lowest percentage of place fields (68.2%, Figure 7). This indicates that on the first day of learning the two contexts there were fewer place fields per number of opportunities on Day 1, but experience leads to an increase in the number of place fields, which could indicate that a more thorough representation has developed.

Additionally, the average place field size for each within context exposure was analyzed across days with a one way ANOVA, and revealed no significant changes over days ($F[7,710] = 0.767$, $p = 0.615$). This suggests the less stable representations of Day 1was not due to larger place fields, which would provide less specific information about the rat's current location, as place field size was the same across days (Figure 8). Together, the size of the place field did not change over days, but the proportion of place fields increased over days with experience.

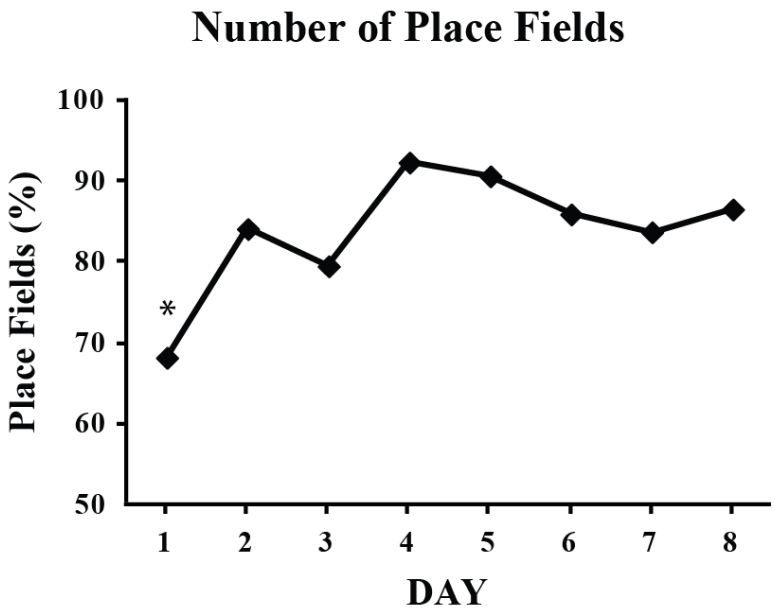

Figure 7. The number of place fields observed, expressed as the percentage of place fields out of the total number of opportunities for a place field (see text), are shown for the eight recording sessions. The * indicates a significant difference compared to all other days.

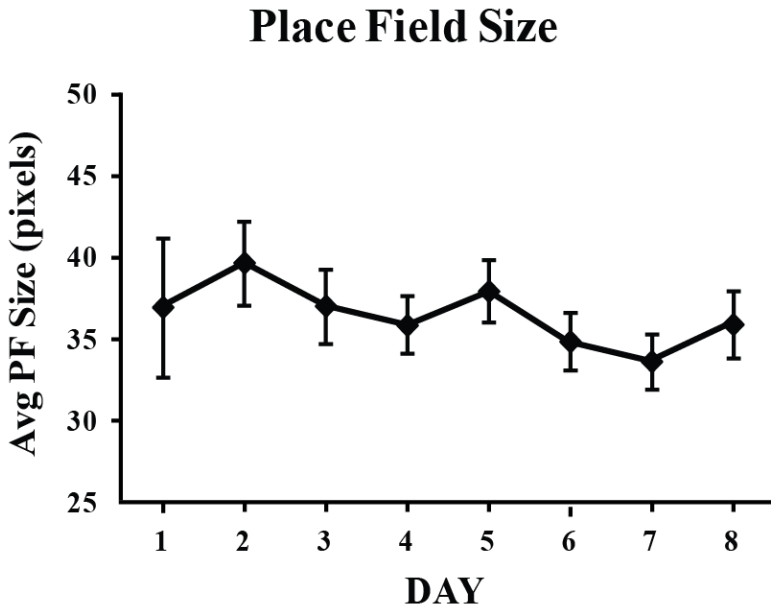

Figure 8. The average size of place fields (in pixels) observed is shown across the eight recording sessions.

Global Remapping Across Repeated Visits to the Same Context. After evaluating the number of place fields and size of the place fields over days, I next evaluated changes in stability of the place fields across days. As mentioned above, there are several types of remapping that involve changes in the spatial selectivity of a cell; I therefore looked to see whether remapping is occurring across repeated visits to the same context, as surprising as that might be. I first assessed whether global remapping changed over days and contributed to the initial place field instability. Although spatial correlation reflects various forms of remapping, global remapping can be measured more directly by asking how far a place field has moved from one condition to another. To do that, the spatial similarity of place fields in each context was calculated using the spatial correlation between the heat maps (see methods) and the shift in the center of mass (COM; Leutgeb *et al.*, 2005; Mehta *et al.*, 1997) of the firing rate distribution across visits to the same context for each day of training (. To be included in the analysis, cells had to meet the place field requirement and have a place field in both within-context exposures. Results from a one way ANOVA revealed a significant effect of COM shift (F,[7,208] = 5.461, $p < 0.001$, Figure 9), and post hoc analyses revealed that Day 1 had significantly more COM shift compared to all other days (Tukey LSD, all $p < 0.05$). The results indicate that global remapping is a contributing factor to place field instability early in learning.

Binary remapping was also analyzed to assess Day 1 stability of place fields. A chi square analysis was run on the proportion of binary cells for each of the two contexts across days leading to two potential data points for each cell on a given day (one for rat #412). The results revealed no significant change in the proportion of binary place cells across days (χ^2(7, N = 429) = 3.749, $p = 0.808$, Figure 10). Therefore the instability seen on Day 1 was not due to having more binary cells within the neuronal representation.

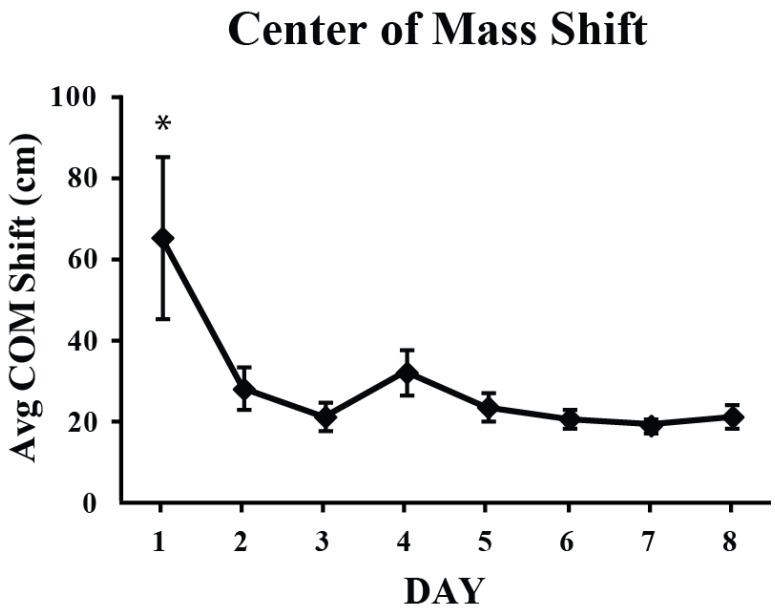

Figure 9. The average COM shift of place fields observed is shown across the eight recording sessions. The * indicates a significant difference compared to the other days of training.

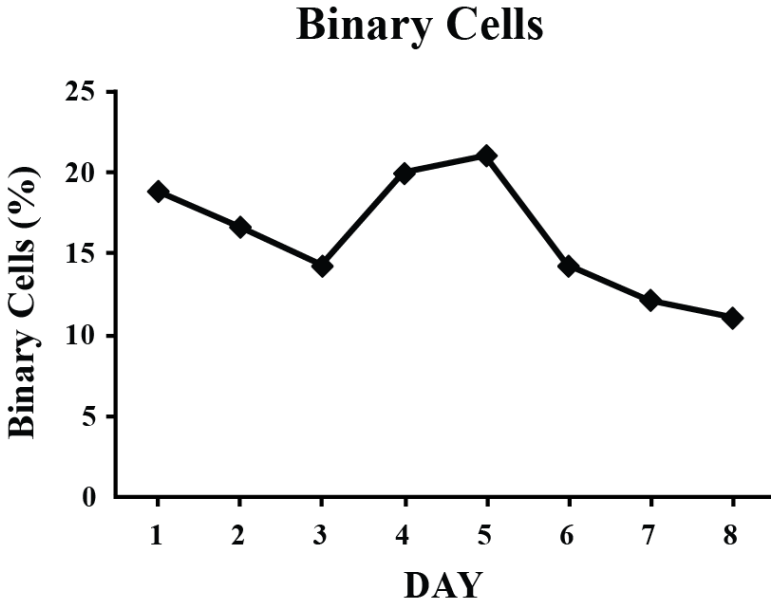

Figure 10. The percentage of binary cells observed is shown over the eight recording sessions.

Rate Remapping Across Repeated Visits to the Same Context. The final form of remapping, rate remapping, was analyzed to assess whether changes in firing rate occurs across repeated visits to the same context contributes to the instability observed early in learning. This was done by calculating a rate remapping index (RRI) by taking the difference between the maximum firing rate and the minimum firing rate during the two within-context blocks (or across context) and dividing the difference by the summed rates. A score of 0 on this index indicates no change in rate, while 1 indicates maximal rate change. An ANOVA was run on the average within-context RRI for each cell across days of learning. The results did not show a significant change in RRI across days (F, [7,194] = 0.709, p = 0.664, Figure 11). Therefore rate remapping does not account for the lack of stability of place fields on Day 1 and did not change over the course of the task.

Place Field Specificity and Firing Rate Across Repeated Visits to the Same Context. Another possible explanation is that place cell instability on Day 1 was due to changes in the specificity of the spatial firing. I first analyzed the infield/outfield firing rate (IF/OF), which was calculated by taking the firing rate of a cell in its place field and dividing it by the sum of the infield and outfield (firing rate outside the place field) firing rates. One explanation could be that as a place cell becomes more stable, it decreases the background firing to form a more stable representation of the preferred location within an environment. Results from a one-way ANOVA revealed that this was not the case, and there was no change in the IF/OF firing rate (F,[7,243] = 1.918, p = 0.068, Figure 12). While this reached significance as a marginal trend, there was no reliable pattern and the firing IF/OF firing rate as it randomly fluctuated across days. This suggests that changes in the IF/OF could not account for the less stable representations on the first day. To evaluate how baseline firing rates changed over exposures to

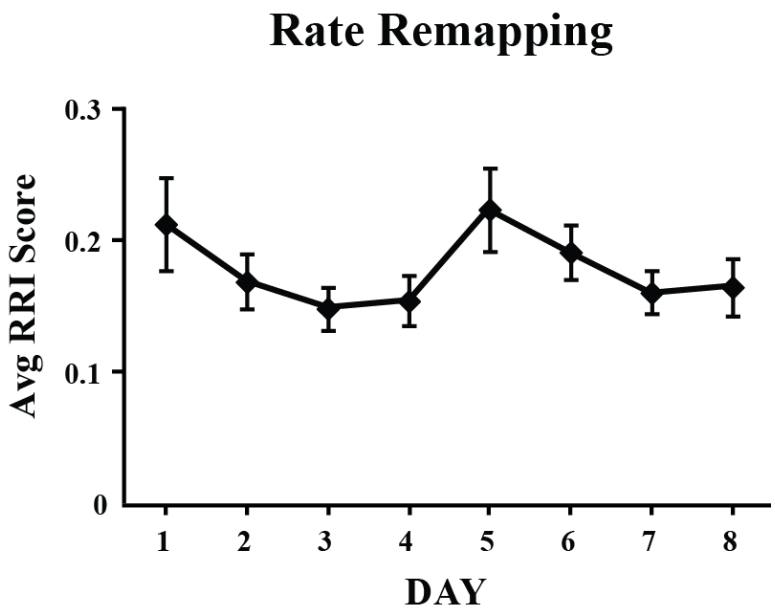

Figure 11. Average rate remapping scores, as measured by the average rate remapping index (see text), observed across the eight recording sessions.

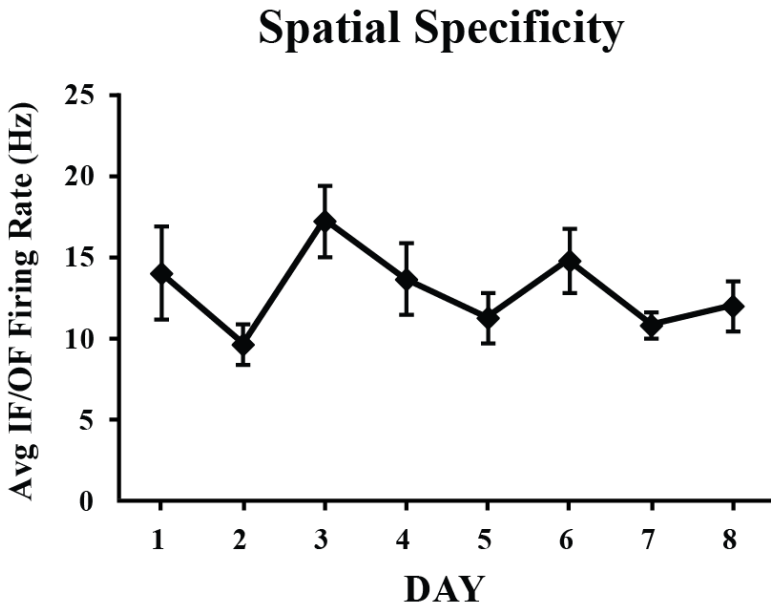

Figure 12. The average spatial specificity, measured by the average infield outfield firing rate, is shown across the eight recording sessions.

the context, overall firing rate was analyzed for each block on each day as a repeated measure ANOVA for the three ABAB rats. The results showed that there was no main effect of day (F[7,172] = 1.038, p = 0.406), no main effect of block (F[1,172] = 1.975, p = 0.150) and no interaction of block by day (F[7, 172] = 1.499, p = 0.130, Figure 13). Therefore there was no significant change in firing rate across blocks or days, confirming that the firing rates of the cells analyzed remained stable.

Spatial Information Content Across Repeated Visits to the Same Context. While COM shifts illustrate changes in the preferred firing locations within a context, and RRI captures the changes of a cell's firing rate within a stable place field, I next quantified the extent to which neurons changed their degree of spatial sensitivity across exposures to the same context over the recording sessions. Spatial information was calculated from a score based on spatial firing data and then z-transformation to account for any spuriously high scores due to low firing rates (See Methods). A cell did not have to meet the criteria as having a place field for this analysis but had to have a significant corresponding Z value > 1.96. To this end, the analysis took into account the average information scores for each cell that had a significant Z value. An ANOVA revealed that there was no significant change in information across days (F[7,246] = 1.907, p = 0.069, Figure 14). Although the analysis did come close to reaching significance, the result appeared to be due to day to day variation in information scores rather than any discernible pattern that could explain the change in stability during the first days of exposure to the two contexts. This suggests that the spatial information content did not change across days and therefore could not account for the spatial instability seen on Day 1 of learning the task.

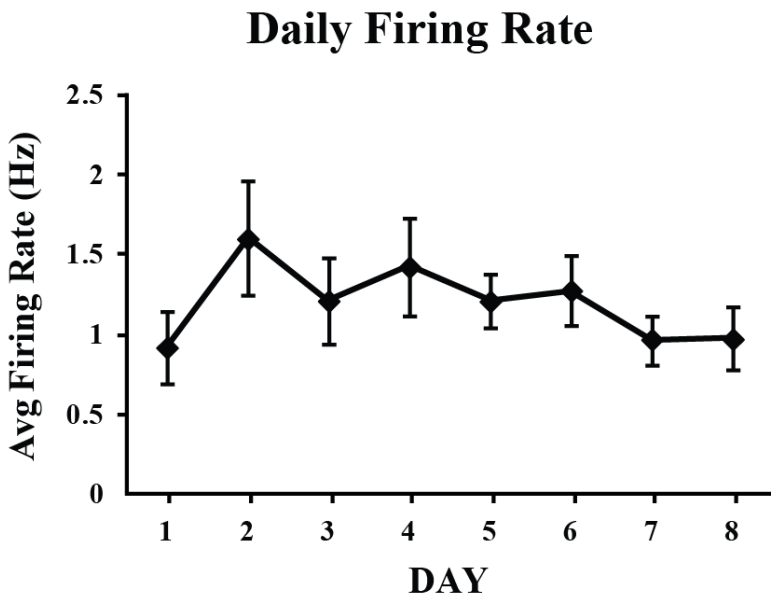

Figure 13. The average daily firing rates is shown for the eight recording sessions.

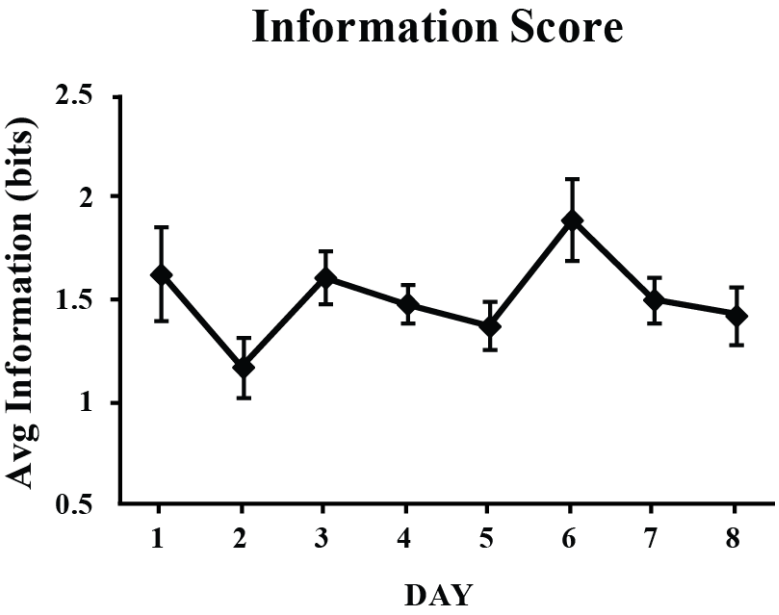

Figure 14. The change in information scores (see methods) observed is shown across the eight recording sessions.

DISCUSSION

In this study, HP cells were characterized during the simultaneous development of distinct neuronal representations while rats foraged for sprinkles in two different contexts. Previous research has shown that place cells develop their spatial specificity within the first several minutes upon entering a new environment and are believed to remain stable upon returning to the environment days, weeks and months later (e.g., Frank *et al.*, 2004; Muller & Kubie, 1987a; Thompson & Best, 1990). Contrary to these previous results, the neuronal representations did not become stable within the first few minutes of exposure to either the white or black context. Our results show for the first time that when two contexts are being learned concurrently, the neuronal representations of those contexts do not start to stabilize until after the first day of training. This was evident by the low within-context spatial correlations. Surprisingly, the low spatial correlations were caused by large scale remapping that occurred across repeated visits to the same context, as indicated by large shifts in preferred firing location (COM shift, Fig. 7) on Day 1. This finding of instability during the initial exposure to multiple contexts has not been seen before, and stands in contrast to previous findings indicating that place fields form and stabilize rapidly when rats learn about a single novel context. Interestingly, Day 1 had the lowest percentage of place fields observed compared to the subsequent days. This suggests that with more place fields the HP is capable of forming a stronger representation of the two contexts. The lack of stability was not caused by abnormally large place fields, place fields that lacked spatial specificity, or place fields that had low spatial information content. Instead, the lack of specificity early in learning seems to be a result of large scale remapping that occurred across repeated visits to the same context, as indicated by large

COM shifts. I therefore conclude that unlike learning a single context, learning two contexts concurrently requires more experience than a single day to stabilize HP neuronal representations.

Recent findings suggest that HP representations might not be as stable as previously thought. One study recorded from the same HP neuronal population in mice over several days of training on a linear track (Jeantet & Cho, 2006). They found that over the course of 6 days place fields became more stable, the spatial information content (information about the subjects location) increased significantly by the 6th day, and that place field size decreased with time. The results show that with repeated exposures to running on a linear track, place cells evolved after the initial visit and continued to stabilize with repeated experience (Jeantet & Cho, 2006). This supports our results suggesting that HP place cell stability requires more than a single day of experience. Although the current results did not find any changes in spatial information or place field size, I did not record from the same neuronal population over the course of the experiment as they did in Jeantet & Cho (2006).

Mankin et. al. (2012) evaluated how HP neurons change after exposures to the same environment in the morning versus in the afternoon for several days. They had rats forage for food in either a square or circular enclosure they recorded over the course of two to three days (Mankin *et al.*, 2012). The rats had been well trained on the task so HP representations would be expected to be stable. They found that place cell populations in CA1 region progressively differed with increased temporal distance between visits to the environments (as evidenced by rate remapping), indicating that events that are more distant in time become less similar even though the spatial and contextual representations remain stable. They suggested that the HP is recruited for temporal coding over time and serves as a means to differentiate experiences that occur in the same environment at different times without changing their spatial specificity

(Mankin *et al.*, 2012). Their results indicate that over extended periods of time, cells are contributing differently to the contextual representation as evidenced by rate remapping. Our results involved larger scale remapping early in learning due to instability in the formation of the contextual representations. The results from Mankin et al. shows that even when HP context representations are expected to be stable, there are still changes that take place in order to differentiate exposures to the same context. It may be that the results from Mankin et al. provide a means for encoding distinct episodic memories.

Evidence from additional studies also shows there are changes in HP neuronal representations over time and that stability wavers. Tayler et a.l (2013) tagged neurons with an activity dependent form of green fluorescent protein, which label active neurons in the HP, prior to training on a contextual fear conditioning task. This provided the opportunity to visualize neurons in the HP in real time and see what cells fire and when they fire while the animal performs a task. Two days following training they found that only 40% of the neurons activated in the HP during acquisition were reactivated when they were tested up to 2 weeks later, yet the rats did not show any impairment in memory (Tayler, Tanaka, Reijmers, & Wiltgen, 2013). Another study analyzed hundreds of CA1 neurons simultaneously using calcium imaging in freely moving rats over the course of 45 days (Ziv *et al.*, 2013). They found that many neurons would exhibit place fields on a given day, but only 25% of those same neurons were active 5 days later and that number dropped to 15% after 30 days, indicating that cells for a given representation come in and out of the representation over time. When the spatial information content of neurons was analyzed using the same cells on Day 1 and Day 30, they found that the cell's spatial information did decline but the information present was enough to accurately predict the mouse's location (Ziv *et al.*, 2013). These results indicate that the HP representations

fluctuate with time and may serve to distinguish individual memories as well as incorporate new information. Changing the neurons that participate in a given memory trace could serve to distinguish similar experiences. For instance, when a rat is exposed to an environment over and over, it would be beneficial to maintain the general representation of that environment but it would also be beneficial to distinguish between different visits to that environment. By maintaining a partial representation with a number of the initial neurons and adding new neurons (or having neurons that change their firing rate) to the memory trace provides a potential means to segregate the different exposures in the same context. Support for this comes from a sequential odor discrimination task that found as a training session progressed the population of neurons recorded became more distinct (Manns, Howard, & Eichenbaum, 2007). Interestingly, the amount of dissimilarity correlated with behavioral performance, the more distinct the population representation the better the rat performed, even though the rats were well trained on the task. The authors suggested that this may be a means for the hippocampus to encode episodic memories.

The evidence now suggests that spatial representations are not as stable during initial training and are continually modified over the course of experience. The existing results suggest that HP representations are less stable than previously thought, perhaps shifting in a systematic way that could support episodic memory. My results show that representations are unstable for the first few exposures and then stabilize after the first couple of days. My results are different from those discussed as in those studies the rats were well trained on these tasks and` in the current study we looked at the development of the neuronal representation. However, our results fit in to the growing body of evidence that HP representations are not static but take time to

develop and after stabilizing there is still fluctuation in the representations to incorporate new information and distinguish similar events.

The findings in the current study provide evidence that when learning two contexts concurrently the neuronal representation in the HP requires more than a single day of experience to develop or at least more than two fifteen minute exposures within a single session. This suggests the HP takes time to 'learn' that the two visits to the same black (or white) box are actually visits to the same context and this happens after the initial day of learning. On Day 1, rats are faced with a novel presentation of two alternating contexts. One possibility is that when returning to a context the second time, the context representation is still malleable and rats have yet to learn that they will experience the same situation (foraging for sprinkles). Due to the malleable state, the HP treats the second exposure as if it were a different context because the HP has not yet learned to generalize the second exposure to the initial exposure. After their experience of learning that the two contexts are in fact the same upon revisiting them (following Day 1) the HP is then capable of generalizing the two within-context exposures as a single contextual representation. Many place fields remapped between visits to the same context on Day 1, which is similar to the results of other experiments that move rats from a known context to a novel context (e.g., Frank *et al.*, 2004). Once the HP has been taught the two contexts are the same, it is then capable of generalizing the within-context exposures as a single representation

The interpretation that the HP is not yet able to generalize the within-context visits as a single representation on the first day is supported by memory consolidation experiments that find plasticity is necessary for a memory to become stable. Long term potentiation (LTP) is induced by a molecular cascade of events within an excited neuron that lead to changes in the

connectivity between neurons, such as increasing the number of synapses or dendrites. This leads to stronger connections between neurons involved in learning and serves to strengthen the memory representation (e.g., Bliss & Collingridge, 1993; Bliss & Lomo, 1970). However, while the initiation of LTP happens almost immediately, it takes hours to fully take hold and make changes to the neuronal connections (Krug, Lossner, & Ott, 1984). There is a wealth of evidence indicating memory consolidation takes time and interference of this process will lead to the extinction of that memory (for review, see McGaugh & Roozendaal, 2009). Our results would suggest that the reason the HP did not generalize between the within-context exposures is because the memory for the within-context representations had not yet had the chance to consolidate the two within-context exposures as one single representation. Once there was substantial time for consolidation, I suggest the HP was then able to generalize across the two contexts and experience them as a single context.

Evidence for this interpretation comes from an electrophysiology study using protein synthesis inhibitors (which block LTP) to evaluate the effects of blocking consolidation on the formation of contextual representations (Kentros *et al.,* 1998). In this task, rats were exposed to one environment several times on the first day to establish a representation of a single context. On day two they are re-exposed to that environment and afterwards the rats received infusions of a protein synthesis inhibitor or saline into the HP. Following a one hour break (to allow the drug to take effect), the rats then received several exposures to the familiar context and to a novel context. The results showed that both groups of rats maintained the representation of the familiar context and both exhibited remapping of HP neurons in the novel context. However, when rats infused with the protein synthesis inhibitor were re-exposed to the novel context on the following day, cells in the HP exhibited a second iteration of remapping. This indicates that blocking LTP

actually inhibited the consolidation of the new context representation. When the rats were re-exposed to the new context again on the following day the HP treated the context as if the rat had never experienced it before (Kentros *et al.*, 1998). This supports the interpretation of the current results, that the HP must undergo consolidation in order to 'learn' to treat the within-context exposures on the first day as the same.

Further support for this interpretation that the HP is unable to generalize a across visits to the same context on the first day of learning comes from previous place cell recording experiments. One experiment trained rats to forage for sprinkles in a cylinder with a white cue card (Bostock, Muller, & Kubie, 1991). Following training, rats were then re-exposed to the cylinder except now the rats were presented with a black cue card in place of the white one. They were then repeatedly exposed to the cylinder with alternating presentations of the white or black cue card. Similar to the current results, they found that during the first two exposures with the new black card, the HP neuronal representations were not stable and did not start to stabilize until the third exposure. They concluded that the HP map for any new environment reaches a steady state only after the rat experiences the new environment enough times. They suggest that there is local plasticity that needs to take place before the representation can stabilize and form a coherent and distinct representation in the presence of the black card (Bostock *et al.*, 1991). Our results are in line with this study in that I also saw an increase in context representation stability following several exposures to two novel contexts.

The current results indicate for the first time that there is instability in HP representations when initially learning two contexts concurrently. I believe that the instability on Day 1 of the within-context representations is due to the HP not having sufficient time to generalize the two exposures as a single context. I suggest that memory consolidation must occur to facilitate the

generalization of the two exposures into one, and therefore there is an increase in stability on the second day of learning followed by a stable representation after the third day. Although no previous studies have evaluated the concurrent development of contextual representations, there is evidence suggesting that the HP requires several exposures to form a stable representation (Bostock *et al.*, 1991; Jeantet & Cho, 2006), a finding similar to the current results. Additionally, disruption of LTP via protein synthesis inhibitors abolishes the contextual representation upon reentry to the same context indicating LTP is a critical component of generalizing a single context across multiple exposures (Kentros *et al.*, 1998). Therefore I conclude that when the HP has to learn about two distinct contexts concurrently, it takes time to stabilize within-context representations and this process is dependent on memory consolidation to generalize across two exposures as the same context.

CHAPTER 3

LEARNING RELATED CHANGES IN HIPPOCAMPAL EVENT RESPONSES DURING A CONCURRENT ODOR DISCRIMINATION TASK

ABSTRACT

The hippocampus has long been known to contain place cells which play a critical role in spatial navigation and spatial learning tasks. However, evidence from humans and rodents has made it clear that the hippocampus is involved in encoding much more then spatial information as it encodes episodic and non-spatial information as well. Several rodent studies have shown that the hippocampus encodes information about specific events (e.g., rewards, odor sampling), sequences, and the passing of time. Although it is known that the hippocampus encodes non-spatial information, there is very little evidence on how these types of responses to non-spatial information develop as learning progresses. The current study used a concurrent odor discrimination task to evaluate what neuronal event responses develop and how they change over the course of learning. Rats were trained on one list of odor discrimination problems over the course of 5 days while neurons in the CA1 region of the hippocampus were recorded. Results indicated there were multiple neuronal responses to specific events (e.g., trial-start and end, odor sampling, etc) and the majority of these event responses significantly decreased in number over the course of learning. I suggest this decrease in responses occurs because as learning occurs, the individual events become less important and the HP encodes the events of a trial as a single sequence which results in the reduction of individual responses. Once the rules of the task have been learned, the rats can focus on the discrimination problems rather than attend to all the events as they occur. The interpretation fits with previous theories on hippocampal function

which state the hippocampus binds relevant information to the context for which it is relevant in order to facilitate learning and generalization across similar experiences.

INTRODUCTION

It is well known that the hippocampus (HP) contains pyramidal cells that are sensitive to spatial locations (place cells) and will fire reliably when a rat is in the location for which that cell is selective (place field) (e.g., O'Keefe & Conway, 1978b; O'Keefe & Dostrovsky, 1971; M. Shapiro *et al.*, 1997). It was therefore initially thought that the HP contains cells that form a mental representation of the environment known as a cognitive map (e.g., O'Keefe & Conway, 1978a; Shapiro, 2001; Shapiro & Eichenbaum, 1999). Since the discovery of place cells, one major area of focus in HP neuronal research has been spatial learning and navigation (e.g., McNaughton *et al.*, 2006; Shapiro & Eichenbaum, 1999; Sharp, 2002) and some theories posit that the main role of the HP in all species is the processing of allocentric spatial information (for review, see Hartley *et al.*, 2013). Although this has been the most popular theory for electrophysiology studies on rodent HP function, there are several opposing theories that suggest the primary role of the HP is relational processing (Eichenbaum, 1998, 2013; Ergorul & Eichenbaum, 2004; Ryan, Lin, Ketcham, & Nadel) and another that suggests the primary role is to form context representations which then bind the relevant information to that contextual representation (Smith & Bulkin, 2014; Smith & Mizumori, 2006a). Due to the vast amount of literature arguing for the spatial processing theory, the current paper will focus on the evidence that the HP plays a much larger role then integrating spatial information.

The original finding of place cells in the HP also noted that in addition to place cells there were cells that corresponded to non-spatial events such as behavioral expectations or bar pressing (O'Keefe & Dostrovsky, 1971). Additional studies focused on non-spatial sensory discrimination paradigms and found cells that fire primarily in relation to discriminative cues and behavioral responses (e.g., Berger, Rinaldi, Weisz, & Thompson, 1983; Deadwyler, Bunn, &

Hampson, 1996; H. Eichenbaum *et al.*, 1987). However, these studies contained a spatial component and therefore could not be ruled as strictly non-spatial events. There was evidence that he HP encodes events during discriminative avoidance learning and eye blink conditioning tasks which was a further indication that the HP is involved in non-spatial tasks (Gabriel & Saltwick, 1980; Weible, O'Reilly, Weiss, & Disterhoft 2006; Kang, Kubota, Proemba, & Gabriel, 1990). More recently, others have analyzed cells that respond to non-spatial components or events during learning of discrimination and context based tasks which have confirmed that cells in the HP encode more than spatial information (e.g., Komorowski *et al.*, 2009; D. M. Smith & Mizumori, 2006b; Wood, Dudchenko, & Eichenbaum, 1999). Interestingly, cells that were found to encode non-spatial events are found in the same population of neurons that exhibit spatially localized firing (place cells, Komorowski *et al.*, 2009; Wiener, Paul, & Eichenbaum, 1989a). Cells responding to specific events undergo similar changes as place cells in that they changed their firing properties (or remap) when there were contextual, behavioral or other task manipulations (Smith & Mizumori, 2006b). This suggests that similar to place cells, event cells alter their firing patterns following similar manipulations that lead to changes in place cell firing and arise from the same neuronal population.

One example of event cells occurring in a non-spatial task compared HP neurons in a place task (in which rats had to visit different corners of the apparatus to obtain a liquid reward) and then an odor discrimination task that took place in the same environment (Wiener *et al.*, 1989a). They found cells that fired in a location specific manner in the place task as well as cells representing specific events (i.e., goal approach cells, odor sampling cells) in the non-spatial odor discrimination task. Interestingly, when they compared the cells recorded in the spatial and non-spatial task they found that many of the cells with place fields in the place task also

responded to specific events when trained in the odor discrimination task (Wiener *et al.*, 1989a). The spatial specificity seen in the place task was no longer present in odor discrimination task as neurons that exhibited increased firing rates in response to spatial locations changed to fire in response to behavioral demands in the odor discrimination task. These responses to specific events clearly indicate the HP encodes information relevant to behavioral demands as opposed to involving a spatial component. This also suggests that the same cells are capable of representing locations or events depending on the demands of the task and further suggests that the term place cell may be an underrepresentation of what cells in the HP truly encode.

Since the initial finding of event cells, there have been multiple other studies indicating the HP encodes non-spatial information. One such study using an olfactory non-match to sample task found multiple types of events and task related responses (Wood *et al.*, 1999). Rats had to learn to dig in a cup of odorized sand when the scent did not match the odor sampled during the previous trial in order to obtain a reward. The study found cells that responded to position, specific odors, when approaching a cup and to the type of trial (match and non-match). Interestingly, there were also cells that responded to the conjunction of events, such as odor and trial type, position and trial type, position and odor and a few that responded when an odor was present at a specific location dependent upon the trial type (match and non-match, Wood *et al.*, 1999). The results indicate that the HP encodes individual task relevant events as well as the conjunction of those events. The conjunctions included location sometimes, but at other times they strictly represented events, such as an odor and trial type (matching versus non-matching). The results of this study and Wiener et al. (1989) suggest that cells in the HP encode the relevant information of the task and indicate that the same cells are responsible for encoding both spatial and non-spatial information.

After it was established that cells in the HP also represent task relevant events, Komorowski et al. (2009) trained rats in a contextually-cued odor discrimination task to evaluate how event cells behave following contextual manipulations. The task took place in two distinct contexts connected by a tunnel and rats would alternate between the two contexts. The same two odors (X,Y) were present in both contexts but in one context one odor was rewarded (X+,Y-) but not rewarded in the alternate context (X-,Y+). They were given a 15 s exploration window before being presented with the samples and also spent at least two trials in each context before switching to ensure they were not simply alternating between the odor choices. They found cells in the HP that initially responded to specific locations (locations were static) and odors, but as the rat learned the task the cells began to incorporate a specific odor in conjunction with the location. These conjunctive cells (as well as cells responding to location and odor only) exhibited different firing patterns depending on the context they were currently performing in (Komorowski *et al.*, 2009). For example, in context A a given cell would fire at one cup when that cup was rewarded, but would exhibit location specific firing only in context B. This indicates that the conjunctive information takes time to develop and occurs as the rats are learning the task, whereas representations of odors and locations develop rapidly. Interestingly, not only did the cells remap between contexts (cells exhibit different firing patterns), but they also found cells that would remap within the same context depending on the position of the two odors. For instance, one cell would fire in context A only when odor X was in the left corner, never when it is in the right corner. It is well known that when cells respond to locations they remain relatively stable in the same environment. However the results of Komorowski et al. indicate that event cells exhibit greater specificity for task relevant events regardless of the environmental context.

Smith and Mizumori (2006) evaluated how cells change following changes in the behavioral demands of the task. They also found that event responses are sensitive to the behavioral demands and not strictly bound to the environmental cues and layout (Gill, Mizumori, & Smith, 2011; Smith & Mizumori, 2006b). In this task rats were trained on a plus maze to approach the end of one specific arm for a reward (east arm) on one half of the trials and then had to switch and approach the opposite arm for a reward (west arm) with the only cue a short delay. They found several different types of event responses, and similar to place cells that change their firing patterns following environmental changes, these cells changed their firing pattern based on changes in the behavioral demands (the change from go east to go west). The cues around the plus maze remained constant throughout the experiment suggesting that these cells were using the behavioral response to distinguish between go east and go west trials. They found several types of event responses such as reward cells that showed a peak in firing rate when they reached the reward location, indicating the HP encodes the receipt of the reward and the location as well as signaling the end of a trial. There were also cells that responded to the start of a trial when the rat was placed on a maze. Rats started the task on random arms, so this type of response could prime the route needed to obtain the reward based on where the animals start position on the maze. There were also cells that fired during the inter-trial interval (ITI), and these events may be related to encoding the previous trial or priming the HP for the upcoming trial location. Although the task requires some navigation on the maze, the important information is which reward arm to visit and rats had to learn to use a win-stay strategy (they had to return to the same reward location as the previous trial) in order to obtain a reward.

Interestingly, the experiment also led to the finding of time cells during the ITI period of this task. Time cells were groups of cells in the HP that fire in the same sequence between the

passing of each ITI period in a reliable and consistent manner. Similar to place cells that fire differently to represent different environments, these time cells would change their preferred firing rate in order to differentiate east and west trials (Gill *et al.*, 2011). These time cells developed on the first day of training and persisted throughout learning. The results were interpreted as a means for the HP to maintain a distinct representation of the behavioral demands necessary for obtaining the reward on the upcoming trials. These time cells may have provided a means for maintaining the proper contextual representation based on the behavioral demands, go east or west. This suggests that cells in the HP known to encode spatial and event information also encode information about time and upcoming events (Gill *et al.*, 2011). The results further support the idea that event cells as well as time cells can change their response patterns or remap following behavioral changes just as place cells change their preferred firing location in response to environmental changes.

Time cells in the HP are also found in a delayed match to sample task have also been found to encode successive moments during the delay period of other tasks as well, suggesting time is a critical memorycomponent encoded by the HP (MacDonald, Carrow, Place, & Eichenbaum, 2013; MacDonald, Lepage, Eden, & Eichenbaum, 2011). For instance, in the delayed match to sample task, neurons in the HP fire sequentially between initial and subsequent sampling such that the entire delay period was encoded between individual trials (MacDonald *et al.*, 2011). Similar to cells responding to a location that alter their firing patterns following a change in the environment, when these cells encode time they also remap (or retime) when the delay period is altered. Additionally, cells responding to time fire during similar tasks when rat's heads were fixed eliminating any movement (MacDonald *et al.*, 2013). Unlike cells that encode a location that requires self generated movement in order to fire, time cells will develop and fill

the gap when the rat is fixed in one location. The results also showed that different populations of time cells were active following different odor sampling sequences depending on the odor. This suggests that different memories are encoded during the delay period and that time cells help to maintain distinct memories for odors when multiple odors are presented (MacDonald *et al.*, 2013). It also suggests that in addition to encoding spatial locations and behaviorally relevant events, cells in the HP also encode information about time when it is an important feature of a task.

Although the HP has long been characterized as having a role in spatial navigation, the fact that the HP also encodes information about events, time and behavioral demands suggests that the HP combines all relevant information for a given situation (e.g., H. Eichenbaum & Cohen, 2014; Smith & Mizumori, 2006a). Similar to findings in place cell studies that show cells remap following environmental changes, cells that encode events and time also change their firing patterns following changes in behavioral demands or delay periods respectively (MacDonald *et al.*, 2011; Smith & Mizumori, 2006b). The cells that encode location, events and time arise from the same population of HP neurons and these neurons have the potential to encode any of these features when the task makes that information relevant.

Few studies have examined how event responses develop with learning (Komorowski *et al.*, 2009; Smith & Mizumori, 2006b; Tort, Komorowski, Kopell, & Eichenbaum, 2011) and none have examined event responses during concurrent odor discrimination learning. The goal of the current study is to document the development of event responses during a concurrent odor discrimination task and to determine whether HP representations change over the course of training. Therefore there are three potential outcomes that could arise while evaluating the event responses during this task: 1) event responses could develop quickly and stabilize on the first day

of learning, similar to place cells (e.g., Frank *et al.*, 2004; Muller, Kubie, & Ranck, 1987; Thompson & Best, 1990), 2) event responses could develop slowly, as subjects learn the task (Gill *et al.*, 2011; Komorowski *et al.*, 2009; Smith & Mizumori, 2006b) or 3) event responses could decrease with time as a means of sharpening the representation (e.g., Guzowski, Setlow, Wagner, & McGaugh, 2001).

METHODS

Subjects, Surgical Procedures, and Microdrives

The subjects were 8 adult male Long Evans rats (Charles River Laboratories, Wilmington, MA) weighing 300–350 g at the time of surgery. Rats were implanted with microdrives containing 24 independently moveable tetrodes aimed bilaterally at the pyramidal cell layer of dorsal hippocampal CA1 region (anterior-posterior [AP] = 4.0 mm; medial-lateral [ML] = ± 2.5 mm, Paxinos & Watson, 1998). Each tetrode consisted of four strands of 12.7 mm platinum/iridium (90/10%) wire (California Fine Wire Company, Grover Beach, CA) platinum-plated to reduce impedance to between 120 and 200 kOhms at 1 kHz. At the end of surgery, each tetrode was lowered 1 mm into the tissue. Following surgery the rats were given an antibiotic (5 mg/kg Baytril) and an analgesic (5 mg/kg ketoprofen). All procedures complied with guidelines established by the Cornell University Animal Care and Use Committee. Rats were allowed at least one week recovery before training resumed and the tetrodes were lowered into the CA1 layer using the amplitude and phase of theta waves, the amplitude and sign of sharp-wave events, and the presence of theta modulated complex spiking cells (Paxinos & Watson, 1998). After recovery from surgery and lowering of the tetrodes, the rats were placed on a restricted feeding regimen (80–85% of free feeding weight) and they began training.

Apparatus and General Training Procedures

The procedure made use of a well-known digging task used to study olfactory memory (Eichenbaum, 1998), in which rats are trained to dig in cups of odorized bedding material to retrieve buried food rewards (45 mg sucrose pellets, Bioserve, Frenchtown, NJ). The details of the apparatus, stimuli, and training procedures have been published previously (Butterly *et al.*, 2012). Briefly, the rats were trained in two wooden chambers (60cm x 45cm) with a removable divider which separated the odor presentation area from an area where the rats waited during the ITI period. Odor cues were presented in ceramic dessert cups (8.25 cm in diameter, 4.5 cm deep) which fit into circular cutouts cemented to the floor of the chamber to discourage the rats from moving the cups or tipping them over. Sixteen pure odorants served as cues. The amount of each odorant was calculated so that they produced an equivalent vapor phase partial pressure when mixed with 50 ml of mineral oil (Cleland *et al.*, 2002) and 10 ml of the resulting odorant solution was then mixed with 2 L of corncob bedding material and stored in airtight containers.

Prior to training, the rats were given two 15-min sessions of acclimation to the chamber. The rats were then shaped to dig in cups of bedding for a sucrose reward. After the rats had learned to reliably retrieve the rewards, they began training on the list of odor discrimination problems. The list contained eight odor pairs (16 different odors) and within each pair, one odor was always rewarded and the other was not. The predictive value of the odors (rewarded or non-rewarded) was counterbalanced across subjects and their locations (left or right side of the chamber) were randomized. The daily training sessions consisted of 64 trials (eight trials with each odor pair, presented in an unpredictable sequence). At the start of each trial, the experimenter placed the two cups containing the odorized bedding into the chamber and removed the divider so the rat could approach the cups and dig until he retrieved the reward. A digging response was recorded if the rat displaced any of the bedding, except for incidental

displacement (e.g., stepping into the cup while walking over it). After consuming the reward, the rat was returned to the waiting area for an ITI of ~15 s while the experimenter prepared the cups for the next trial. The rats were given daily training sessions until they reached a behavioral criterion of 90% correct choices on two consecutive sessions. After reaching criterion the rats were then trained on a second list as described in Bulkin, Law and Smith (Appendix).

Data Collection

Neuronal spike and video data were collected throughout the task with Cheetah Data Acquisition System (Neuralynx, Bozeman, MT). Signal from the electrodes were amplified 2,000-10,000 times, filtered at 600 Hz and 6 kHz, and digitized. All waveforms exceeding a user-defined threshold were stored along with their time of occurrence offline analysis. Standard spick sorting techniques were used to distinguish multiple unit records into single units (Spikesort, Neuralynx). Waveform features used for sorting included spike amplitude, spike width, waveform principle components, and waveform area. The rat's position was monitored by digitized video (sampled at 30 Hz) of an LED array attached to the rats head. Video data were also used to establish the start and end of trials, odor sampling, digging start and stop times, errors, and the receipt of rewards for each trial.

Data Analysis

All analyses were performed using custom software written in the numerical computing environment Matlab (Mathworks, Natick MA). Analyses were restricted to cells with an average firing rate of less than 5 spikes per second across the session (i.e., discarding any putative interneurons). The chamber the experiment took place in was divided into four areas; the ITI area, the area where the training took place (these two areas were separated by a divider) and the two cups where odor sampling took place.

Unit changes in event related activity were assessed by constructing peri-event time histograms (PETHs) which were calculated by binning the data in 100 ms bins centered on three events during the trial: the start and end of the trial (defined by the position of the barrier which separated the ITI side from the odor sampling side) and odor sampling (defined by the moment the rat's nose broke the plane of the cup). Odor sampling event times were identified by manual flagging (creating time stamps) of raw video data sampled at 30 Hz. Trials lasting longer than 30 seconds were discarded from all event related analysis, however, performance was still included for these trials (these trials always contained errors, and the errors were always made within the first 30 seconds of the trial). In order to identify cells with significant event responses, T-tests were used to compare the firing rate of the neuron before and after the event time stamp. All significant events were visually inspected and any significant event cells that had a firing rate of less than one spike per second or fired less than 50 spikes in either time window of the PETH were discarded. The event time windows compared 1s prior to the event to the 1s after the event except for the trial-start responses which used a 500ms windows pre and post event stamp.

Because a several important task events took place in very close temporal proximity, most responses could not be classified using a single simple analysis. Instead, a defined sequence of decisions was used to assess potential event responses of each cell to ensure there were no overlapping responses and to catch all responses the cell exhibited. It was not possible to flag reward responses as I could not see the exact moment when the rat received the reward, therefore reward responses were not analyzed. The rules for each event are as follows:

Trial-start Events: The divider served as the marker for trial-start responses and the sampling period for this event was a 1000 ms time window (500 ms on either side of the time stamp). Once the divider had been removed and the rat crossed the divider line a start response

was flagged. If there was a significant increase in the firing rate after the time stamp, the cell was classified as a trial-start response (Figure 2D). If there was a significant increase in firing rate prior to the time stamp, the time window was shifted -1000 ms and another T test was run. Therefore, a decrease in firing rate had to have two significant T-tests to be classified as a trial-start response. Offsetting the time window for the T-test served to rule out any cells that simply fired continuously during the ITI period but stopped firing when the trial started, which would otherwise be erroneously detected as an "off response" to the trial-start. Examples of trial start event responses can be seen in Figure 2A. There was a tendency for there to be a peak in firing rate centered directly on the event which resulted in a non-significant result that was clearly present (Figure 2B). Therefore, any cell that had a non-significant trial-start response was offset -250 ms and another t-test was performed to reassess the trial-start response. If this resulted in a significant result the cell was classified as having a trial-start response (Figure 2C).

Cup Approach and Odor Sampling Events: For sampling events, the event flag was time stamped when the rat's nose arrived over the cup containing the digging medium and consisted of a 2000 ms time window (1000 ms on both sides of the time stamp). A significant decrease in firing rate prior to the timestamp was classified as a cup approach response (i.e. the neuron fired as the rat approached the cup and stopped firing when he arrived at the cup). A significant increase in firing rate following the time stamp were classified as a general odor sampling event (Figure 4). There were three types of cup approach responses that were considered for our analysis, a general cup approach, first cup approach and last cup approach. A first cup approach was only considered if there was not a trial-start response. The last cup approach only analyzed the last sampling event excluding those cases where the first cup was also the last cup sampled.

The general cup approach analyzed all sampling events on a given day. For examples of the different cup approach responses, see Figure 3.

Trial-end Events: The ITI side served as the marker for trial-end responses (time stamped when the rat crossed over to the ITI side) and the sampling period for this event was a 2000 ms time window (1000 ms on either side of the time stamp). A significant increase in firing rate before the time stamp resulted in a trial-end event response (Figure 5A). If the cell significantly increased its firing rate after the time stamp, it was then subjected to a second t-test with an offset of +1000 ms (instead of -1000 ms) and had to have a second significant t-test result to ensure a cell did not simply fire throughout the ITI. Figure 5d shows examples of trial-end event responses with an increased firing rate after the time stamp. Similar to the trial-start responses, trial-end responses also tended to peak at the time stamp (Figure 5B). Therefore any non-significant t-test was then tested again with the time stamp shifted -500 ms. If this produced a significant result it was classified as having a significant trial-end response (Figure 5C).

Individual Odor Responses: To determine whether cells responded to specific odors as opposed to simply having a generalized response to odors, all the trials for each specific odor were also subjected to a t-test similar. The odor sampling time stamp was used for this analysis and a t-test was performed for each of the 16 odors individually determine whether there was a significant increase in firing rate following the odor sample timestamp. Each cell has the potential to respond to no odors, one odor or multiple odors.

Analysis

After classifying the event responses as described above, the change in these event responses was analyzed across days of learning. It is generally difficult to record the same neurons across multiple days; therefore not all rats had data on every day of training. A list of

how many cells contributed to the analysis by each rat is shown in Table 1. To analyze the change in event responses, the proportion of the recorded population that exhibited various kinds of event responses was analyzed using chi squared analyses.

Histology

Following the final day of recording, rats were deeply anesthetized and transcardially perfused. The brains were removed, cryoprotected, and sectioned into 40-µm coronal slices. The sections were stained with cresyl violet in order to verify the placement of electrodes were in the CA1 region of the HP. Each section of the HP with tetrode tracks was collected for analysis and all of the tetrode wires were by following small angular deviations from the tetrodes across multiple sections until the track ended. The end location was determined as the placement of a given tetrode. Recordings from a tetrode were included in the analyses if the deepest position of the tetrode was in or just below the CA1 region.

RESULTS

A total of 763 neurons were recorded over 4-5 days of training on a list of odor discrimination problems. Interneurons were excluded from the analysis leaving a total of 668 neurons that were evaluated. As previously described, HP pyramidal neurons responded to several task relevant events and those that did not have event responses may have had place fields or responses to events that were not classified. Those events and the development of those events are classified below. The numbers and percentages of neurons that exhibited various kinds of responses are listed in Table 2.

Behavior

All of the rats were trained until they reached the behavioral criterion (at least 90% correct for two consecutive days) on the discrimination problems. The rats took an average of 4.24 ± 0.11 (Mean ± SEM) training sessions to reach the criterion. A one way ANOVA revealed

RAT ID	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	TOTAL
1714	5	12	17	41	N/A	75
1747	30	29	35	25	N/A	119
1716	59	54	40	56	N/A	209
1762	24	22	28	30	36	140
1755	15	15	X	18	32	80
1678	5	X	X	19	43	67
1680	7	X	X	9	23	39
1638	X	X	X	15	N/A	15
TOTAL	145	132	120	213	134	744

TABLE 1. This table includes all of the rats in the study and the number of neurons they contributed on each day of training as well as the total number of cells contributed. X indicates a day of training where there were not enough neurons to record the session. N/A indicates the animal had already reached criterion and data was not collected for this experiment on that day.

Event Response	Trial-start	Trial Stop	First Cup Approach	Last Cup Approach	Gen. Cup Approach	Gen. Odor Response	Specific Odor Response	Multiple Events	None
Cell #	206	321	5	78	78	67	95	275	213
(%)	(30.8)	(48.1)	(0.7)	(11.7)	(11.7)	(10.0)	(14.2)	(41.1)	(31.8)

TABLE 2. This table contains the total number of cells that exhibited a specific event response or had multiple event responses along with the percent of cells that exhibited a response. The data includes all days of training and is the percent out of 668 total pyramidal neurons. Note that many neurons responded to more than one event, so the percentages do not sum to 100%.

that rats learned the task over the course of training and showed a significant increase in performance ($F[4,33] = 65.31$, $p < 0.01$, Figure 1).

Kinds of Event Responses

Trial-start Events. There were several different types of trial-start event responses. The first type occurred when a cell had an increase in firing rate that was time locked just before the trial-start time stamp (Figure 2A). Although the divider had not yet been lifted for the official start of the trial, the rats consistently received anticipatory cues that the trial was about to start. This included the placement of the two cups containing odorized bedding into the box on each trial as well as the investigator moving their hand and grasping the divider to raise it and initiate a trial. I suspect that in this case, this response acted as an anticipatory trial-start response. The second type observed had an increase in firing rate time locked directly at the time stamp (Figure 2B). These types of responses tended to be sharp in their response at the moment that a trial is initiated. This type of response suggests these cells may correspond to the raising of the divider and entering the behavioral section of the box. The third type of trial-start response occurred as an increase in firing after the start time stamp (Figure 2D). This type of response may be responding to when the rats observe the two cups after the divider had been raised and they know they have to make a decision. A trial-start event always signaled the start of a trial and may prime the memory system for the sequence of events that are about to occur.

Cup Approach events. There were three potential types of cup approach events: first cup, last cup (excluding those cases where the first cup was also the last cup sampled) and general cup approach (first and last) responses. First cup approach event responses were the least likely of the three and there were only a total of 5 first cup approach cells on all days of learning and therefore will not be addressed further (for examples, see Figure 3A). This is most likely due to

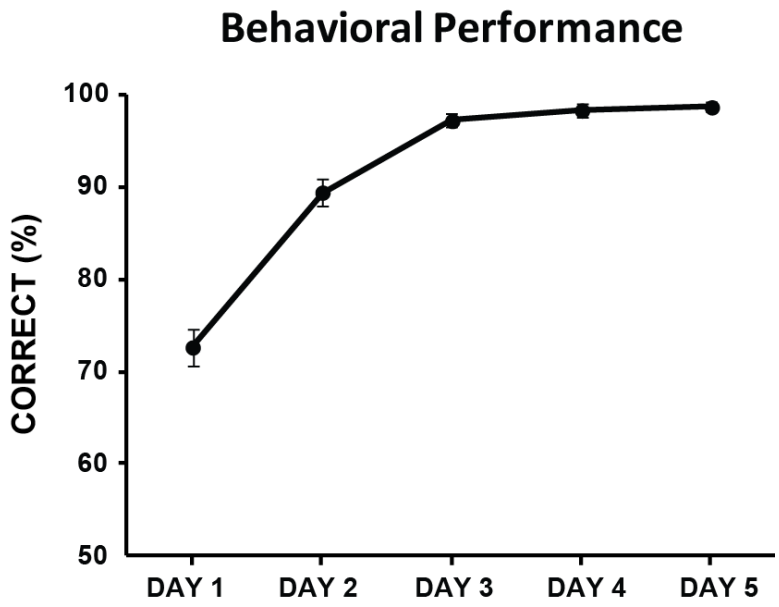

Figure 1. Change in behavioral performance during the concurrent odor discrimination task across days of learning.

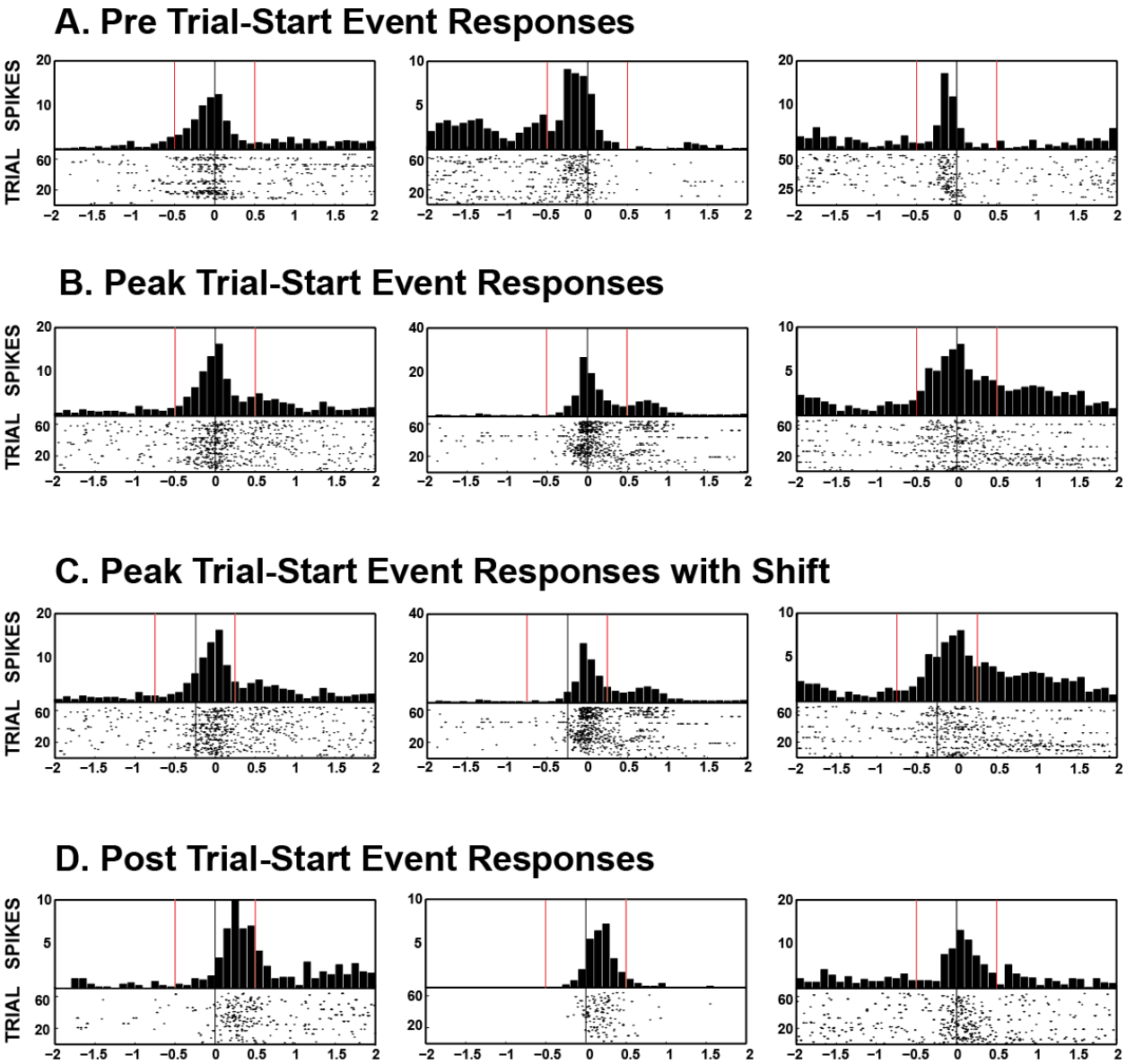

Figure 2. Perievent time histograms illustrating trial-start related firing of individual neurons recorded during learning. For each plot, the firing was summed across the 64 trials on a given day of training, with raster displays illustrating the trial-by-trial neuronal firing (1 row of tick marks per trial). Four seconds of data are shown, from 2 s before to 2 s after the event. To focus the analysis on firing that was time locked to the event, the analyses were restricted to 500 ms before and after the event (outlined by red lines). Examples of pyramidal neuron event responses at the time each trial started. A) Examples of significant pre trial-start responses. Note that the rats could anticipate the start of the trials because they could hear the cup being placed in the apparatus and see the experimenter preparing to remove the divider (see Results). B) Examples of peak trial-start responses that did not come out as significant. C) The same cell examples of peak trial-start responses, the same as those in B, marked with the -250 ms time shift which led to a significant trial-start response. D) Examples of significant post trial-start responses.

the trial-start response occurring close in time to the first cup approach response and there could not be a first cup approach if there was a trials start response that overlapped (all plots were visually inspected for accuracy). Additionally, there was a tendency for first cup approach responses to also have last cup responses and were therefore classified as a general cup approach responses. Last cup approach responses were much more likely to occur (for examples, see Figure 3B). The last cup sampled was always a rewarded cup and may indicate that as they approached the last cup (after initial learning) rats knew a reward would be present. There were also cells that had a general cup approach event responses for which cells increased their firing rates in response to approaching all of the cups (for examples, see Figure 3C). General cup approach responses occurred just prior to odor sampling events and therefore may act as a priming mechanism to the rest of the brain that a decision needs to be made upon arrival to dig or not to dig.

Odor Sampling Events. The general odor sampling events are different from cells that responded to specific odors. A general odor sampling event required there to be an increase in firing rate following the sampling event regardless of the specific odor being sampled. Therefore it did not take into consideration specific odor responses. There were a host of cases that showed general odor sampling events that were both non-selective to individual odors (for examples, see Figure 4A) and those that did respond to specific odors (for examples, see Figure 4B-D). Cells with a general odor sampling event response are responding to the sampling event itself possibly to guide their decision to dig or not to dig. Some of these cells not only had a general odor response to sampling event but also to specific odors. Additionally, there were cells that also had specific odor responses that did not have a general odor response. While these were few in

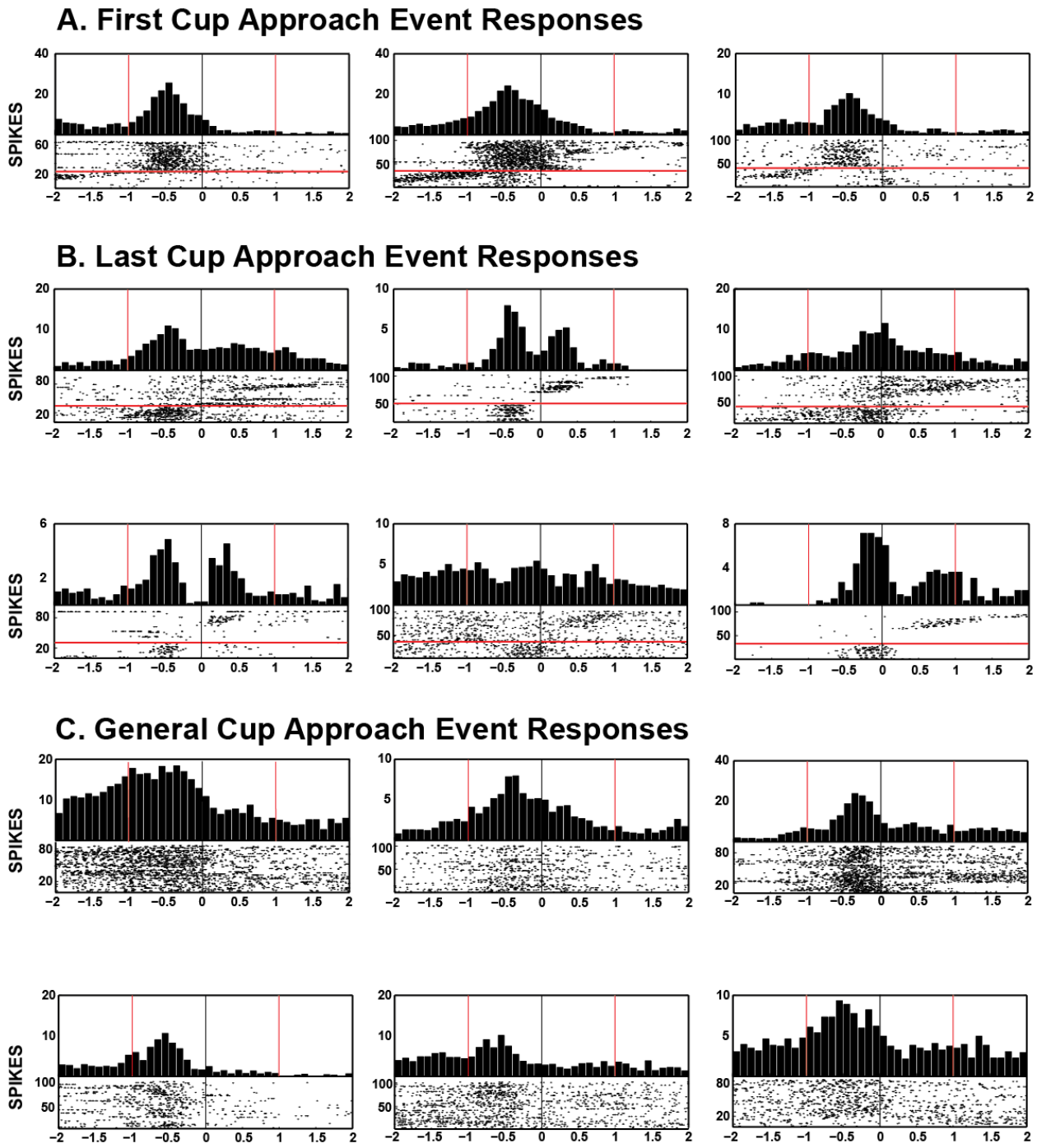

Figure 3. Plot described in Figure 2. Examples of pyramidal neuron event responses at the time an odor was sampled. Rasters are sorted to separate approaches to the first and last cups (excluding those cases where the first cup was also the last cup sampled). The horizontal red line in A and B separates firing around the first cup a rat sampled on a trial (above the line) and the last cup sampled on a trial (below the line). A) Examples of cells that responded preferentially when the rat approached the first cup. Note the greater firing above the red line. B) Examples of cells that responded preferentially when the rat approached the last of multiple cups. Note the

greater firing below the red line. C) Examples of cells that responded when the rat approached any cup.

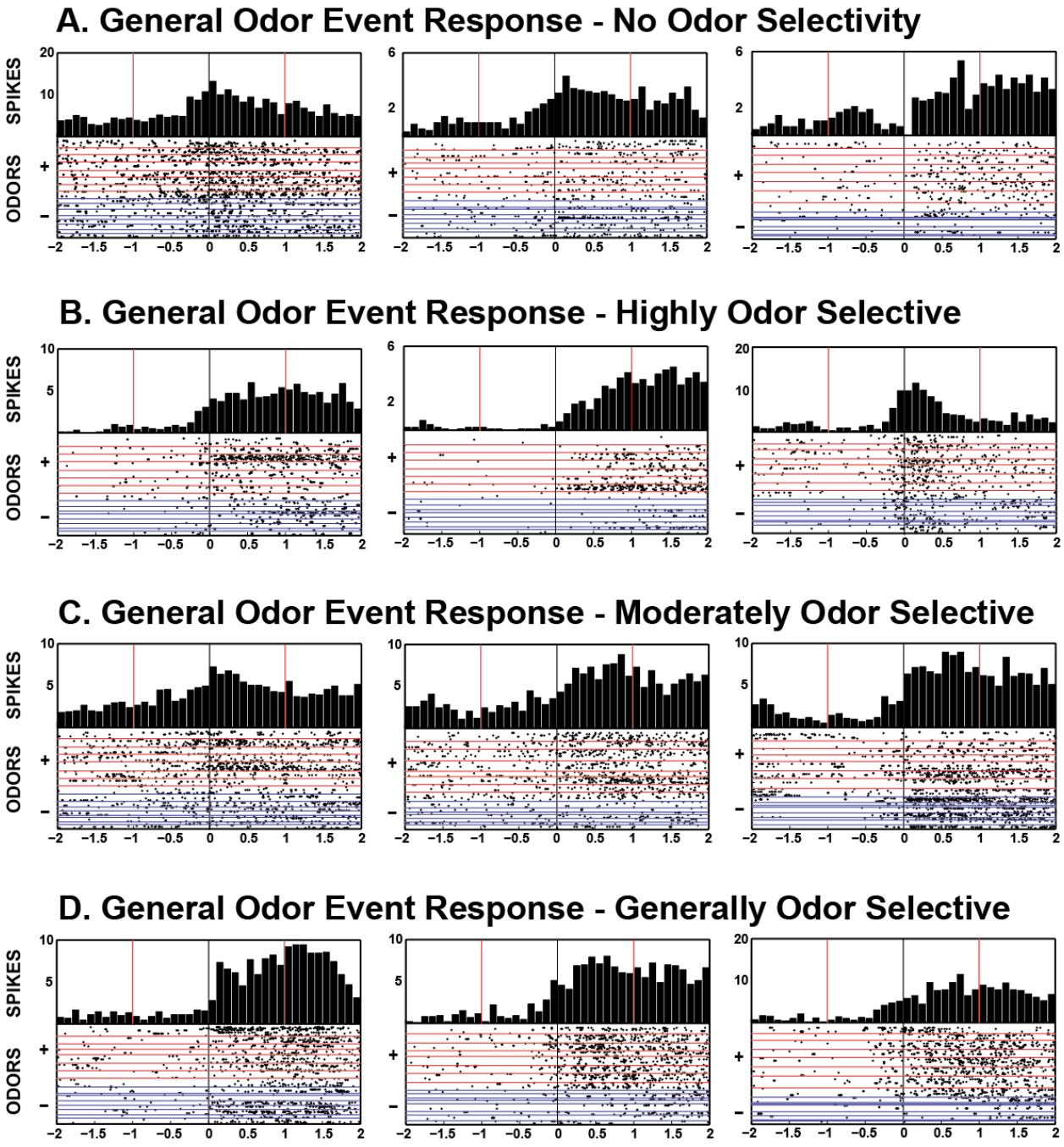

Figure 4. Plot described in Figure 2. For each plot, the firing was summed across the number of sampling events on a given day of training, with raster displays illustrating the neuronal firing for individual odors that were sampled (1 row of tick marks per trial). The red bars indicate sampled odors that were rewarded (+) while the blue bars indicate sampled odors that were not rewarded (-). Examples of pyramidal neuron event responses at the time an odor was sampled. A)

Examples of cells that had a general odor response but did not exhibit a response to specific odors. B) Examples of cells that had a general odor response and were highly selective to specific odors. The first two plots are examples of cells that had significant responses to one odor while the third plot is an example of a cell that had a significant response to two odors. C) Examples of cells that had a general odor response and were moderately selective to specific odors. The three plots are examples of cells that had a general odor response as well as to 3, 4 and 5 specific odors respectively. D) Examples of cells that had a general odor response and were generally selective to specific odors. The three plots are examples of cells that responded to odors generally as well as to 6, 7 and 8 specific odors respectively.

number, it may suggest that while general and specific odor responses can co-occur they are not dependent upon each other.

The cells that increased their firing rate when the rat sampled specific odors varied in the number of odors they responded to ranging from a single odor up to 8 specific odors. I classified the types of odor responding cells as highly selective (responding to 1 or 2 odors, Figure 4B), moderately selective (responding to 3-5 odors, Figure 4C) and generally responsive (responding to 6-8 odors, Figure 4D). However, the most common type were cells that exhibited highly selective odor responses, which on the day with the lowest proportion of this type of response still made up at least 65% of cells with an odor response. There were 3 cells with the broadest response to odors (8 odors) and these cells did not appear until the third (1) and fourth (2) days of learning. Cells that responded to specific odors primarily responded to the rewarded odors indicating that the HP may encode the most relevant odors relating to a reward rather than the non-rewarded odors which signal to withhold a dig response. While cells responding to different odors is a replication of several other studies (Komorowski *et al.*, 2009; Wood *et al.*, 1999), this is the first time they have been described in a concurrent odor discrimination task.

Trial-end Events. Trial-end responses were the most numerous of all the event responses recorded (for examples, see Figure 5). As with the trial-start responses, there were also three types of trial-end responses. One type of response exhibited an increase in firing rate just prior to the end time stamp and may be related to the reward since the rats receive the reward just prior to the end of a trial (Figure 5A). Another type of trial-end response occurred as an increase in firing rate directly at the end time stamp (Figure 5B). The final type of response occurred as an increase in firing rate just following the trial-end time stamp (Figure 5D). In general a trial-end response may serve inform the memory circuit that the trial has ended and one interpretation of

these responses is that the preceding events need to be bound together as a single trial representation (or sequence of events). Along these lines, perhaps the trial-end response that occurs after the timestamp is sending a signal to prime the HP for the next trial.

Transient Event Responses. There were also other event responses that I observed which did not occur as frequently as those just described. The first were event responses that I found tended to either start responding to a particular event as the trials progressed and was not present during the first several trials or was present from the beginning of the session but then stopped responding to that event. The firing at the time of these event responses was significantly different for the first and second half of the session. For example, there were cells that responded to the start of a trial, the approach of a cup, sampling of an odor and end of trial that either increased or decreased their responding during a session (for example, see Figure 6A & B). However, I could not distinguish any sort of development as learning progressed or change in proportion of these types of cells across days. In addition to these half responses, there were a few cells that had multiple event responses which would switch what event they respond to during a session of training. For example, Figure 6C shows a cell that has a trial-end response that started out strong at the beginning of the training session but lost its trial-end response later in the session. As the trial-end event decreases in responding, the cell began to respond to the first cup approach towards the end of the session. Figure 6D shows the opposite, a cup approach response that became a trial-end response. While there weren't a lot of these types of cells, they tended to occur mostly on Day 1 which also had the largest number of cells with multiple event responses.

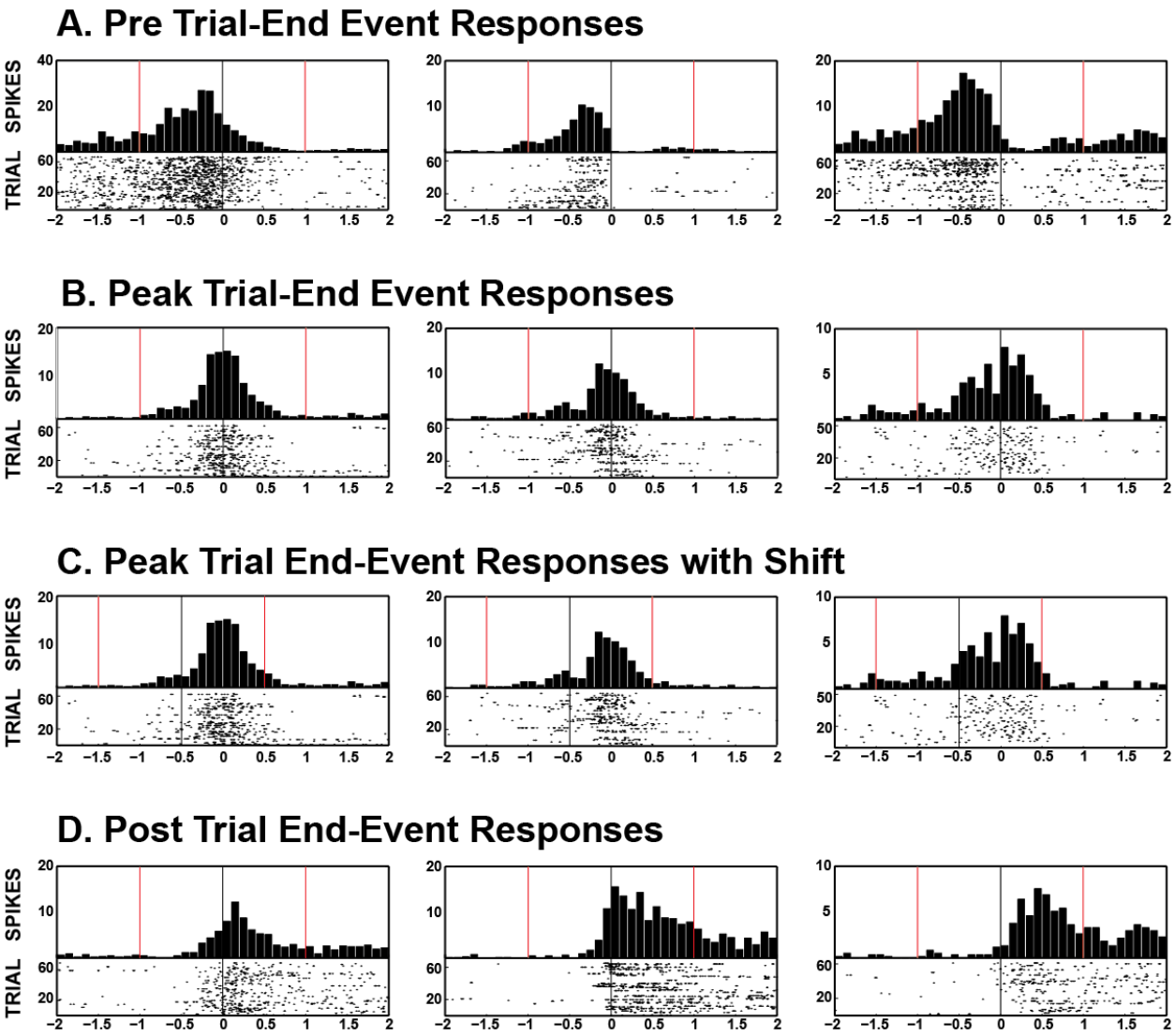

Figure 5. Plot described in Figure 2. Examples of pyramidal neuron event responses at the time each trial-ended. A) Examples of significant pre trial-end responses. B) Examples of peak trial-end responses that did not come out as significant. C) The same cell examples of peak trial-end responses, the same as those in B, marked with the +1000 ms time shift which led to a significant trial-end response. D) Examples of significant post trial-end responses.

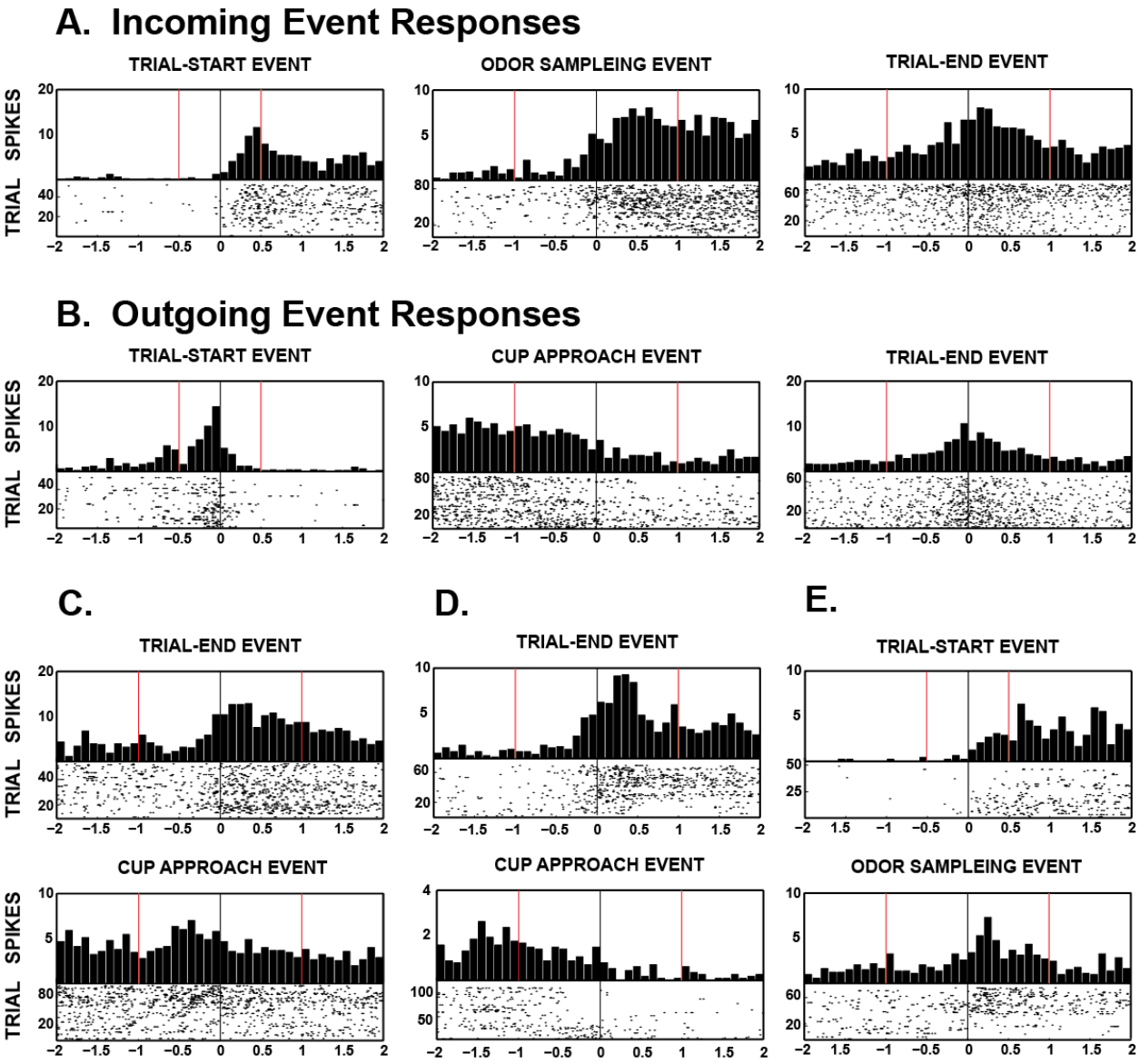

Figure 6. Plot described in Figure 2. A) Event responses that were not present in the early trials (bottom rows of the raster) but start to respond as the trials progressed (top rows of the raster). B) Event responses that were present in the early trials and ceased responding as the trials progress. C) A trial-end event response that started out strong at the beginning of the training session but decreased in responding as the trials progressed (top plot) and start to fire in response to the first cup approach towards the end of the session (bottom plot). D) Example of the opposite of C, a cup approach response (bottom) that became a trial-end response (top). E) Example of a cell that started out responding to the start (top) at the beginning of training and then started responding to general odor sampling (bottom) events later in learning.

Differential Sampling Responses. The position of the rewarded cup was randomized, so the rats approached the rewarded odor first on about half the trials just by chance. Since the rat obtained the reward at that cup, the second cup was never investigated and there was only a single sampling event for those trials. However, on many trials the rats sampled more than one odor and I classified the last cup as the last sampling event (excluding those cases where the first cup was also the last cup sampled). Therefore we performed a t-test on the odor sampling event comparing the firing rate of cells after the first cup when that cup is rewarded and the firing rate after the last of multiple. This produced a comparison of rewarded odor sampling events. There were a number of cells that increased their firing rate in response to the first odor sample when it was the rewarded one or cells that increased their firing rate when they reached the last cup, but responded to these two trial types differently. These cells appear to encode different types of information; they encode the conjunction of odor and reward as well as when the odor was presented, first or last (see Figure 7). There were a few interesting cases late in training where cells would increase their firing rate for the first or last rewarded sample only, but only to one specific odor (Figure 7B). The specificity of these responses later in learning may suggest early in learning, cells are merely responding to rewarded odors generally but as they learn the task they increase their specificity and respond to only one odor rather than several. There were not many cells of this type so I could not quantify any of the responses or their development during learning.

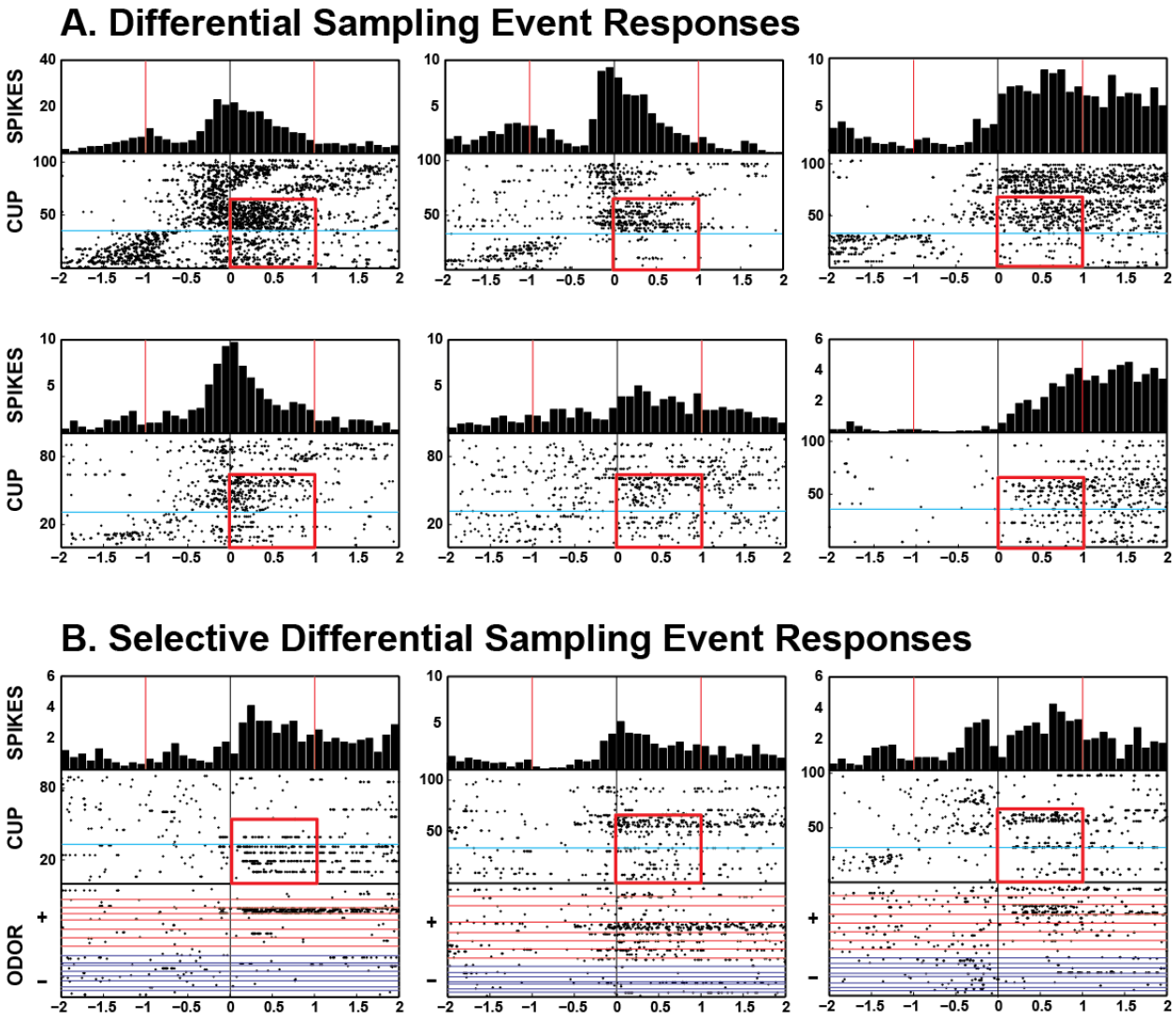

Figure 7. The perievent histograms are the same as those described in previous plots. In the middle raster plot the samples are sorted so that the blue line separates the first cup arrival (above) and the arrival at the last cup (excluding those cases where the first cup was also the last cup sampled, below). The raster tics in the red box above the blue line indicated trials where the reward was obtained on the first sample, and below the blue line are the last cup visited. A) Examples of six cells that responded to odor sampling events significantly more when sampling the first cup (red arrow) than when sampling the last cup (blue arrow). B) In these plots there is an additional raster that separates each odor that was sampled as well as the rewarded odors (within the red lines) and unrewarded odors (within the blue lines). Examples of three cells that responded to odor sampling events differentially when comparing the first cup odor sampling event when it was rewarded to the last cup sample of multiple samples. Additionally, these differential odor sampling responses all responded significantly to one odor. The first example shows a cell that fired for one specific odor but only if it was encountered after a previous sample (blue arrow). The other two examples are cells that fired for one specific odor only if it was the first odor encountered.

Changes in Event Responses

Trial-start Responses. There were 206 cells that had a trial-start event response over the 5 days of learning. The development of trial-start event responses was analyzed across days to determine whether there were significant changes in the expression of start responses. A chi squared analysis was run comparing the proportion of trial-start responses over days of learning. The results revealed a significant decrease in trial-start responses as learning progressed ($\chi^2(4) = 10.38$, $p < 0.05$, Figure 8). This indicates there were fewer cells responding to the start of a trial as learning progressed.

Cup Approach Responses. First cup approach responses did not occur enough to analyze changes as learning progressed. Last cup approach responses were much more likely to occur, and there were a total of 78 cells that responded to this event type. A chi squared analysis on the development of last cup approach event responses showed no significant change ($\chi^2(4) = 3.66$, $p = 0.45$, Figure 9). General cup event responses had a total of 78 cells that responded to this type of event. A chi square analysis on the development of general cup approach responses revealed a significant decrease in the proportion of responses across days ($\chi^2(4) = 15.87$, $p < 0.01$, Figure 10). This indicates there were fewer cells responding to approaching a cup as learning progressed.

Odor Sampling Responses. There were a total of 67 cells that exhibited general odor sampling responses across the 5 days of learning. A chi squared analysis on the development of general odor sampling event responses revealed a significant decrease in the proportion of cells responding to this even ($\chi^2(4) = 12.45$, $p < 0.05$, Figure 11). This indicates there were fewer cells that responded generally to odor sampling events as learning progressed.

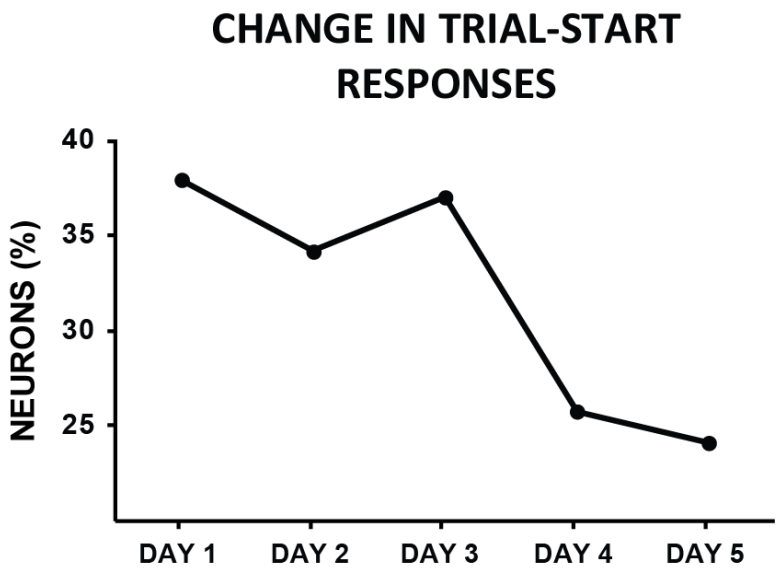

Figure 8. The plot represents the significant change in the percentage of neurons that had trial-start event responses over the five days of training.

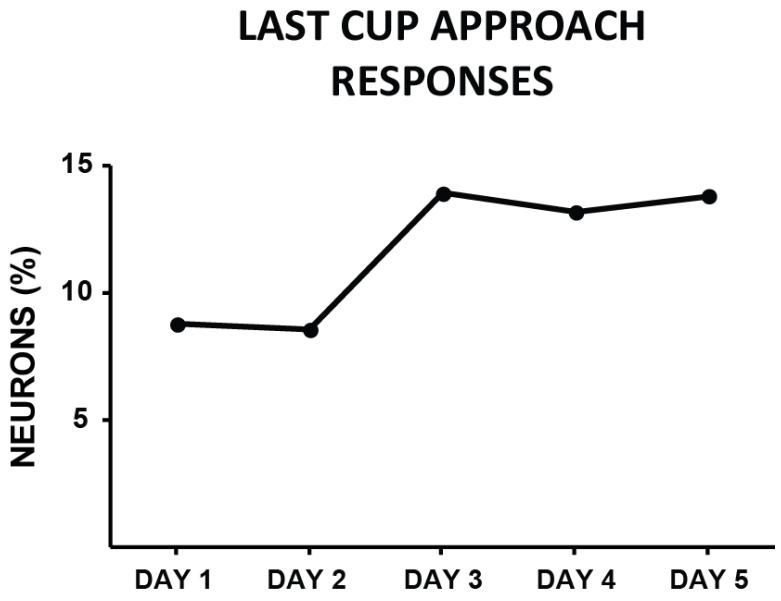

Figure 9. The plot represents the percentage of neurons that had last cup approach event responses over the five days of training (excluding those cases where the first cup was also the last cup sampled). This percentage did not change significantly across the training days (see text).

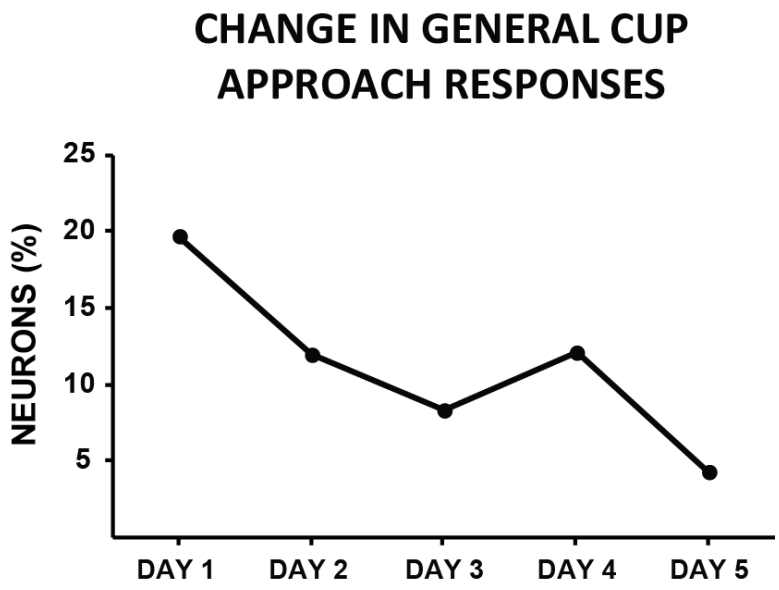

Figure 10. The plot represents the significant change in the percentage of neurons that had general cup approach event responses over the five days of training.

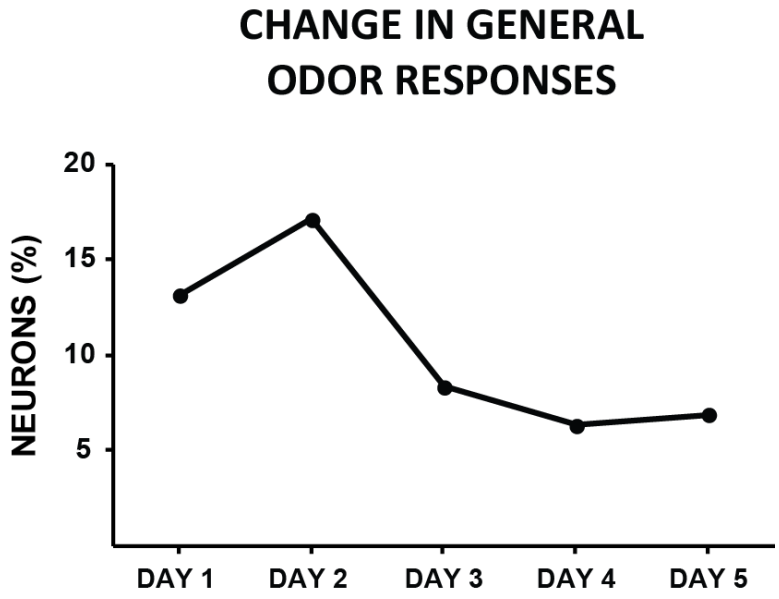

Figure 11. The plot represents the significant change in the percentage of neurons that had general odor sampling event responses over the five days of training.

Trial-end Responses. Trial-end responses were the most numerous of all the event responses recorded and there were 321 cells that responded to trial-end events over the 5 days of training. A chi square analysis on the development of trial-end event responses revealed a significant decrease in the proportion of responses across days ($\chi^2(4) = 14.89$, $p < 0.01$, Figure 12). As with the previous types of event responses, there was a decrease in trial-end event responses as learning progressed.

Cells with single and multiple events. Many of the cells recorded had two or more event responses. Overall, there were 275 cells with multiple event responses compared to 180 cells with single event responses and 213 with no event responses. A chi squared analysis was performed to compare the change of cells with no event response, a single event response and multiple event responses over the days of learning. The analysis revealed a significant decrease in the proportion of cells with multiple event responses while the proportion of cells with no event response increased and proportion of cells with single events did not change ($\chi^2(8) = 23.25$, $p < 0.01$, Figure 13). Interestingly, the most common two events encoded by cells were trial-start and trial-end event responses which accounted for 49.8% of the multiple event cells across days and did not vary by day. It is interesting that trial-start and end event responses are the most common to co-occur as these two events bracket the behavioral sequences that take place during a trial and could serve as a means to bind the trials events as a single sequence of events.

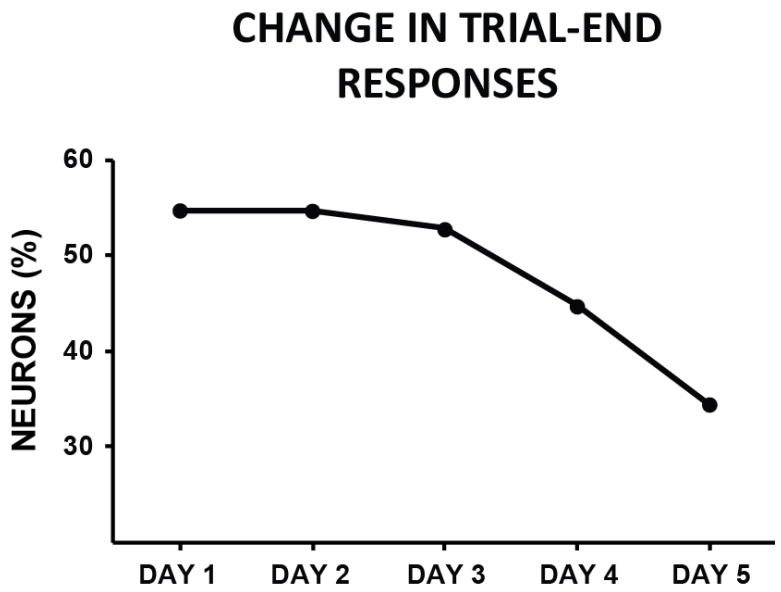

Figure 12. The plot represents the significant change in the percentage of neurons that had trial-end event responses over the five days of training.

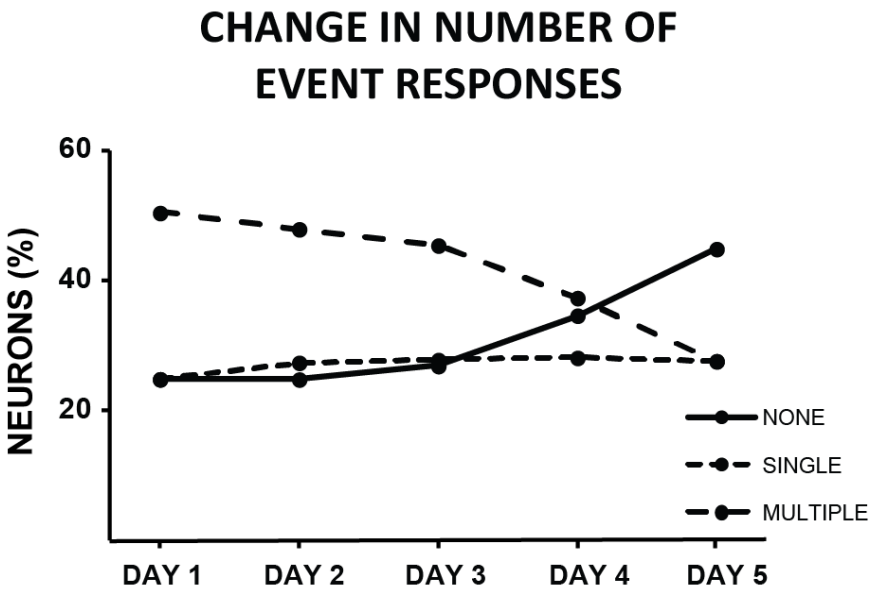

Figure 13. The plot represents the change in the percentage neurons that had no event responses, a single event response and multiple event responses over the five days of training.

DISCUSSION

I found that many hippocampal neurons responded to pertinent task events during the concurrent odor discrimination task. Although some of these event responses have been reported previously (e.g., odor selective responses, Wood *et al.*, 1999), this is the first report from a concurrent discrimination task involving many odor cues while learning progressed. Additionally, I observed a number of responses that have not been reported before, including a large number of trial-end responses. Surprisingly, I found that the proportion of neurons that exhibited many of these kinds of responses declined as learning progressed. This is in contrast to previous studies of event responses that found increases in the number of responses as learning progressed (e.g., Komorowski *et al.*, 2009; Smith & Mizumori, 2006b). Additionally, unlike place cell experiments that find neuronal representations develop in the first few minutes and remain stable upon revisiting a known context (Frank *et al.*, 2004; Muller *et al.*, 1987; Thompson & Best, 1990, but see chapter 2), I did not see stability in the proportion of event responses following the initial day of learning. My results showed that the event responses decreased as learning progressed, which is in line with previous research showing that the number of cells active (as measured by IEG positive cells) during a spatial cognition task decrease with improvement in the task (Guzowski *et al.*, 2001). This suggests that as learning progresses, the representation of the task becomes sharper as a result of learning and therefore requires fewer neurons to perform the task efficiently. While this outcome was not expected, this is a common finding in motor learning tasks (e.g., Lohse, Wadden, Boyd, & Hodges). The results indicate that the HP was creating a more concise representation of the task as performance increased. Therefore, I suggest the HP encodes a broad contextual representation that binds together the relevant event, spatial, contextual, behavioral and temporal (sequential) information to form a

coherent memory (e.g., Eichenbaum & Cohen, 2014; Smith & Mizumori, 2006a). This idea will be discussed later in the chapter.

Event Responses

Cup approach responses were observed across all days of learning and could be a general signal that serves to prepare the HP that a decision to dig or not is forthcoming. While cup approach responses have been previously reported (e.g., Wood *et al.*, 1999), they have not been previously evaluated quantitatively. The proportion of cup approach cells that Wood et al. found (20.4%) were comparable to the proportion I found (24.5%); however our approach cells included when the rat approached the first cup on a trial, the last cup on a trial or a general cup approach response. In the previous report, rats were presented with one cup at a time in a non-match to sample task and therefore they could not assess the types of approach responses in this task. These previous results were also recorded in rats that were well trained in the task so the finding that the number of approach responses decrease concurrently as learning progressed has not yet been described. In the Wood et al. paper and the current experiment, rats had to learn when approaching a cup they had to make a decision to dig or not to dig. This suggests that the approach cells send a signal to the memory circuit that a decision is approaching and this signal helps to prime the system for a decision making process. While there was no significant change in last cup approach responses, there was a small numerical increase in the number of these event responses on the final day compared to the first day. It was hypothesized that these responses would increase as learning progressed because on the latter days of training, rats are performing at a high level and when they approach the last cup it follows an error or correct rejection implying they will receive a reward. While this is merely speculation on a non-significant result, this was the only event response that did not show a decrease in proportion across days.

There were also cells that responded generally to the odor sampling event and to specific odors. While specific odor responses have been reported previously (e.g., Eichenbaum *et al.*, 1987; Wiener, Paul, & Eichenbaum, 1989b; Wood *et al.*, 1999), this is the first time they have been evaluated in a concurrent odor discrimination task. The cells that responded to general odor sampling events did not always have a response to specific odors although most odor sampling event cells did respond to specific odors. Although I found that most odor specific cells preferred rewarded odors, I could not tease apart the responses to the non-rewarded odors as they overlapped heavily with the cup approach of the next odor after an error or a rejection. For instance, the rats would may times sample an odor with an extremely brief sniffing motion and then move to the next cup, creating too much overlap to assign it as a non-rewarded odor response and not a cup approach response for the next sample. Similar to previous reports of cells responding to more than one odor, I found that most cells responded in a highly selective manner, a moderately selective manner and to most of the odors. Although I did not find any significant change in the specific odor responses across days, there was a decrease in the number of cells that had a general odor sampling response, just as with the other event responses.

The current results showed many cells that responded to trial-start events. There were different types of start responses (increases in firing rates before, at and after the timestamp) that have not been previously reported. I believe these differences in peak firing rate at the start of the trial have to do with trial relevant events preceding the start time stamp, such as placement of the cups or raising of the divider. Interestingly, our results found a decrease as learning progressed in trial-start event responses while a previous study found that trial-start events increased as learning progressed (Smith & Mizumori, 2006b). This was most likely due to the differing task demands. In Smith and Mizumori (2006), rats were trained to always go to the

east arm on a plus maze to obtain a reward and then halfway through had to switch and always approach the west arm. Therefore, rats had to initially learn the task by trial and error. Rats in that study always started from a different arm on the maze making the information about where the rat starts the trial a very important component in finding the goal arm. In the current study the behavioral demands never changed; they always were presented with 2 cups of odorized bedding for which they had to decide to dig or not, making the trial-start a static event that occurs on every trial in the same manner. Therefore, our results suggest trial-start responses are part of a sequence in a more general contextual representation of the task.

Trial-end responses have not previously been described before. However, previous studies have found cells that respond to a reward or that respond to arrival at an ITI period (Smith & Mizumori, 2006b), which may be functionally similar to trial-end responses found in the current results as they signaled the end of a trial. Therefore our task, which has discrete trials that end when the rat moves to the ITI area, provides the opportunity to find trial-end event responses that are not confounded with a reward and which occur in response to the rat's behavior as opposed to being physically moved to an ITI platform. In the scope of the current experiment, as mentioned earlier, I suggest the trial-end event responses not only represent the end of the trial but also signals to the HP that the previous sequence of events need to be combined as a single trial representation.

Taken together, I suggest that the trial-start and trial-end responses could serve as a signal that triggers event segmentation. Event segmentation is an automatic process that serves to separate ongoing information into meaningful events (for review, see Zacks & Swallow, 2007). Evidence suggests event segmentation is automatic, events form the units of memory encoding, and that there are specialized neural mechanisms which identify event boundaries by tracking

significant changes (Zacks & Swallow, 2007). This would suggest that the trial-start and –end responses could serve as the event boundaries that bind an individual trial together. Along these lines, early in learning the individual events are all encoded because it is not yet known which events will be crucial in the overall learning of the task. As learning progresses, the individual events become embedded into a single trial representation allowing for a more concise representation of each trial (as well as all the trials in a training session) rather than representing every event that occurs. Since the events are being combined or chunked for each trial and each trial contains the same general events (start, end, approach, sample) this could result in a reduction in the neural load. Trial-start and –end events were the most common event responses both individually and when cells had multiple event responses. This concept of forming a more concise representation with multiple events as a single sequence will be discussed further below.

Learning Related Changes in Event Responses

In contrast to previous results on the development of event responses that found increase in responses with development (Komorowski *et al.*, 2009; Smith & Mizumori, 2006b) our results are in line with results from Guzowski et al. (2001) which found a decrease in the number of cells that are active as rats learn a spatial task. I found event responses decreased as performance increased, suggesting the representation of the task becomes sharper as a result of learning and therefore requires fewer neurons to perform the task efficiently. Guzowski et al. (2001) trained rats on a spatial version of the water maze task and measured the number of cells that were active across learning sessions by evaluating three different immediate early genes (IEGs). They found that as animals learned the task, the number of IEG positively labeled cells decreased across as performance on the task increased. This suggests that as learning occurs, the HP requires fewer cells to contribute to the ongoing maintenance of the memory. Similarly, Tayler et al (2013)

tagged neurons with a permanent form of GFP that labeled active neurons in the HP. Two days following training they found that only 40% of the neurons activated during acquisition were reactivated when they were tested up to 2 weeks later, yet the rats did not show any impairment in memory (Tayler *et al.*, 2013). Another study analyzed hundreds of CA1 neurons simultaneously using calcium imaging in freely moving rats over the course of 45 days (Ziv *et al.*, 2013). They found that many neurons would exhibit place fields on a given day, but only 25% were active 5 days later and that number dropped to 15% after 30 days. This indicated that neurons were dropping out of the representation, but they also found other cells that took their place. The original cells of the representation still maintained enough spatial information to predict the mouse's location based on the remaining active cells. This further suggests that as memories become more stable, they do not require the same number of active neurons or the exact same cells as when they initially learn the task.

The current results along with those just discussed support the idea that during the initial formation of a memory, the HP recruits a multitude of cells that become less active (or cells come in and drop out of the initial representation) as learning progresses (e.g., Guzowski *et al.*, 2001). This indicates that the HP was creating a more concise representation of the task as performance increased. This process may involve the initial formation of the contextual framework where learning occurs that then gets updated with new information on subsequent sessions which transforms distinct events into single sequences within a trial, possibly through event segmentation (Zacks & Swallow, 2007). Sparse coding of HP neurons has been proposed as a means of reducing neuronal computational costs while maintaining informative processing (e.g., Karlsson & Frank, 2008) and supports the current results and those from Guzowski et al. (2001). This is accomplished by recruiting the minimal number of neurons necessary to form a

memory representation which also leads to a reduction in the number of neurons needed for maintaining and updating the memory with new information. For instance, one study showed that less than half of neurons in the CA1 region of the HP are active in a familiar environment. However, as that environment becomes more familiar the number of active cells in the CA1 region decline in such a manner that cells with a firing rate greater than 12 Hz are enhanced while those below are silenced over time (e.g., Karlsson & Frank, 2008). This leads to a sparse representation with very strong spatial tuning. The current results mimic this pattern of activity in that the number of cells responding to events on a given day decreases as the task becomes more familiar and learning occurs.

Experimental evidence from rodents (e.g., Lee, Griffin, Zilli, Eichenbaum, & Hasselmo, 2006; Smith & Mizumori, 2006b; Wood *et al.*, 1999) and humans (Brown & Golod, 2010; Brown & Stern, 2014; Kuhl, Shah, DuBrow, & Wagner, 2010; Kumaran & Maguire, 2006) indicates that sequential associations are combined with contextual information in the HP which allows for retrieval of specific episodes even when there may be overlapping interference from other representations. For example, rats were trained on an sequential odor task in which they are presented a series of cups filled with odorized sand and then presented with two of the odors for which they need to chose the odor that occurred first (or last) in the sequence to obtain a reward (Agster, Fortin, & Eichenbaum, 2002). Rats were capable of performing this task well above chance. However, if they received lesions to the HP rats could no longer perform the task above chance levels suggesting memory for sequences is HP dependent. Human studies have also shown that the HP is necessary for remembering sequential relationships of scenes or stimuli (e.g., Ekstrom & Bookheimer, 2007; Jenkins & Ranganath, 2010). One study compared amnestic patients with HP damage and control participants in both a procedural and declarative

sequential task (Hopkins, Waldram, & Kesner, 2004). They found the amnestic patients were capable of learning the procedural sequence task but were severely impaired in the declarative sequence task compared to controls. There is a wealth of emerging evidence suggesting the HP plays a pivotal role in encoding episodic memories as sequential events along a temporal scale (for review, see Eichenbaum, 2013, 2014).

The interpretation that the HP encodes the individual events in a trial as an overall sequence concurrent with learning has previously been proposed (Eichenbaum, 2013; Smith & Mizumori, 2006a). Smith and Mizumori (2006) proposed a hierarchical encoding of HP neurons that encodes information at different levels of complexity, such as individual events within a trial, all the events on a single trial, the correct behavioral response for a trial and all of the trials on a given day leading to an overall generalized context representation. In accordance with this idea, I suggest that early in learning the HP encodes all of the features and events of the task. As performance increases, the nonspecific components of a trial (such as starts, approaches, etc.) become incorporated within the contextual frame and update the initial memory. Therefore as learning progressed, the individual events became less specific, becoming segmented into single sequences rather than individual events and the correct behavioral component (the odor cue to dig or not) becomes the prominent feature. Once in place, this contextual framework would allow for updating any new learning that occurs during the task. When rats first learned the task it was by trial and error to find the reward until the rats learned one odor within a pair was always rewarded and the other is not. If new odor pairs (that do not overlap with the initial odor sets) were presented to the rats while everything else remained stable, the rats would be able to learn the discrimination more quickly as the contextual framework for the task is already in place (a finding described previously, e.g., Tort *et al.*, 2011). The HP would merely have to update the

general contextual representation with new information about the rewarded and non-rewarded odors.

This generalized contextual framework I propose has been described previously as a schema, which is a mental representation of preconceived ideas, such as a framework representing some aspect of the world or of a situation. It is well known that memories are not stored in isolation, and it has been suggested that memories are instead incorporated into schemas (McKenzie & Eichenbaum, 2011). As proposed in this view, schemas are made up of semantic and episodic memories and involves the interleaving of new information with previous memories leading to the formation of new memories (McKenzie & Eichenbaum, 2011). Originally proposed by Bartlett (Bartlet, 1932), the idea has been adapted to a consolidation view which posits that with rapid synaptic modification in the HP that slowly modifies connections within the cortex, the HP supports memory formation for a brief period after learning. During this period, system reactivations incorporate new information by modifying a pre-existing schema which serves to connect related memories (McClelland, 2013; McClelland *et al.*, 1995; McKenzie & Eichenbaum, 2011).

Experimental evidence of schema modification has been demonstrated in a task where rats learned several paired associations (food flavor/location) and found that new paired associations could be added to the memory representation within a single trial (Tse *et al.*, 2007). However, when they had to learn new paired associations in a new context the learning was more gradual. They suggested that it is much easier to update a pre-existing schema then it is to produce a new one. Interestingly, when IEG activity was measured in the HP there was not a significant increase in IEG positive cells when rats had to retrieve the well known memories for paired associations, but there was a significant increase when the rats had to learn new paired

associations or update the task schema (Tse *et al.*, 2011). A similar study recorded from the HP while rats traversed a circular track where they had to learn new goal locations in an environment where there were already multiple goal locations established (McKenzie, Robinson, Herrera, Churchill, & Eichenbaum, 2013). They found cells that fired for the current goal locations and as rats learned new locations many of the neurons active for the original goal locations also fired for the new locations with a similar population ensemble representation. As rats became familiar with the new locations, the activity patterns of the new and original goals began to diverge and became distinguishable within the population ensemble representation. This suggests that schema modification involves the assimilation of new memories into preexisting neural network in the HP that supports the incorporation of new and existing memories (McKenzie *et al.*, 2013).

I believe that our results are due to the HP generating a schema as they learn the odor discrimination task that gets updated with each learning session. On the first day of training, rats are learning by trial and error and as the trials progress they begin to form a schema for trials. After the first day of training, rats start to merge the schemas for events on individual trials into a global contextual representation (or schema) consisting of all 64 trials. As the schema becomes more complete, the HP requires less neuronal support to maintain and update the schema. Future research should focus on the development of these schemas as well as the mechanisms for updating existing schemas to gain a more full perspective on schema formation and modification.

GENERAL CONCLUSIONS

The current experiments all examined different aspects of context dependent memory processes and indicate there is still a lot to learn about how contextual memories are encoded and how they help to overcome interference. Although there have been multiple experiments evaluating the role of the HP in contextual memory, the AT has yet to be evaluated whether the AT is also critically involved. Similarly, there is an abundance of information about how the HP develops context representations in one environment (O'Keefe & Dostrovsky, 1971), or when switching between a well known and novel context (Frank *et al.*, 2004), but how two context representations develop concurrently had yet to be examined. The final chapter aimed to evaluate what event cells arise during a concurrent odor discrimination task and how those event responses change over the course of learning. The majority of studies describing event responses take place after the behavioral task is well known (e.g., Wood *et al.*, 1999) and very few have looked at changes over the course of learning (e.g., Smith & Mizumori, 2006b). Therefore, although there is a lot of information on how contextual information is processed in the HP, there remain many areas that have not been explored. The current dissertation aimed to address some of these key components missing in the contextual learning literature.

The first chapter highlights that like the HP and RSC, the AT plays a critical role in the formation of contextual memories (e.g., Keene & Bucci, 2008a; Kim & Fanselow, 1992; Phillips & LeDoux, 1992). There was a long period of time where it was believed the learning and memory hub was the HP, however, over the last couple of decades it has been made clear that the HP does not work alone but as part of a limbic memory circuit (e.g., Aggleton & Brown, 1999; Aggleton & Nelson, 2014; Aggleton *et al.*, 2010). It had been known that the HP and AT were involved in very similar memory functions, such as episodic (e.g., Mair *et al.*, 1979; Scoville &

Milner, 1957; Swaab *et al.*, 1998; Victor *et al.*, 1971), and spatial memory (e.g., Eichenbaum *et al.*, 1990; Mair *et al.*, 2003; Mitchell & Dalrymple-Alford, 2006; Morris *et al.*, 1982; Olton *et al.*, 1979), but this was the first example that the AT is also critically involved in using contextual information just as the HP is (Butterly *et al.*, 2012). This provides further evidence that the HP is not the center of memory and that without a functioning AT there would still be debilitating memory impairments even with an intact HP. Future research should focus on the potential differences there are in supporting contextual memories between these two structures.

The second chapter was the first study to analyze the development of two contexts concurrently. The results showed that early in learning the HP representation of the two contexts is unstable and takes multiple exposures to stabilize. I suggest LTP must occur for these two representations to stabilize and this stabilization allows for the HP to generalize across visits to the same contexts and treat them as a single context. Recent evidence has emerged suggesting, along with this experiment, that hippocampal representations are not as static as once thought. For instance, other experiments have shown fluctuations in the neuronal representations of rats that have been well trained in tasks (e.g., Jeantet & Cho, 2006; Mankin *et al.*, 2012), suggesting potential mechanisms that distinguish similar events or episodes. However, my experiment indicates instability early in the development of those representations when there are two contexts that have to be encoded concurrently that stabilizes after several exposures to the environment.

The final chapter was the first to analyze event responses that are encoded by the HP in a concurrent odor discrimination task. I found several different types of event responses and showed the number of event responses decrease with learning, a finding that has not been previously reported in HP electrophysiology studies. I suggest this is due to the individual

events being transitioned into an overall trial sequence, and that the sequences of trials on a given day become part of a generalized context representation. My results suggest that the generalized context representation stabilized as learning progressed. The results add to the growing body of evidence that there are not cells in the HP that encode specific information (e.g., place cells, time cells, event cells) but that the cells in the HP encode the relevant information about any situation.

The last two chapters are closely related and may seem at odds with each other on the surface. The second chapter indicates increased stability across days as evidenced by less remapping and more place cells following the initial day of training while the third chapter found that the number of event responses decrease as learning progressed. However, I suggest that both results occur as a result of stabilizing the representation and the difference are due to the task demands. In the second experiment the rat was being moved from context to context with an intermittent ITI period. Therefore the prominent feature is the context itself which is the manipulation in that study. Experiment three had rats performing an odor discrimination task where the prominent feature is the odor pair for each discrimination problem. Although I found multiple event responses, they decreased with learning and I suggest they were being encoded into the general sequence of an overall trial which could allow for more focus on the task and less on the individual events. There is experimental evidence and theoretical proposals that support these interpretations. Therefore both experiments indicate that the HP is creating a more stabile representation as learning progressed, but highlights different ways this can occur within the HP.

Context is a necessary component for both the encoding and recall of HP dependent memories. The current research adds to the growing body of research on contextual memory by

introducing the AT as a critical component in the contextual memory circuit and also introduces two different ways in which context memories can stabilize depending on the task. Future studies can build off the current results therefore strengthen our understanding of how contextual information is encoded by the limbic memory circuit. For instance, the RSC has been indicated as a component in the memory circuit that also plays a role in contextual learning. Understanding how the HP, AT and RSC work in concert during contextual learning will provide critical insight on how context-dependent memories are encoded and retrieved at the circuit level. The current results have added that the AT is critically involved and provided two new lines of physiology results on the development of context representations.

APPENDIX

PLACING MEMORIES IN CONTEXT: HIPPOCAMPAL REPRESENTATIONS PREVENT INTERFERENCE

David A. Bulkin, L. Matthew Law, David M. Smith

ABSTRACT

For learning to be effective, intrusions of interfering memories must not impede retrieval of useful information. To solve this problem, memories are closely linked to the contexts in which they were learned. Visiting a context triggers the retrieval of appropriate memories while inhibiting the retrieval of inappropriate memories. The hippocampus is essential for this process: animals with hippocampal lesions can learn, but are unable to use context to prevent interference. We investigated the mechanics of the hippocampal activity that supports this behavior by recording ensembles of neurons while training rats on two conflicting sets of odor discrimination problems, either in the same context or in two distinct contexts. When rats learned two conflicting sets of odor discrimination problems in two distinct environments, they formed two distinct hippocampal representations. These rats were insulated from interference, and so performed better, than rats trained on both sets of problems in a single environment. Moreover, trial by trial variation in activity was related to performance: on trials where an old representation was dominant rats made many errors, but on trials associated with a new representation errors were rare. Our results show how hippocampal activity provides a context code that can be used to assuage the effects of interference.

INTRODUCTION

Context is a fundamental organizing feature of memory. Information is best remembered when subjects are tested where they learned (Balsam and Tomie, 1985; Godden and Baddeley, 1975; Smith, 1988), a phenomenon so integral to memory that merely providing the instruction to think about the learning context is sufficient to improve recall (Smith, 1979). Context has a particularly important role in mitigating mnemonic interference (Bouton, 1993; Smith, 1988; Smith and Vela, 2001). Subjects that learn two lists of word associations or nonsense syllables experience less interference (i.e. show better recall) when the items are learned in distinct environments than when both lists are learned in the same environment (Bilodeau and Schlosberg, 1951; Dallett and Wilcox, 1968; Greenspoon and Ranyard, 1957; Watts and Royer, 1969)

The neural basis of the link between context and memory almost certainly involves the hippocampus (for review, see: Nadel, 2008; Smith, 2008). Context specificity of various kinds of memories is disrupted following lesions of the hippocampus , and an intact hippocampus is critical for the use of context to overcome interference (Butterly et al., 2012). The underlying mechanism seems to involve hippocampal place cells, which respond based on the spatial position an animal occupies (O'Keefe and Dostrovsky, 1971). These neurons alter their location of preferred firing following a change context (or 'remap'; for review, see Colgin et al., 2008), spatial or not (e.g. Eschenko and Mizumori, 2007; Kennedy and Shapiro, 2009; Smith and Mizumori, 2006b; Wood et al., 2000), and thus provide a population representation that identifies context.

Two important developments in the study of hippocampal neurophysiology raise new questions about the mechanics of hippocampal context representations. First, is the uncovering of

a broad spectrum of nonspatial features that govern hippocampal activity. Indeed, O'Keefe and Dostrovsky's initial report of place cells (1971) noted many neurons had activity determined by a variety of behavioral correlates. Since then, systematic study has indicated remarkably rich tuning of hippocampal neurons to nonspatial features (e.g. Eichenbaum et al., 1987; Muzzio et al., 2009; Pastalkova et al., 2008; Wood et al., 1999). Second, recent studies have demonstrated that neural activity is exquisitely patterned across ensembles of hippocampal neurons (e.g. Dupret et al., 2013; Jezek et al., 2011; Kelemen and Fenton, 2010) and the clear implication is that critical information is contained in the hippocampal population. Ensemble spatial activity seems to remap all at once following a contextual manipulation, providing a statistically independent representation that is ideal for associating with context-specific memories (Smith and Bulkin, 2014).

Does non-spatial activity show a similar type of remapping as spatial activity, generating a new and statistically independent population code when a new context is encountered? And how does the ensemble code support learning and memory? In the present study we trained rats on a proactive interference task in which they learned two sets of conflicting odor discrimination problems. Rats learned these two sets either in the same context, or in two distinct contexts. In this task, just like in tasks with human subjects learning lists of words, rats that learn the new odor problems in a new context are better equipped to resolve interference. Our previous work has shown that rats learning these two sets in two distinct contexts show a lower error rate than rats learning both sets in the same context (Butterly et al., 2012; Law and Smith, 2012).

We quantified the difference in hippocampal activity patterns as rats learned the two conflicting problem sets in the same context, and compared it with the difference when the problem sets were learned in different contexts. We observed a variety of response types in

individual neurons, including position and event locked activity and mixtures thereof. Because neurons exhibited such an array of responses, the present study afforded a unique opportunity to examine context-triggered remapping of spatial and event responses at the population level. As such, rather than applying a classification strategy, we subjected neurons to a series of spatial and temporal analyses that were agnostic to response type. Rats that learned new odor-reward associations in a new context had neural firing that was completely different in the two contexts, while those that learned new associations in the old context showed persistent activity patterns. This remapping yielded an ensemble representation that was statistically independent in the new context, a result that was apparent in both position- and event-locked analyses. Moreover, the trial-to-trial variance in representational similarity was predictive of behavioral performance: rats were least likely to make errors when hippocampal activity was most distinct. Taken together, these results show how hippocampal ensembles work to provide a new and statistically independent code, across a heterogeneity of constituent neuronal response types. Hippocampal activity thus provides a context representation that can be associated with memories, priming context-appropriate behaviors and preventing interference from inappropriate behaviors.

RESULTS

We trained rats on a proactive interference task in which they sequentially learned two sets of odor discrimination problems. On each trial, a removable divider was lifted, and rats encountered a pair of cups filled with odorized digging medium. Rats quickly learned that one odor in each pair was associated with a reward, and dug in the appropriate cup for a buried sucrose pellet, rejecting the unbaited cup. Once the rats reached asymptotic performance on the first set of odor problems, training commenced on a new set (Figure 1A; Appendix Supplement Figure 1A,B). To maximize the potential for interference, the second problem set contained some

of the odors used in the first set (with their valences reversed) paired with novel odors. Thus, the rats had to master new odor associations that were in direct conflict with the ones previously learned.

Four rats learned the new set in the same environment as the first (same context, SC), and three rats learned in a new environment (different context, DC). Replicating the results of our previous experiments (Butterly et al., 2012; Law and Smith, 2012; Peters et al., 2013), rats in the SC group experienced substantial interference, evidenced by a higher error rate on the first session of learning the second set of odor problems (Figure 1B; $T(5)=5.05$, $p<.01$; See also Appendix Supplement Figure 1C).

To investigate the nature of hippocampal representations and their utility in interference prevention, we recorded dorsal CA1 neurons from rats as they switched from the well learned initial problem set to the new, conflicting problem set. We then compared neuronal ensemble activity in the SC (62 units) and DC (59 units) conditions. Specifically, we recorded the neuronal activity on the final session of the first problem set and the first learning session of the second problem set (A_{final} and $B_{initial}$ respectively; the shaded region in Figure 1A). By investigating the relative change across these two critical sessions, we were able to see precisely how hippocampal activity patterns might support interference reduction in DC rats.

When the new learning took place in the same context, neuronal response patterns remained stable (Figure 2 B,D; Appendix Supplement Figure 2). Neurons in DC rats, however, showed a complete reorganization of activity (Figure 2 A,C; Appendix Supplement Figure 3).

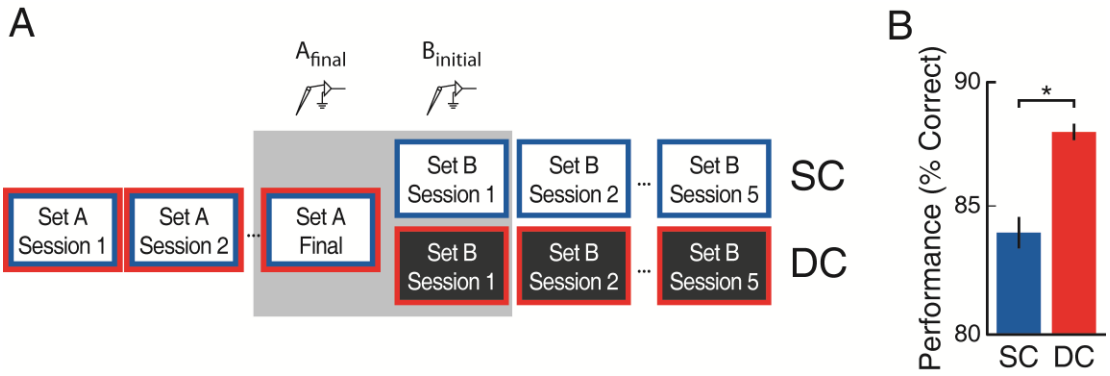

Figure 1. Proactive Interference Task. Rats were trained on a proactive interference task in which they learned two sets of odor discrimination problems. They first learned to discriminate within 8 pairs of odors over the course of 4-5 sessions, until they reached a criterion performance level of 90% correct choices. Next they learned a new set of problems either in the same environmental context (SC; blue) or in a different environmental context (DC; red). Recordings were taken throughout training, but analyses focused on a set of neurons that were stably recorded as the rats switched from one set to the next (A_{final} and $B_{initial}$; shaded region in A). Rats that learned the new set in the new context performed better. B shows the average performance on Set B. Error bars indicate SEM across rats.

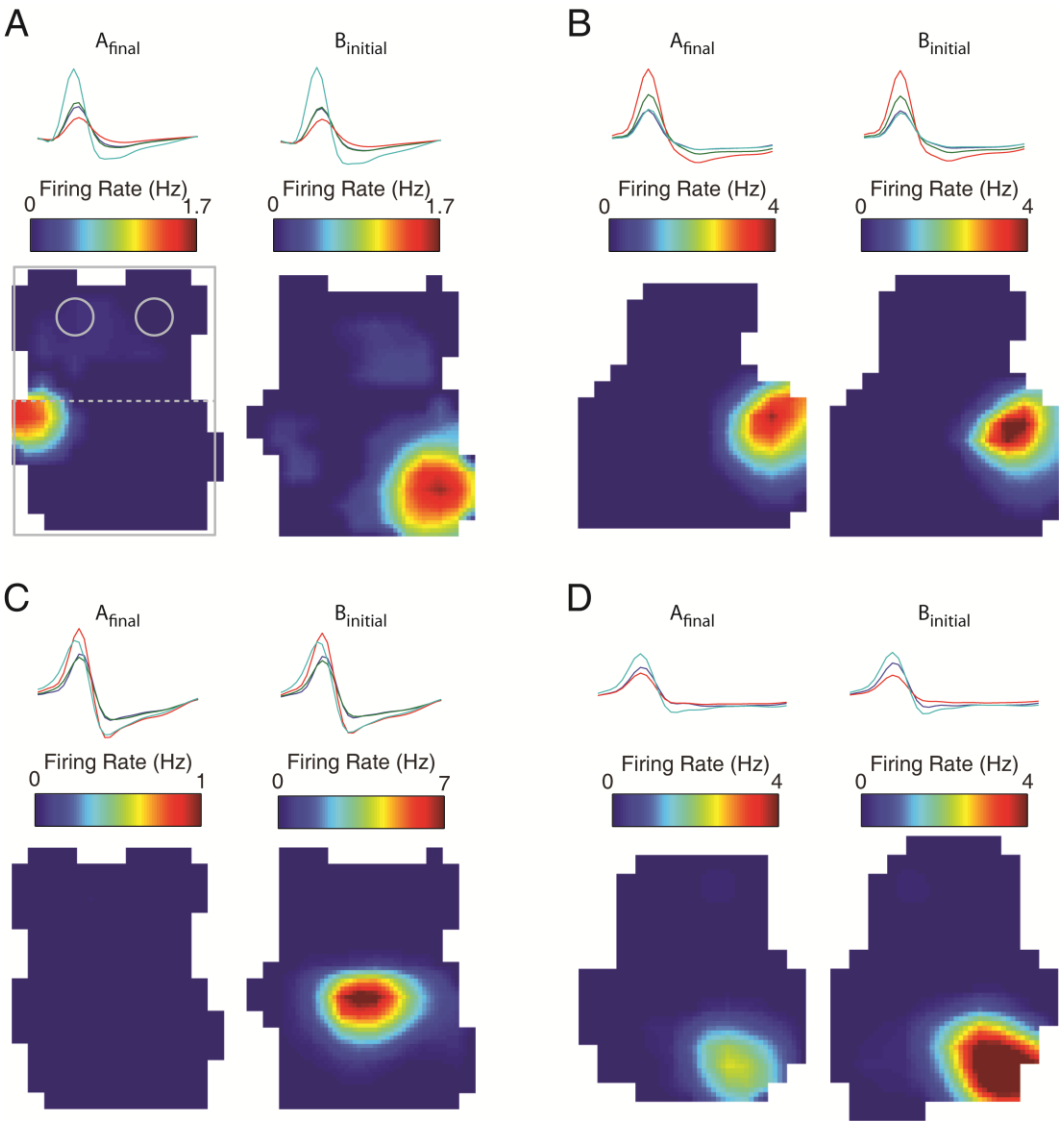

Figure 2. Examples of Spatially Tuned Neurons Across the Critical Sessions. Each of the panels shows the average action potential waveforms (top; the four colors indicate the waveform recorded on the four wires of the tetrode) and firing rate as a function of space (bottom) for example units on the final session of Set A (A_{final}; left) and the initial session of Set B ($B_{initial}$; right). The cells in (A) and (C) were recorded from a rat in the DC group, and show completely different firing patterns in the two sessions. (A) shows an example of a cell that had a place field in both sessions, but in different locations. (C) shows an example of a cell that showed a place field in $B_{initial}$ but was essentially silent in A_{final}. The cells depicted in (B) and (D) were recorded from rats in the SC group, and showed relatively stable firing across the two sessions. While there was variation in rate (the example shown in D more than doubled its infield firing rate) the preferred firing location was highly similar. Overlaid on the left heatmap in A is a diagram indicating the location of a removable divider (horizontal broken line) and the locations where cups containing odorized bedding were placed (see Methods, and Supplementary Figure 1 for more details).

This change in firing pattern could take several forms. The neuron depicted in Figure 2A showed clear spatial tuning but moved its place field. During A_{final}, this neuron fired preferentially on the left side of the apparatus, just behind the divider (indicated by the grey broken line; see Appendix Supplement Figure 1 for a more complete diagram of the apparatus). When the rat entered a new context, in $B_{initial}$, firing was mainly on the right hand side of the apparatus, far from the divider. The neuron depicted in Figure 2C showed a binary response pattern: this cell formed a well-defined place field during $B_{initial}$ but was essentially silent during A_{final}. Note that this cell did show a small number of action potentials during this session (14 spikes during A_{final}) and we were able to confirm the waveform shape across sessions using data from a third, neutral context (where this cell fired 239 times; see Appendix Supplemental Methods). In contrast to the remapping seen in DC rats, spatial tuning in SC rats was unchanged across the sessions. The spatial firing maps in Figure 2B and 2D show tuning that was stable, these neurons fired in the same locations during both sessions. Interestingly, while neurons recorded from SC rats did not change their preferred firing locations, many cells (like the one depicted in Figure 2D) showed large differences in firing rate (i.e. rate remapping). This may have occurred in response to the new problem set or merely the passage of time (see Mankin et al., 2012).

In addition to spatial sensitivity, many units showed discharge patterns that were tightly locked to important temporal events that occurred during the trial. Such event related firing has been described previously (Wood et al., 2000), and event responses seem to undergo remapping comparable to spatial responses (Smith and Mizumori, 2006b). The neurons depicted in Figure 3B and D, recorded from SC rats, showed stable responses time-locked to odor sampling in both sessions. In contrast, the neurons shown in Figure 3A and C, recorded from DC rats, indicated event locked firing in only one of the two sessions ($B_{initial}$ and A_{final} respectively). Thus, we

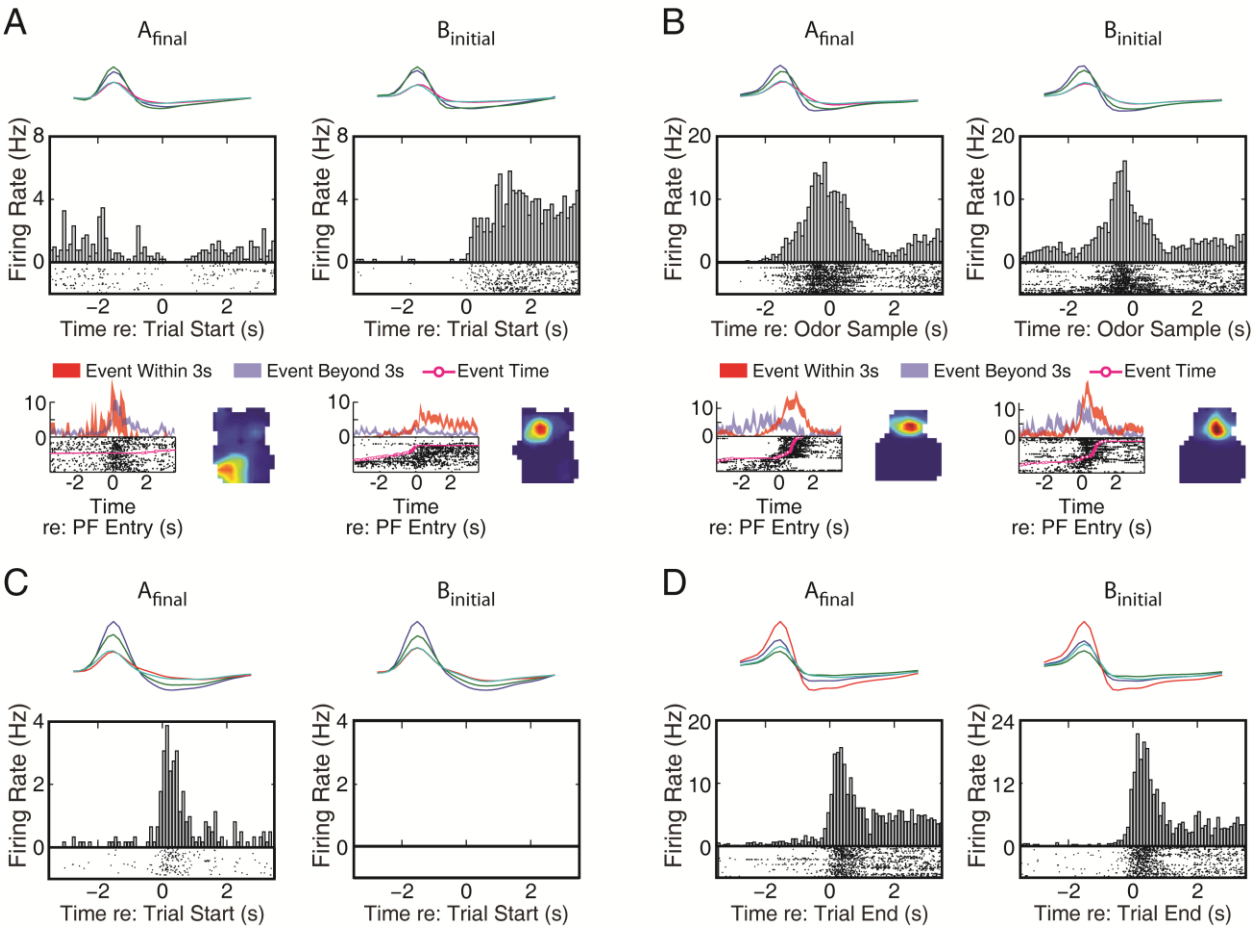

Figure 3. Examples of Event Tuned Neurons Across Critical Sessions. PETHs and rasters depicting activity of example neurons that were strongly influenced by trial events. The cell in (A), recorded from a DC rat, showed a response immediately following the start of the trial on $B_{initial}$ (right) but not A_{final} (Left). The cell in (B), recorded from an SC rat, showed a response as the rat sampled the odors during both sessions. Below A and B are panels indicating spatial firing for the same cells/sessions above and PETHs/rasters aligned on the rats' entries into the place fields. The rasters have been sorted by the relative time of the event (magenta line; the start of the trial in A and the arrival at the cup in B). Note that the more clearly spatially tuned response (the left inset in A) showed a dominant increase at place field entry, while more event related activity showed a firing increase that was better time locked to the event. The accompanying histograms are split into traces containing data when the event was within 2.5 seconds of place field entry (red) or more than 2.5 seconds from place field entry (blue). The shaded region indicates SEM across passes through the place field. Panels C and D show additional examples of event responses from DC and SC rats respectively.

considered an additional type of remapping that consisted of a change in response type (from spatial to event-related, or vice versa). Indeed, just like when considered spatially, looking at activity with respect to events showed that neurons in SC rats had highly similar responses across sessions while those neurons in DC rats showed unrelated response profiles (Appendix Supplemental Figures 4-5).

Distinguishing event driven from spatially driven responses is challenging because the rat visited a restricted set of locations at the times when events occurred. For instance, neurons that fired as the rats sampled odors (like the cell depicted in Figure 3B) formed an apparent place field around the location of the cups containing odor stimuli. Importantly, the activity was not purely determined by the location of the rat. To confirm the specificity of the cells, we defined a place field based on the apparent spatial sensitivity. We then constructed raster plots (shown in the insets below 3A-B, and Appendix Supplemental Figure 6) using each journey the rat took through the field (i.e. each row of the raster is aligned on entry into the place field). Finally, we sorted the rasters based on the temporal proximity of the event in question. Thus, while the spatial firing maps in Figure 3A (right) and Figure 3B suggest a place field, the corresponding rasters show that activity was more closely locked to the time the rat sampled the odor (magenta line). This stands in contrast to the pattern seen in the more purely spatial response shown in the left panel below Figure 3A, this cell increased its activity as the rat entered the place field (i.e. time locked to 0 in the raster), regardless of the temporal proximity of the trial start. The accompanying histograms show average firing rates for passes through the place field that were within 2.5 seconds of the relevant event (red trace) or more than 2.5 seconds away from the relevant event. The difference between the shapes of these curves indicates a strong modulation

based on event time. We observed a range of modulation, spanning a spectrum from purely spatial to purely event related responses.

Because both spatial and temporal factors influenced activity, and the context manipulation led to changes in both domains, we conducted parallel analyses in which we binned the data based on spatial location and two important task events, the start and end of the training trials. No studies have investigated the effects of context on event modulated activity by applying formally similar analyses to both kinds of responses. Moreover, in many kinds of experiments (e.g. maze studies) the design precludes disentanglement of spatial and event-related responses because important task events always occur at the same location. This fundamental confound can make a response appear locked to space when it is actually governed by temporal factors (Kraus et al., 2013). In fact, hippocampal neurons are routinely found to be responsive to a specific combination of spatial and non-spatial factors (i.e. conjunctive responses; Allen et al., 2012; Ferbinteanu and Shapiro, 2003; Frank et al., 2000; Muzzio et al., 2009; Wood et al., 2000). Thus, we adopted an agnostic approach to the analysis wherein we analyzed all of the neuronal responses separately in a spatial framework and a temporal (event-aligned) framework without any a priori classification of the responses. We first treated all of the neurons as spatial, and quantified the extent of change across the critical two sessions. Then, we treated all of the neurons as event-responsive, and applied an analogous set of analyses. This approach allowed us to investigate remapping while remaining agnostic to whether an individual neuron was receptive to the rat's position, trial events, or some combination of the two.

Changes in Spatial Firing

To examine the various types of changes in response patterns we quantified the extent of change between A_{final} and $B_{initial}$ in neurons recorded from SC and DC rats. First we compared

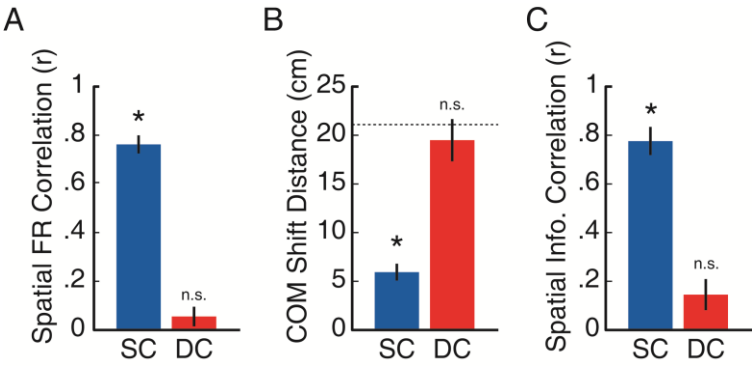

Figure 4. Quantification of Spatial Response Stability Across Neurons. Correlations between the firing rate in each spatial bin across the two sessions are shown in A. Neurons in SC rats (blue) showed well correlated spatial firing, while neurons in DC rats (red) showed uncorrelated spatial firing. Panel B shows the shift in center of mass of spatial firing rate (COM) across the two sessions. Stability of neurons recorded from SC rats is indicated by relatively small changes in COM, while DC rats showed chance relationships between COMs across the sessions. The broken horizontal line indicates chance COM shift, which was estimated by calculating the average COM distance between neurons. C compares the correlation between normalized spatial information across the two sessions for SC and DC rats. The extent to which a neuron exhibited spatial responsivity was related across the two sessions in SC rats: neurons that showed high spatial information in one session were likely to show high spatial information in the other. In DC rats, there was no relationship between a given neuron's spatial information across sessions.

the average firing rates in each two-dimensional spatial bin between the sessions. In the SC condition, the spatial response patterns of neurons were highly correlated across sessions (Figure 4A). In the DC condition however, there was no relationship between the spatially binned activity across the two sessions (SC compared with DC: $T[90]=12.95$, $p<.01 \times 10^{-19}$; SC r compared to 0: $T[45]=20.62$, $p<.01 \times 10^{-21}$; DC r compared to 0: $T[45]=1.37$, $p=0.18$).

Low spatial correlation values in DC-rats could arise from each of the types of change depicted in Figures 2-3. To investigate changes in response that were driven by a change in the preferred firing location, we compared the center of mass of spatial firing rate distributions (COM; Leutgeb et al., 2005; Mehta et al., 1997) of neurons across sessions. To be considered for this analysis cells had to show spatially restricted activity in both sessions (i.e. a contiguous region with at least twice the firing rate within the field as outside of the field, with an area less than 30% of the apparatus, and at least 100 total spikes within the field; 39 units in SC rats, 24 units in DC rats). Importantly, we did not exclude apparent place fields that were evidently due to event related firing which happened to coincide with a particular location (e.g. odor driven responses occurring at the cup location, such as in Figure 3B).

The distance between COMs from A_{final} to $B_{initial}$ was larger in DC rats than SC rats (Figure 4B; $T[61]=6.70$, $p<.01 \times 10^{-6}$). Furthermore, the COM shifts for the neurons of rats in the DC condition were as large as the average distance between the place fields of two different neurons (one sample t-test; DC $T[24]=.92$, $p=.37$; SC $T[38]=17.51$, $p<.01 \times 10^{-17}$). In short, in DC rats, the preferred firing location of a neuron in one context was completely unrelated to the preferred location in the other context.

To quantify the extent to which neurons changed their degree of spatial sensitivity we computed a spatial information score for each unit/session and compared these values for

individual units across sessions. This approach asks whether neurons that have responses which are tightly linked with space in A_{final} continue to have strong spatial sensitivity in $B_{initial}$. Spatial information was calculated based on the average spatially binned firing rates and then z-transformed using the methods described in (Markus et al., 1994; See Supplementary Methods). Neurons recorded from SC rats showed similar spatial information scores across sessions (Figure 4C; $T[3]=13.42$, $p<.001$): those units that displayed highly spatially sensitive responses during A_{final} continued to display highly sensitive responses in $B_{initial}$ and neurons that were agnostic to space remained as such. None of the DC rats showed a relationship between spatial information scores of neurons across contexts (Figure 4C; group t-test on $r>0$: $T[2]=2.27$, $p=.11$; $p>.05$ for each individual rat). In other words, neurons that were spatially sensitive in one context were equally likely to be sensitive or insensitive in the other.

Changes in Event Locked Firing

Because we saw evidence for activity that was time locked to trial events (such as the responses depicted in Figure 3), our next step was to quantify the extent to which this activity changed across the critical sessions in the two groups. We did this by applying an analogous set of analyses to those which we used for space, but anchored to the timing of events rather than the location of the rats. Just like with the spatial measures described above, we performed these analyses on all neurons rather than categorizing individual cells spatial- or event-selective. To investigate changes in an event locked frame of reference we constructed peri-event time histograms (PETHs) by binning unit response data aligned on the start and end of each trial. Figure 5 shows the average PETH of each neuron, sorted based on the time of peak activity during A_{final}. Though these PETHs are aligned on two critical task events, they actually include responses to several others, including the approach and sampling of odors (just after trial start)

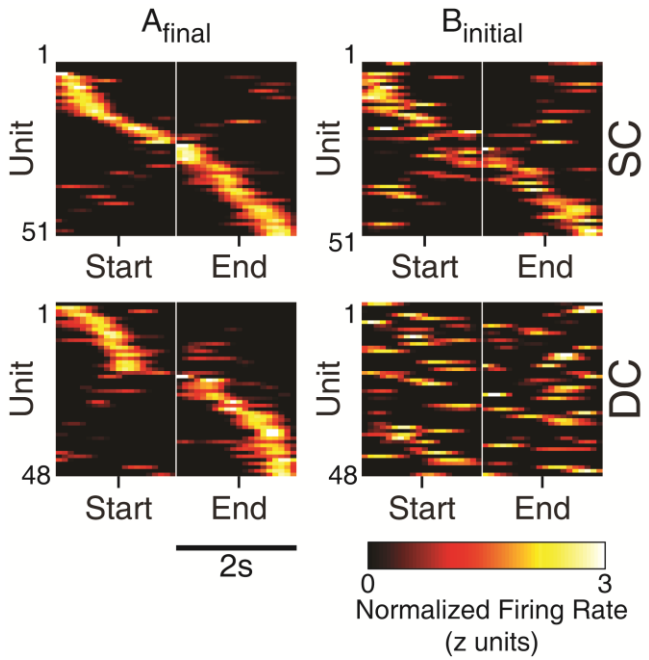

Figure 5. Normalized Firing Relative to Events for All Neurons. Firing rates aligned on the trial start and trial end are indicated in color, each row within a panel shows the average normalized rate of an individual neuron (z-scored using the mean and standard deviation of the average PETH). The rows were sorted based on the time of peak firing during A_{final}, and then the same sort order was applied to $B_{initial}$ data. Neurons in SC rats retained much of their temporal structure when the rats learned the new problem set (upper right). This structure was completely abolished in neurons recorded from DC rats (lower right). Note that these figures were not limited to event modulated responses, evidence of neurons with place fields is seen in the 'X' shape caused by the rats' tendency to be in similar locations at the start and end of trials.

and the retrieval of reward (just before trial end). When rats learned the new problem set in the same context (top row of Figure 5) the pattern was largely preserved: neurons that showed elevated activity before/after the start/end of trials continued to show elevated activity at similar times. Neurons recorded from DC rats, however, completely changed their event-related firing. When the PETHs were sorted by peak firing time on A_{final}, and the same sorting order applied to $B_{initial}$, the temporal structure was completely abolished (bottom panels of Figure 5).

Similar to the position-locked analyses, which included apparent place sensitivity that likely arose from event sensitive firing, the event locked analyses included apparent event responses that likely arose from spatial firing. For instance, a cell with a place field near the divider (like the cell depicted in Figure 2C) would show an apparent event response as the rat passed through the field around the start of the trials, and again at the end of the trials. Indeed, this sort of activity can be seen in Figure 5: several of the rows show firing that is symmetrical around the midline of the plot. By applying both sets of analyses to all cells, ignoring the 'cause' of firing, we were able to quantify remapping without categorizing cells based on the factors shaping their receptive fields.

We first applied a binwise correlation, analogous to the spatially binned correlation shown in Figure 4A. For each neuron we compared the average activity in each of the twenty 100ms bins surrounding the start and end of trials across the two critical sessions (Figure 6A). Event related firing was well correlated in neurons recorded from SC rats (one sample t-test compared to 0; trial start: $T[45]=8.18$, $p<.01 \times 10^{-7}$; trial end: $T(44)=9.22$, $p<.01 \times 10^{-9}$) and showed no relationship in DC rats (trial start: $T[38]=.16$, $p=.87$; trial end: $T[45]=.30$, $p=.76$). These data indicate that when neurons were considered in an event-locked reference frame (as in

the spatial frame), the ensemble as a whole underwent a complete reorganization of activity for rats that learned the new odor set in a new context.

Next, we investigated the extent to which units shifted the event-related firing time, using an analysis that was analogous to the spatial COM shift shown in Figure 4B. We defined a PETH COM by fitting Gaussian curves to the PETH data (aligned on trial start and end, +/- 2 seconds, 100ms bins), and identifying the time of the peak (see Supplemental Methods and Supplemental Figure 5). To be included in the analysis, both sessions had to be best fit by a Gaussian that had positive amplitude (i.e. a peak in response; 95% confidence interval). The shift in peak times was smaller in SC rats than DC rats (Figure 6B; trial start: $T[61]=4.09$, $p<.001$, trial end: $T[65]=4.60$, $p<.0001$). As with the spatial COM, the PETH peak shifts for neurons of rats in the DC condition were as large as the average shift between the peaks of two different randomly selected neurons (one sample t-test, trial start: $T[26]=.37$, $p=.71$; trial end: $T[31]=1.02$, $p=.32$). In contrast, COM shifts for rats in the SC condition were significantly smaller than chance (trial start: $T[35]=11.57$, $p<.01x10^{-9}$; trial end: $T[34]=7.30$, $p<.01x10^{-5}$).

Just as cells with spatially tuned responses could remap either by changing the location of peak activity or the degree to which they were tuned to space, event sensitive neurons could change either their peak activity time (with respect to an event) or the degree to which they showed event locked activity altogether. For each neuron we calculated an event related information score (Figure 6C) for the trial start and trial end using a method analogous to the approach used for spatial information (i.e. considering how informative firing was about the time of an event, rather than location; see Supplemental Methods for details). This approach asks whether neurons that have responses which are tightly linked with an event in one session continue to be associated with an event in the next. Similar to spatial information scores, the

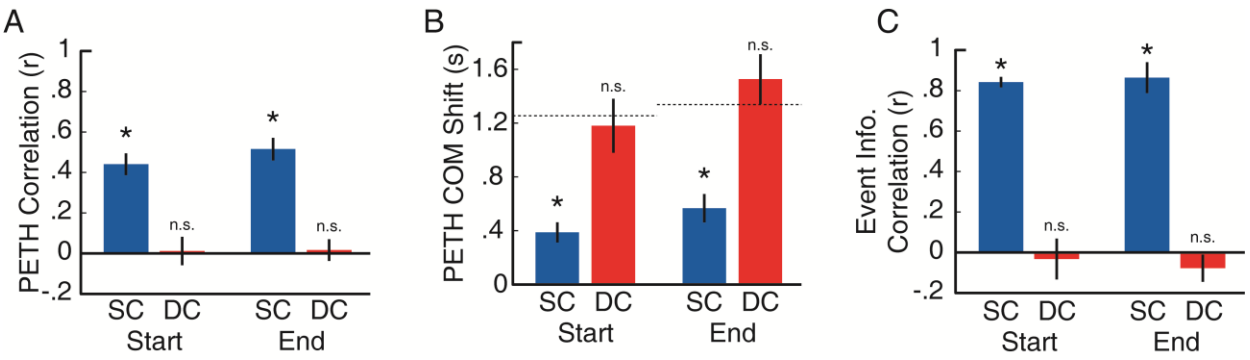

Figure 6. Quantification of Event Response Stability. Panel A shows the correlation between average PETH values (across A_{final} and Binitial) for trial start and end (analogous to the spatial analysis shown in Figure 4A). In SC rats (blue), neurons were likely to show correlated PETHs across the change in problem sets while DC rats (red) showed no such relationship (see also in Figure 5). Shift in the PETH COM (B), for trial start and end. PETH COM shift was defined by fitting a Gaussian to firing rates and taking the difference in peak locations for those fits which had positive amplitudes (95% confidence intervals) in both sessions (analogous to the spatial analysis shown in Figure 4B). As with the spatial COM shifts, SC rats showed small changes in PETH COM, while DC rats showed chance relationships between COM across sessions. Panel C shows the relationship between the normalized event-related information content across the two sessions (analogous to the spatial analysis shown in Figure 4C), for trial start and end. Neurons in SC rats carried similar quantities of information about an event from one session to the next, but in DC rats the amount of information was totally unrelated across sessions.

information about events was highly correlated across sessions in neurons recorded from SC rats (trial start: $T[3]=32.55$, $p<.0001$; trial end: $T[3]=11.32$, $p<.01$) but not DC rats (trial start: $T[2]=.32$, $p=.78$; trial end: $T[2]=1.15$, $p=.37$). Thus, neurons that had firing that was informative about proximal events retained this information in SC rats, but in DC rats the degree event-related information in one session was unrelated to the degree of event-related information in the other.

Changes in Population Activity

The above analyses verified that spatial and event representations persisted in SC rats and underwent complete remapping in DC rats. However, recent work has highlighted just how much of the ensemble signal is either inaccessible, or obscured, when measured at the level of individual units (Dupret et al., 2013; Jezek et al., 2011; Kelemen and Fenton, 2010). We next compared ensembles as a whole, on the same critical days as the single unit analyses, using metrics sensitive to interactions between units (i.e. covariance).

We began with a discriminant classification approach to predict location and temporal epoch relative to task events using each rat's neural ensemble responses. We first divided the apparatus into four spatial quadrants, and in a corresponding manner divided the trials into four epochs. Unit activity was binned to form population vectors containing the firing rates of simultaneously recorded neurons, and labeled with the associated quadrant/epoch. We then trained linear discriminant classifiers using a subset of vectors from A_{final}. To avoid errors associated with overfitting, and to estimate confidence intervals on the performance of the models, we repeated the classification process 10000 times, randomly selecting subsets of training and test data for the classifiers on each iteration. We then defined the performance of the model using the median of the iterated hit rate distribution, with confidence intervals based on

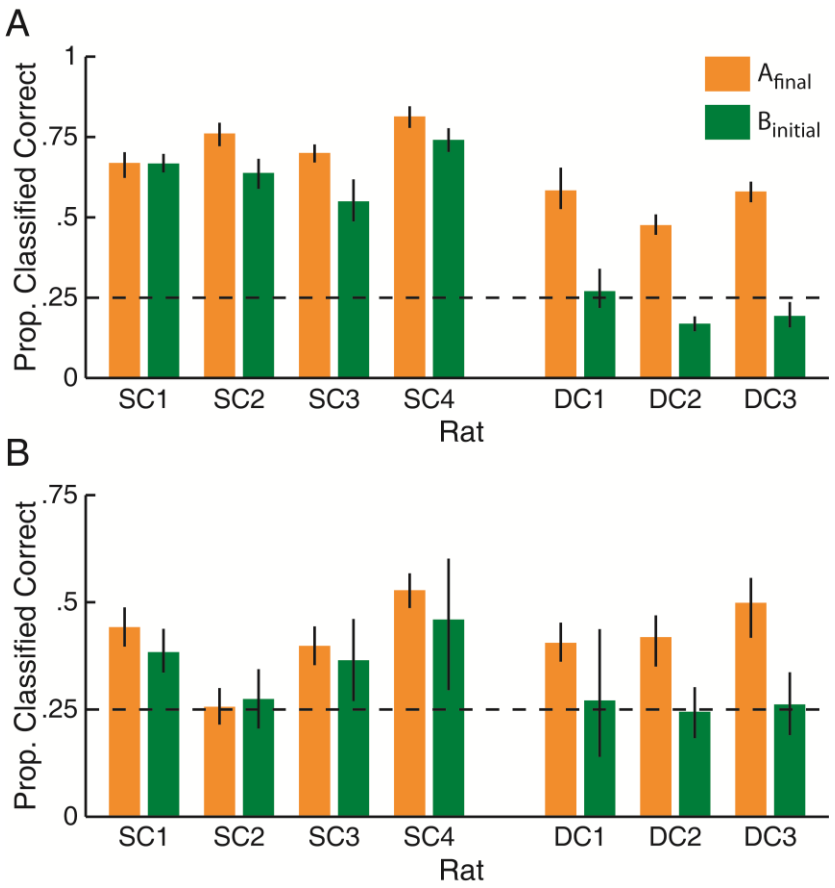

Figure 7. Classification of Population Responses Within and Across Sessions. Linear discriminant analysis was used to classify neural responses associated with one of four spatial quadrants (A) or one of four temporal epochs (B). The classifier was trained on a subset of one half of the data in A_{final}, and then tested on the remainder of the neural data (orange bars) as well as on one half of the neural data in $B_{initial}$ (green bars). An iterative process was used, randomly selecting the training and testing subsets, to estimate the performance of the classifier. Heights of the bars indicate median hit rates from this process, error bars range from 5% to 95% of the distribution of hit rates. Error bars not overlapping with .25 (horizontal broken line) indicate that the classifier performed above chance. When the classifiers were trained with data from A_{final} and tested with data from $B_{initial}$, they continued to perform above chance in SC, but not DC, rats. In one rat (SC2) the classifier did not exceed chance performance within the A_{final} session, and so it was unsurprising that it remained at chance when applied to $B_{initial}$ data.

the 5^{th} and 95^{th} percentiles.

When the classifiers were trained and tested using data from the same session, they generally performed well above chance (orange bars in Figure 7). In SC rats, the classifier continued to perform well when trained using data from A_{final} and tested on the responses in $B_{initial}$. In DC rats, however, the performance of the classifier fell to chance when applied across sessions. These results indicate that the population codes for space and temporal epoch persisted in SC rats across sessions, but in DC rats the code was rendered useless.

To measure the dissimilarity between ensemble representations on a trial-to-trial basis, we computed whole trial length population vectors marking the average firing rate of each neuron on each trial. We then computed the distance between each $B_{initial}$ trial to the average vector for the set of A_{final} trials using Mahalanobis distance, which was normalized to the number of neurons in the sample to define a distance index (MDI). Mahalanobis distance quantifies the distance of an n-dimensional point (the population vector from a $B_{initial}$ trial) to the average of a group of points (the vectors from all A_{final} trials), scaled by the covariance of the group. This provides a metric that is sensitive to changes both in global rate as well as interactions between neurons, and it has been used previously for quantifying dissimilarity of ensemble responses (Hyman et al., 2012; Manns et al., 2007; Sheinberg and Logothetis, 1997).

The average MDI was far greater in DC rats than SC rats (Figure 8A; $T[5]=3.35$, $p<.05$). The finding that this metric was sensitive to the remapping of ensemble activity is actually somewhat surprising: trial durations were long and variable (mean=10.0 seconds, SD=4.5 seconds). Furthermore, the trajectory of the rat, the specific odors encountered, and even the number of odor sampling events, varied from one trial to the next. The result is consistent with recent work showing that despite a complex set of relationships between hippocampal codes

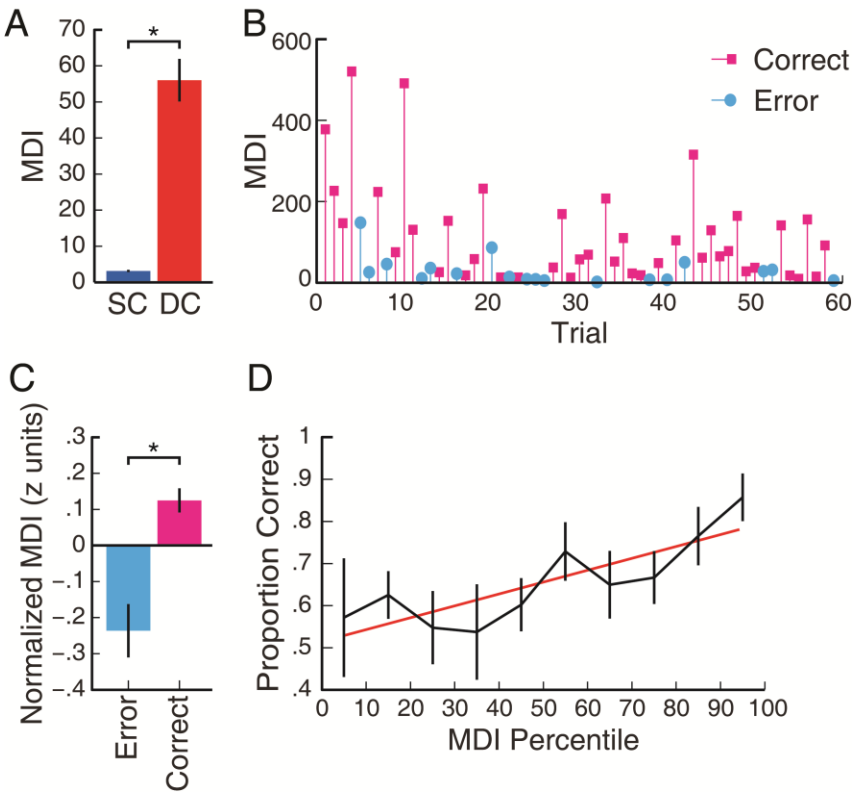

Figure 8. Ensemble Distances between A_{final} and $B_{initial}$ Trial Representations. Dissimilarity, quantified as the distance between each $B_{initial}$ population vector (i.e. the average firing rate of each neuron throughout entire trials) and the set of A_{final} population vectors was calculated using Mahalanobis distance, and then normalized to the number of neurons to yield a distance index (MDI). (A) Ensembles showed far greater dissimilarity in DC rats (red bar) than SC rats (blue bar). Error bars indicate SEM across rats. (B) shows MDI for each trial in an example DC rat, with correct trials indicated by magenta squares and error trials indicated with cyan circles. On trials with high MDI, the rat rarely made errors. (C) shows the average z-scored MDI across rats was lower for error than correct trials. Here the error bars indicate SEM across rats. (D) shows the proportion of correct trials, across rats, for each decile of MDI. Each rat was most likely to make an error when MDI was at the low end of that rat's distribution. The red line indicates a linear regression fit to the data (r^2=0.12; $p<0.005$).

within contexts, distinct codes are identifiable for distinct contexts (McKenzie et al., 2014).

Interestingly, in both context conditions, MDI was smaller on those trials in which rats made errors. Figure 8B shows each trial's MDI in a $B_{initial}$ session from a DC rat. When the MDI was high (i.e. the representation was most distinct), the rat virtually never made an error (cyan circles). When the MDI was low (i.e. the current representation was more similar to the old, A_{final} representation), interference dominated and the rats performed near chance (Figure 8C-D). Interestingly, the precise quantity of representational dissimilarity alone was not sufficient to prevent interference: SC rats showed distributions of MDI values that were far lower than DC rats. However when considering the relative MDI (either using z-transformed data or percentile division) it was clear that correct performance was associated with more distinct representations than incorrect performance (Figure 8C; $T[6]=3.38$, $p<.05$). Taken together, these results show how the remapping of hippocampal ensemble representations provides a context signal that can be used to alleviate the effects of interference.

DISCUSSION

Our results confirmed the hypothesis that hippocampal representations were more distinct, and performance was better, when rats learned conflicting sets of odor problems in two contexts than when they learned both sets in the same context. These results are consistent with a framework in which separate representations allow rats to form new memories unfettered by intrusions of memories from conflicting memories, thus reducing the number of errors. On error trials, the representations were more similar to the hippocampal states observed on the first set than on correct trials. In other words, when an old representation was more dominant, rats were more likely make an error.

Previous experiments using the same proactive interference task showed that temporary inactivation of the dorsal hippocampus had a remarkably specific effect: it selectively impaired the ability to use contextual information to resolve interference (Butterly et al., 2012). Temporary lesions impaired performance in the DC condition, but had no effect on performance in the SC condition. Additionally, when rats were trained on a low interference variant of the task (i.e. no conflicting odor cues), temporary lesions had no effect. Thus, hippocampal activity is crucial for the ability to retrieve the odor memories that belong to the current context without interference from previously learned odor associations that belong to a different context.

Our results support this idea and are consistent with the idea that hippocampal firing patterns influence memories stored in extra-hippocampal, presumably neocortical, locations. Learning is undoubtedly associated with plastic changes in sensory cortical regions (Ghose, 2004; Harris et al., 2001). Moreover, many kinds of memory are preserved following hippocampal damage (Butterly et al., 2012; Eacott and Norman, 2004; Eichenbaum et al., 1988; Honey and Good, 1993; Phillips and LeDoux, 1992), yet become highly sensitive to hippocampal damage when a contextual component is present (for review, see: Nadel, 2008; Smith and Mizumori, 2006a). Work in human patients with hippocampal pathology has indicated that semantic memory (which is context independent) is preserved while episodic memory (which includes contextual information; see (Tulving and Markowitsch, 1998)) is completely abolished. Coupled with the idea that the hippocampus serves as an index (Teyler and DiScenna, 1985, 1986) that activates memories that are stored elsewhere (i.e. neocortex), it is now clear that one of the key functions of the hippocampus is to supply an ensemble representation of context that can serve to prime extra-hippocampal memories. This provides a signal that both primes

context-appropriate memories, and suppresses inappropriate (i.e. potentially interfering) memories (Smith and Bulkin, 2014).

In addition to differences between SC and DC rats, we observed trial to trial variance in representation that was predictive of performance. How can two statistically independent representations, as we observed in DC rats, vary in their similarity? Several recent studies have demonstrated that hippocampal representations, at the population level, can alternate on a moment to moment basis (Dupret et al., 2013; Jezek et al., 2011; Kelemen and Fenton, 2010). One possible explanation of our data is that errors occurred on trials with transient appearances of the A_{final} representation. However, identifying this sort of pattern in the present study was impossible, due to the numerous and partially dependent dimensions influencing neural responses.

The tendency for SC Rats to show more a distinct representation of $B_{initial}$ correct trials may similarly have been due to transient occurrences of a new, as yet unseen representation. Despite a lack of environmental context manipulation, hippocampal neurons have previously been shown to change activity patterns following non-environmental changes (e.g. Eschenko and Mizumori, 2007; Kennedy and Shapiro, 2009; Smith and Mizumori, 2006b; Wood et al., 2000) suggesting that a new representation would eventually appear even in the same environment. Alternatively, differences between correct and error trials might have arisen from a representation that was less strongly driven on error trials. Others have suggested that cells which show robust firing on correct trials fire less on trials in which the rat makes an error (Robitsek et al., 2013). We saw no evidence for systematic differences between rate on correct and error trials, and reduced activity on error trials would likely have made representations more distinct while our results indicated that they were less so. However, because this study was

designed such that a robust context signal would make SC rats less likely to succeed (because of interference), a weakly activated representation might have been associated with correct performance.

Although we have interpreted the distance results as representation changes that drove behavioral differences, it is possible that the variation in behavior between correct and error trials led to divergence in activity. However, behavioral distinctions leading to representational differences would predict an effect in the opposite direction. Because trials on A_{final} were performed correctly by design (>90% correct) the behavior on $B_{initial}$ trials were more similar when the rat performed correctly. Yet it was the $B_{initial}$ error trials that showed more neuronal similarity. If ensembles showed very different responses when the rat made an error, the distance effect would have been in the opposite direction.

Indeed the behavioral and physiological complexity in the present experiment presented a unique set of analytic challenges. The location of the rat at any given moment, the order and the timing of odor sampling, were all left experimentally uncontrolled – we left these decisions to the rat. This approach provides a behavior that is simultaneously difficult and interesting to interpret. We generally applied a strategy of 'agnostic' analyses, subjecting all cells to measures of both spatial and temporal response property change. We did not segregate the data based on a classification of cells into one response type or another, but rather considered the extent to which activity changed, assayed from a ariety of viewpoints. Whether or not the complex receptive properties of individual neurons are integral to memory function remains an important and open question. We used population metrics to answer questions about the relationship between physiology and behavior directly, in the absence of a specific response field or pattern. Our data suggest that a useful context signal can be read out despite heterogeneity among unit response

types as well as variability in behavior from one trial to the next. In short, no matter how we considered the data, decreased interference following a context change was related to a new, and statistically independent, representation.

EXPERIMENTAL PROCEDURES

Behavior

Seven adult male Long-Evans rats were trained on a proactive interference task that has been described previously (Butterly et al., 2012). Rats learned two sets of eight odor discrimination problems, either in the same environmental context or in two different contexts. Rats learned the first set until they reached asymptotic performance (>90% correct on two consecutive sessions) and then began learning the second set. Three of the rats learned the second set in a different context and four learned the second set in the same context. See the supplemental methods for details of the apparatus and training procedures. All procedures complied with the guidelines established by the Cornell University Animal Care and Use Committee.

Surgery and Electrophysiological Procedures

Moveable electrode arrays containing 16 insulated platinum iridium tetrodes (composed of 4 17µm wires; California Fine Wire, Grover Beach, CA) were implanted bilaterally just above the dorsal hippocampus (3.5mm posterior and 2.5mm lateral to bregma). Following recovery from surgery, tetrodes were slowly lowered into the CA1 cell layer and rats began training on the first list of odors. Because the focus of the study was on changes in activity across two critical sessions (A_{final} and $B_{initial}$;Figure 1A), tetrodes were advanced over initial training on the first odor set but then left in place at least 24 hours before the final recording session on the first odor set (A_{final}) in order to maintain recordings as the rats began the new set ($B_{initial}$). Multi-unit

potentials were sorted into constituent units using standard clustering techniques and were matched across sessions by applying cluster boundaries from one session to the other with manual adjustments to account for drift (Mankin et al., 2012). To help ensure that records were maintained across the two days of recording, particularly when neurons could become silent due to the context change, we recorded a 15 minute block of data in a neutral context where rats foraged for scattered rewards following each day's behavioral training in the main task.

Data Analysis

All analyses were performed using custom software written in the numerical computing environment Matlab (Mathworks, Natick MA). Analyses were restricted to cells with an average firing rate of less than 3 spikes/second across the session (i.e. discarding any putative interneurons). Separate analyses with the inclusion of these neurons, or with higher thresholds for firing rate did not qualitatively change any of the differences between SC and DC conditions.

Unit changes in spatial activity across sessions were assessed by binning firing rates based on the rat's location to compute a spatial firing activity map for each cell/session. Pearson correlations were computed between the spatially binned firing rates across sessions. For those units that displayed spatial sensitivity in both sessions we computed the center of mass of spatial firing rate as the weighted average of the binned position of the rat, with weights determined by binned firing rate. For a unit to be considered spatially sensitive it had to have a spatial firing map with a contiguous region (i.e. place field) with at least twice the firing rate within the field as outside of the field, a field area less than 30% of the apparatus, and at least 100 total spikes within the field. Spatial information content was computed and normalized following the methods described in (Markus et al., 1994; see also Supplemental Information) and compared across sessions using Pearson correlation.

Unit changes in event related activity were assessed by constructing peri-event time histograms (PETHs) aligned on the start of the trial (when the rat crossed into the trial area) the sampling of odors (when the rat's nose was directly above a cup) and the end of the trial (when the rat returned to the inter-trial waiting area). Analogous to spatial analyses, Pearson correlations were computed between the PETHs, and information scores were calculated using PETH binned data in lieu of spatially binned data.

Classification was performed by training a linear discriminant model. To estimate confidence intervals on classifier performance, and to avoid problems of overfitting, an iterative process was used. A random subset of half of the data from one session was selected as the training set, and the performance of the classifier was tested against the remainder of the data from that session as well as a randomly selected set of half the data from the other session. The process was repeated 10000 times, and the performance was calculated for each iteration. Performance of the classifier was considered above chance if 95% of the distribution of classifier hit rates were above a chance value (taken as the reciprocal of the number of classes).

Similarity between representations was calculated using a distance index (MDI). For each trial, the average firing rate of each unit was tabulated to form a population vector for that trial. The covariance across A_{final} population vectors was calculated. Next the distance to the mean A_{final} response was calculated for each $B_{initial}$ trial, in units of A_{final} covariance. This Mahalanobis distance (i.e. distance normalized by covariance) was then additionally normalized by the number of units in the sample (for comparison across rats with different sample sizes) to form the MDI.

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APPENDIX SUPPLEMENT

Training Procedures and Apparatus

The training procedure followed our previously reported methods used to probe the use of context to prevent interference in rats (Butterly et al., 2012; Law and Smith, 2012; Peters et al., 2013). Training took place in wooden chambers with a 60cm by 45cm floor and a removable divider (Supplementary Figure 1A). One side of the chamber served as an inter-trial waiting area, the other contained two plastic cup holders for the presentation of stimuli. Contexts were differentiated by a number of multimodal cues: the enclosure's color and substrate (wood or a black rubber lining), the surrounding walls (black painted walls or white blinds), the frequency content of a 65dB continuous background masking noise (white noise or pink noise), the ambient odor left by wiping out the chamber with baby wipes prior to each training session (unscented or scented, Rite Aid, Inc.). Additionally, the rats were transported in covered cages to the experimental area by different methods (via a cart or carried by hand).

Before training began, each of the rats was exposed to each of the contexts for two 15 minute sessions. Subsequently rats were shaped to dig in cups of odorized bedding for buried food rewards (45 mg sucrose pellets, Bioserve, Frenchtown, NJ). For these shaping sessions a distinct odor was used, that was not incorporated anywhere else in the experiment. Once the rats had learned to reliably dig for rewards, they began training on the first set of odor discrimination problems (Figure 1A). Each set consisted of eight odor pairs (16 individual odors) with one of the odors in each pair always baited with a reward and the other always unbaited. Each session consisted of 8 trials of each odor pair, presented in an unpredictable sequence, with the location of the rewarded odor cup (left or right side of the chamber) randomized.

Trials began when the experimenter lifted the divider and the rat encountered the two cups. The rat approached the cups and searched for a reward. Subsequently, the rat was returned

to the intertrial waiting area and the divider was replaced while the experimenter prepared the cups for the next trial. Trials were marked as errors if the rat dug in the unbaited cup, any displacement of bedding was considered a digging response. Training sessions continued until the rat reached a behavioral criterion of 90% correct choices, or a minimum of 4 training sessions had elapsed. All rats achieved criterion performance by the fifth day of training. Sessions were generally conducted daily, however 1-3 days without training were interleaved in order to maximize the opportunity to obtain large and stable populations by the time of the final training session on the problem set.

Once the rat reached criterion performance, training began on a second problem set (Figure 1A, Supplementary Figure 1B). This set contained 8 odors from the first set, with their predictive value reversed, paired with 8 previously unencountered odors. To prevent rats from adopting a strategy based on odor novelty, half of the new odors were rewarded and the other half were unrewarded. Training on the second set continued for five sessions regardless of performance. The effects of the context manipulation were assessed by submitting the data to a one-way repeated measures analysis of variance (ANOVA) with group (SC or DC) as the between subjects factor and set 2 session number (five levels) as the within subjects factor.
A third, neutral context, was used to probe for activity as electrodes were lowered into hippocampus (before training began) and after each session to increase the chances of identifying cells that were not active during the main experiment (i.e. cells that were only active on one of the two critical sessions). In this context rats foraged for chocolate sprinkles in a one meter square PVC box, with a unique configuration of each of the context cues used in the main experiment. Data from this part of the experiment were used only for unit clustering, and were not subjected to any of the analyses of context effects.

Data Analysis

Spatial firing maps were constructed by calculating firing rates in 4.5cm square bins spanning the apparatus. The data were smoothed by convolution with a 4 bin Gaussian kernel with unity sum. Spatial bins that contained less than one second of occupancy (following smoothing) were discarded. For display purposes only, firing rate maps were interpolated linearly with 3 points between each sampled data value. Bin by bin correlations between sessions were applied only to bins that were visited in both contexts.

Peri-event time histograms (PETHs) were calculated by binning and averaging the data in 100 ms bins centered on three events during the trial: the start and end of the trial (defined by the moment the rat crossed an imaginary line just beyond the removable divider) and odor sampling (defined by the moment the rat's nose was directly above the odor cup). Odor sampling times were identified by manual flagging of raw video data sampled at 30 fps. Trials lasting longer than 30 seconds were discarded from all event related analysis, however performance was still included for these trials (these trials always contained errors, and the errors were always made within the first 30 seconds of the trial). For display only, the data shown in Figure 5 were smoothed with a 1 second Gaussian window, and normalized to z units.

To calculate normalized information scores, an information score was calculated as described in (Markus et al., 1994)):

$$I = \sum P_i \frac{R_i}{R} \log_2 \frac{R_i}{R}$$

For spatial information, $P_i$was the probability for occupancy of bin i, R_i was the mean firing rate for bin i, and R was the overall mean firing rate. For event related information content, P_i was fixed at the reciprocal of the number of trials (each bin of the PETH was 'visited' the same

number of times), values for R_i and R were calculated in the same way as for spatial data except that they were calculated using temporal bins (+/- 2 seconds around each event, 100ms bins) rather than spatial bins. Normalized values were calculated using an iterative process (Markus et al., 1994). A distribution of 500 pseudo information values was calculated by randomly offsetting spike times using uniform random values ranging from 5 to 100 seconds. The values used for comparison between sessions were z transformed using this distribution (i.e. the number of standard deviations from the mean of the distribution of pseudo information values).

The event COM analysis was performed by binning data in 100ms windows in a +/- 2 second range around trial start and end events, and fitting the following Gaussian curve using a nonlinear least-squares approach (Matlab Curve Fitting Toolbox):

$$y = Ae^{-\left(\frac{x-\mu}{c}\right)^2} + d$$

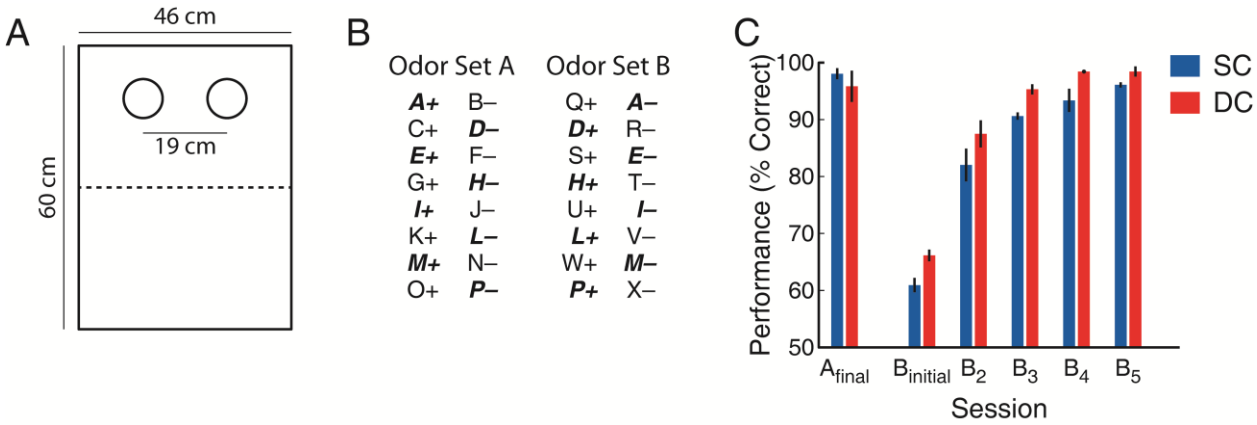
The parameters *c* and *d* were unrestricted. The parameter A, which indicates the amplitude of the Gaussian, was restricted to positive values, and the parameter μ which marks the peak time of the Gaussian was restricted to the range of the histogram (i.e. +/- 2 seconds). Initial values for these coefficients were taken from the range and time of maximum average binned firing rates. Following identification of a fit, confidence intervals on parameters were estimated using the inverse R factor from QR decomposition of the Jacobian, the degrees of freedom for error, and the root mean squared error (i.e. the Matlab function confint). Only those fits which had a positive amplitude (95% confidence interval on the coefficient *A* greater than 0) in both session were included. The difference in the parameter μ across sessions was taken as the shift in COM, the 95% confidence interval on this parameter is plotted in Supplemental Figure 5B.

Classification was performed using linear discriminant analysis with uniform priors. For epoch classification each trial was divided into four time windows: a 1 second period preceding the trial, the period between the start of the trial and the arrival at the first cup, the following 400 ms, and a period extending until 400ms after the arrival at the final cup (Supplementary Figure 3). Firing rates were divided into 100ms bins, and each bin was labeled with the epoch. Accuracy of the model was tested by creating 10000 subsets of the data, each time selecting half of Set A trials to train the classifier, and the remaining trials as well as half of the Set B trials to test the classifier. Shuffling allowed us to prevent spuriously high success rates due to overfitting, and creating subsets using randomly selected trials rather than bins allowed us to avoid spuriously high success rates due to the high dependency of firing rate from one bin to the next.

Spatial classification took an analogous approach, dividing the apparatus into 4 quadrants (to match the number of epochs in the event related classification). Data were divided into 1 second bins, and each bin was labeled with the quadrant identity. Bins in which the rat occupied multiple quadrants were discarded from analysis. To shuffle the data, each session was divided into 100 evenly distributed blocks throughout the session, and the blocks were randomly shuffled. All other methods corresponded exactly to the epoch classification.

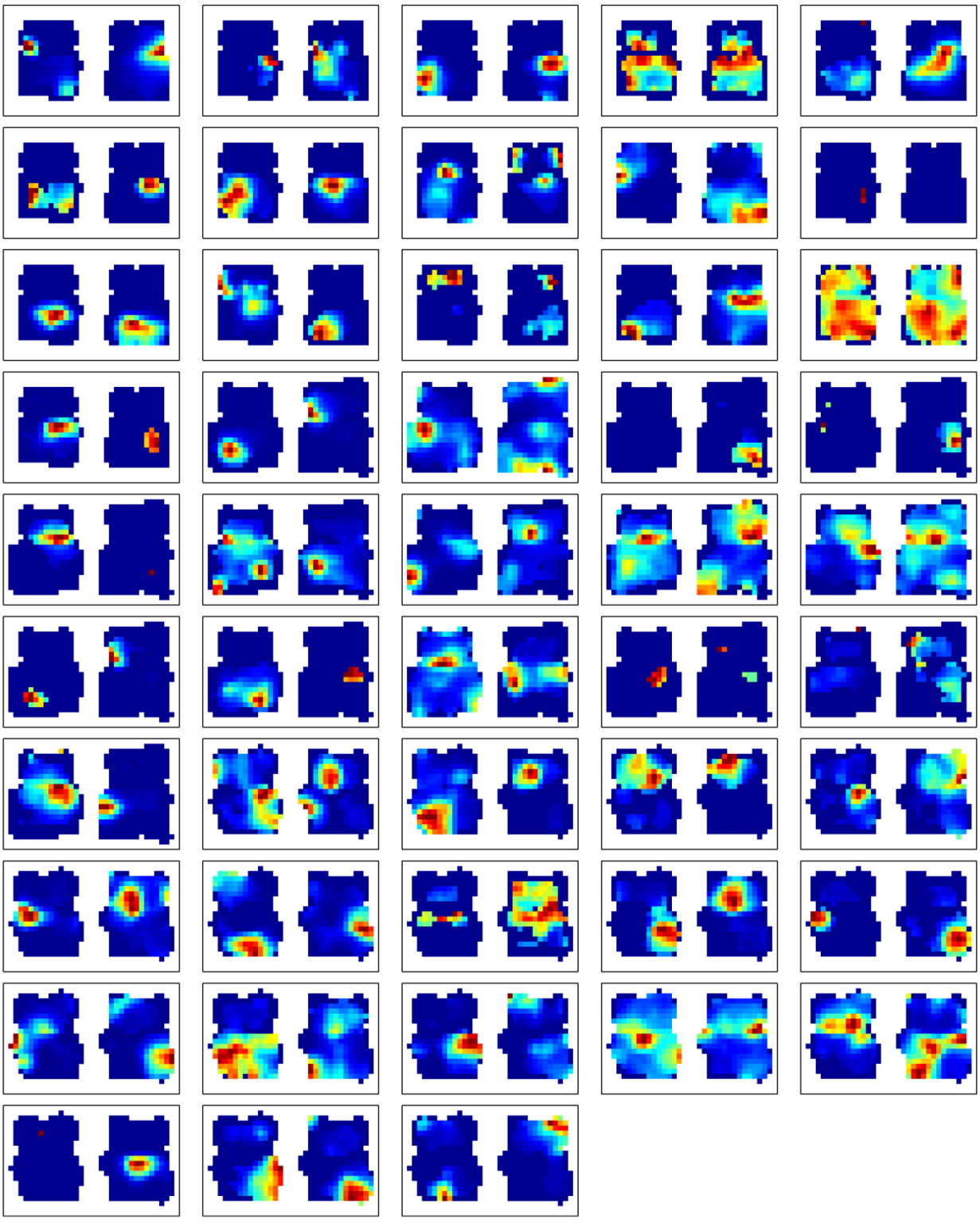
Histology

Following the experiment the rats were deeply anesthetized and perfused transcardially with 4% paraformaldehyde. Their brains were removed, sectioned at 40 μm, mounted on slides, and stained with cresyl violet. The position of the electrodes were confirmed to be in the cell layer of CA1 in dorsal hippocampus.



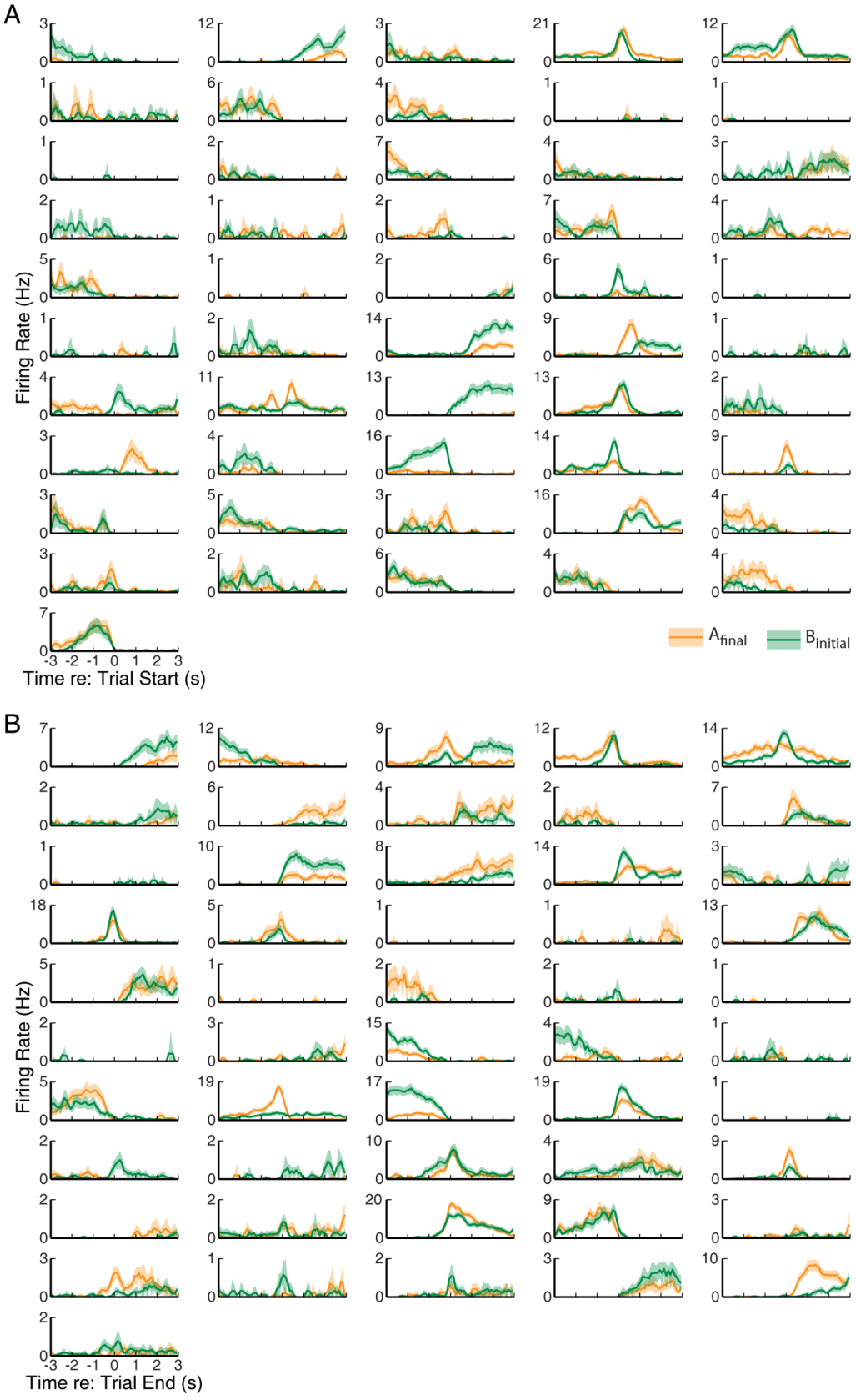
Supplemental Figure 1. Task Design and Performance. A diagram of the behavioral enclosure is shown in (A). An inter-trial waiting area (bottom) was separated from the discrimination training area (top) using a removable divider (broken line). Fixed holders were used to position two ceramic cups (8.25 cm diameter, 4.5 cm deep) containing the odorized bedding. While distinct enclosures were used for the two contexts, their geometrical dimensions were identical. (B) shows a schematic of the odor discrimination problem sets. Each set consisted of 8 odor pairs, with a reliably rewarded (+) and reliably unrewarded (–) odor within each pair. 8 of the odors were shared between the two sets (italicized), four of these shared odors were unrewarded on Set A and four were rewarded on Set A. The predictive valence of the common odors was reversed on Set B. (C) shows the average performance of SC and DC rats on A_{final} (greater than 90% by design), and each session of learning on Set B. Although both groups of rats learned the new, conflicting problem set, performance was significantly improved when rats learned the new problems in different context.

Supplemental Figure 2. Spatial Firing Maps of All SC Units. Each panel shows activity maps depicting the average spatial firing rates on A_{final} (left) and $B_{initial}$ (right). The figure includes all putative pyramidal cells recorded from SC rats. Firing rates are scaled to the minima and maxima for each unit/session.

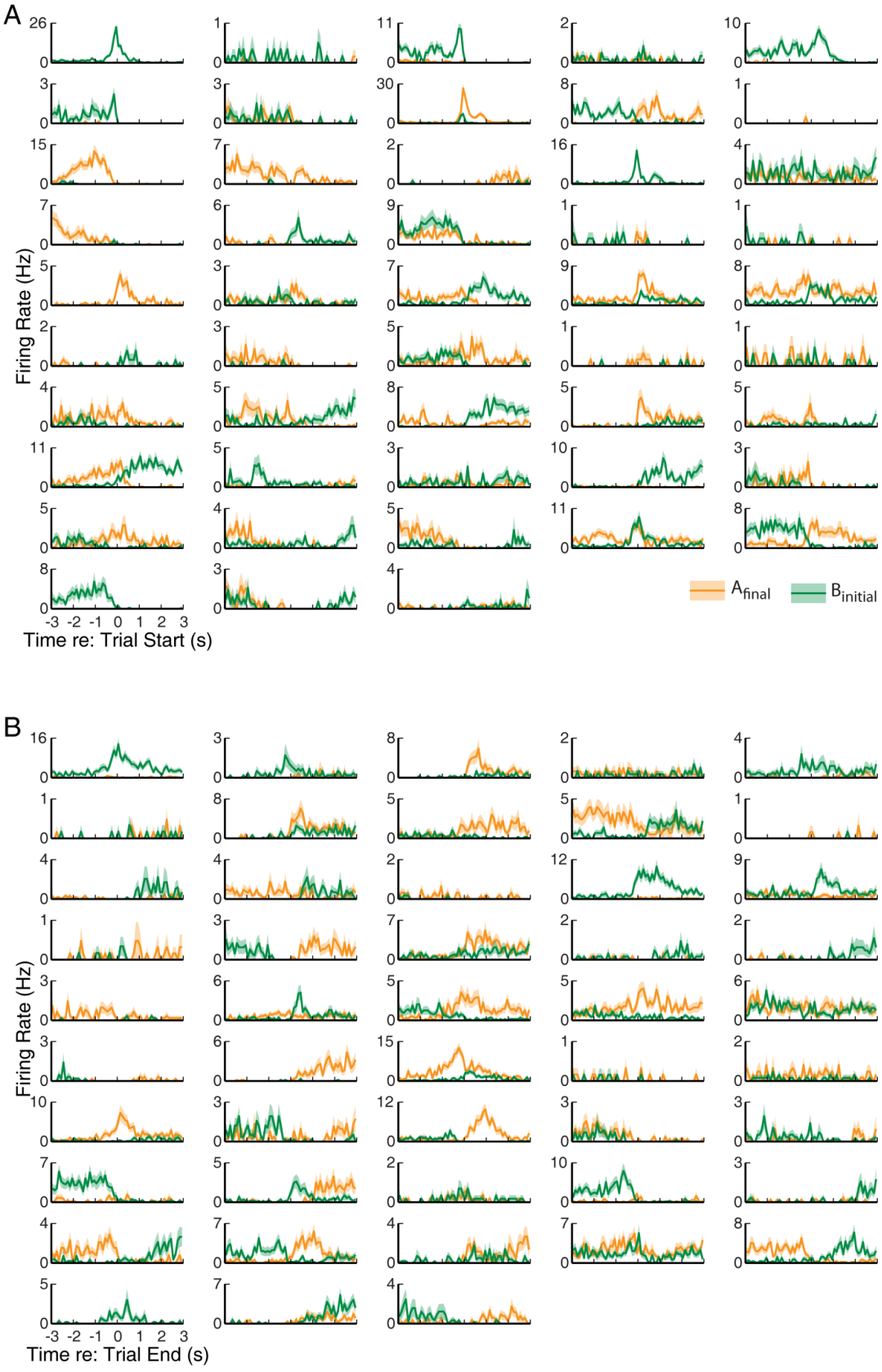


Supplemental Figure 3. Spatial Firing Maps of All DC Units. Each panel shows activity maps depicting the average spatial firing rates on A_{final} (left) and $B_{initial}$ (right). The figure

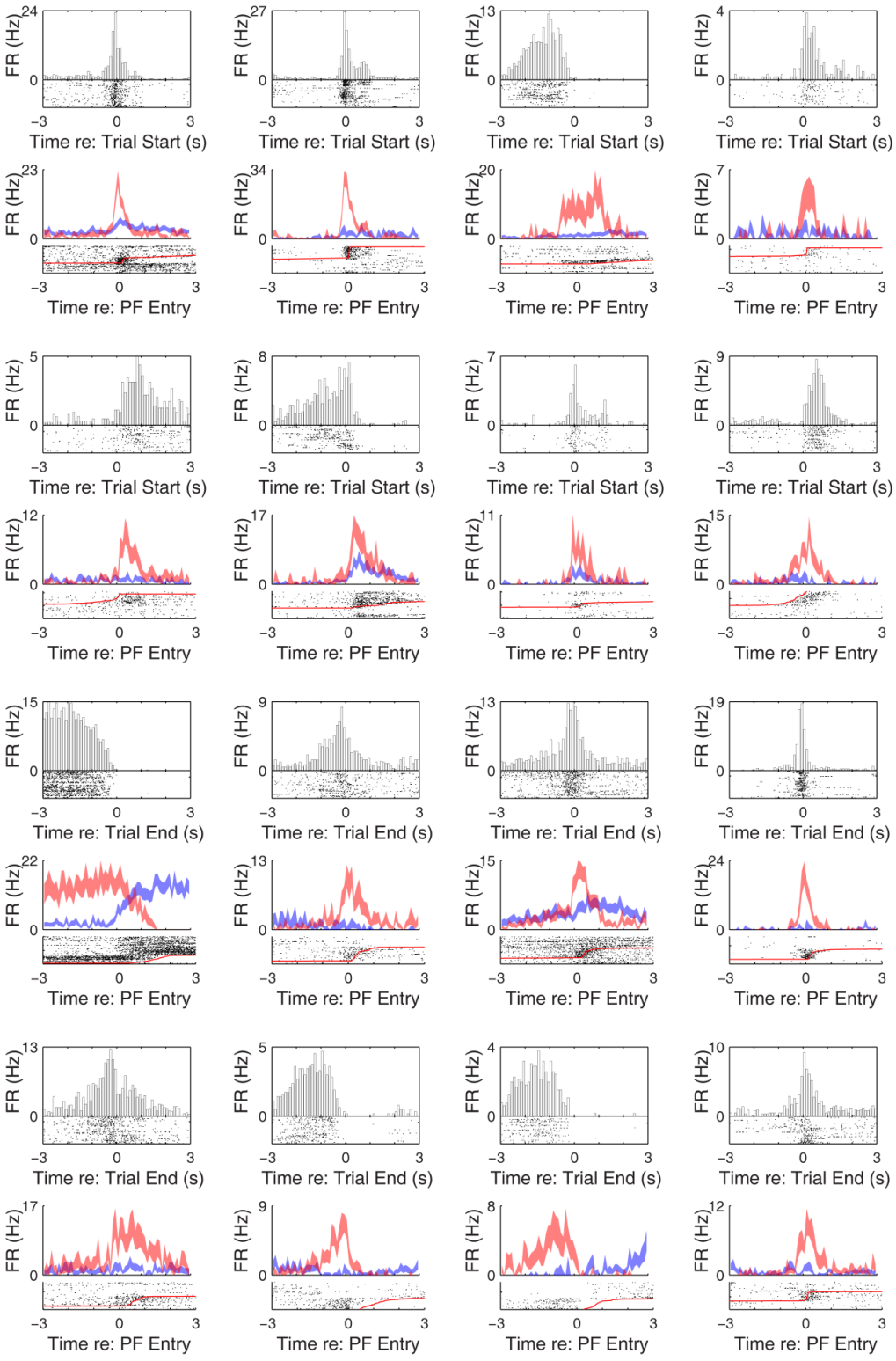
includes all putative pyramidal cells recorded from DC rats. Firing rates are scaled to the minima and maxima for each unit/session.


A
Firing Rate (Hz)
Time re: Trial Start (s)
-3 -2 -1 0 1 2 3
A_{final}
$B_{initial}$
B
Firing Rate (Hz)
Time re: Trial End (s)
-3 -2 -1 0 1 2 3

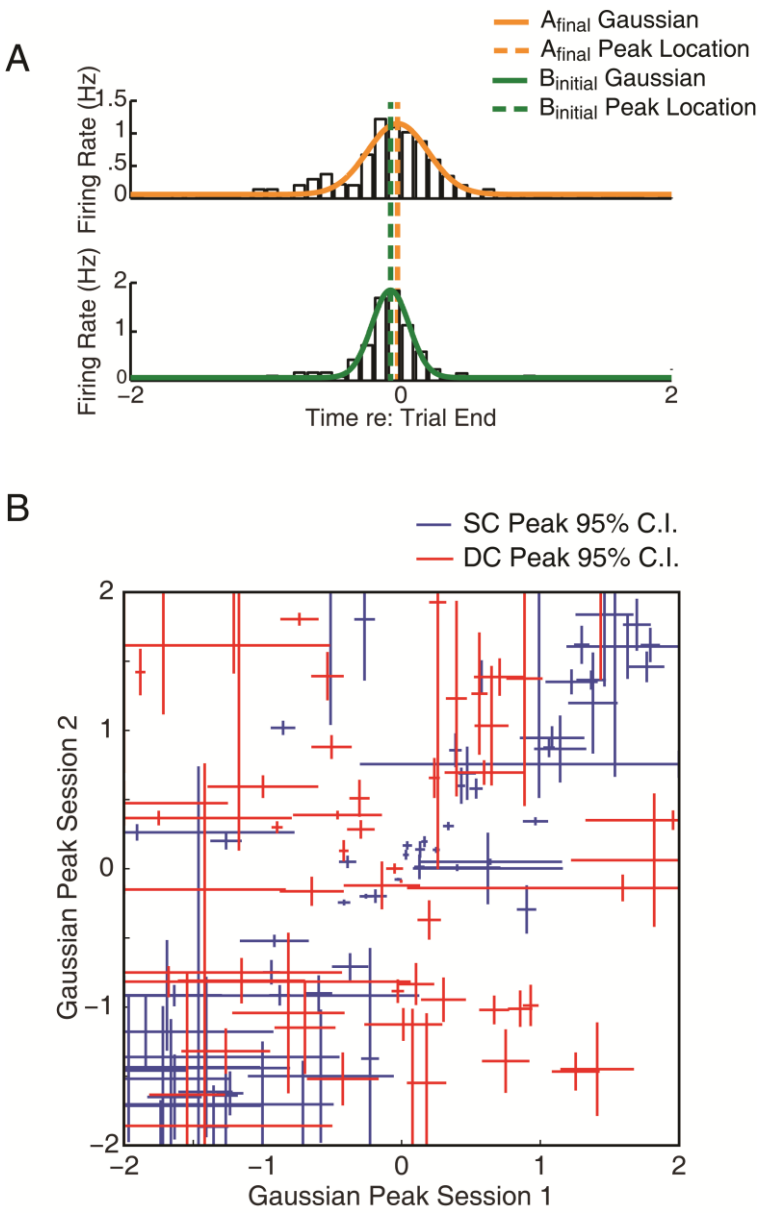
Supplemental Figure 4. Event Aligned PETHs of All SC Units. Each panel shows event aligned histograms depicting the average event-locked firing rates on A_{final} (orange) and $B_{initial}$ (green). The figure includes all putative pyramidal cells recorded from SC rats. (A) shows data aligned on the start of trials (when the rat crossed from the inter-trial waiting area to discrimination training area), while (B) shows data aligned on the end of the trials (when the rat returned to the inter-trial waiting area). For simplicity, the data are shown aligned to two time points, the trial start and end, plus or minus 3 seconds. However, neurons responded to a variety of task events encompassed by these epochs, including the approach to the cups, odor sampling, reward retrieval, etc. The units depicted in these panels correspond to those displayed in Supplemental Figure 2.


A
Firing Rate (Hz)
-3 -2 -1 0 1 2 3
Time re: Trial Start (s)
A final
B initial
B
Firing Rate (Hz)
-3 -2 -1 0 1 2 3
Time re: Trial End (s)

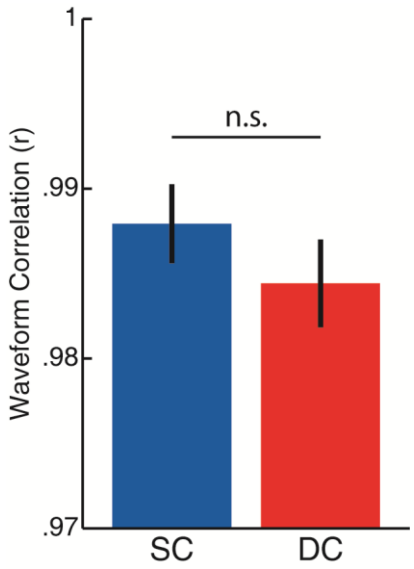
Supplemental Figure 5. Event Aligned PETHs of All DC Units. Each panel shows event aligned histograms depicting the average event-locked firing rates on A_{final} (orange) and $B_{initial}$ (green). The figure includes all putative pyramidal cells recorded from DC rats. (A) shows data aligned on the start of trials (when the rat crossed from the inter-trial waiting area to discrimination training area), while (B) shows data aligned on the end of the trials (when the rat returned to the inter-trial waiting area). The units depicted in these panels correspond to those displayed in Supplemental Figure 3.



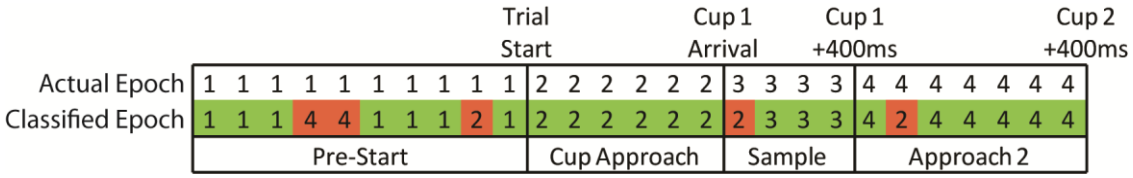
Supplemental Figure 6. Example Event Responses. Example PETHs and raster plots aligned on the start and end of trials (top panel in each pair) and aligned on entry into the corresponding place field (bottom panels in each pair) for 12 example units. Plotting conventions follow the insets in Figure 3 A-B.



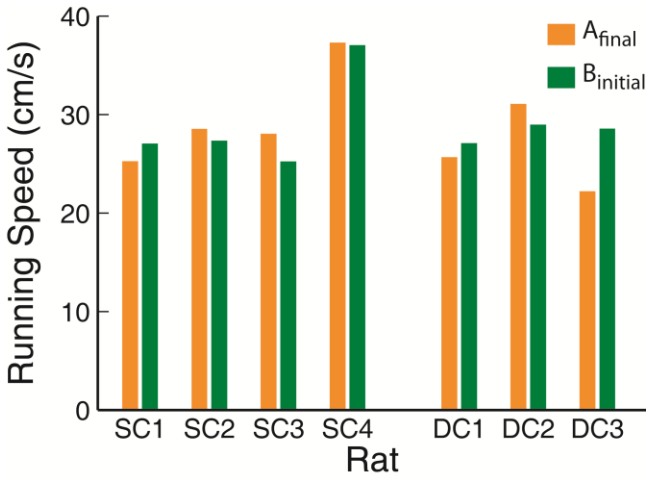
Supplemental Figure 7. Event COM Shifts. Panel A illustrates Gaussian fits to PETHs for an example unit recorded from an SC rat. The COM was defined as the peak location of the Gaussian (broken vertical lines), for units that had Gaussian fits with positive amplitudes (95% confidence intervals > 0) in both sessions. Panel B shows 95% confidence intervals on estimates of the event COM for SC rats (blue) and DC rats (red). Note that data from SC rats frequently falls near unity (i.e. similar peak locations across sessions) whereas DC rats show no relationship across sessions (SC: r=.82; DC r=-.07) .



Supplemental Figure 8. Correlation between Spike Waveforms. Matching of units across sessions was performed by applying the cluster boundaries of one session to the next, and then manually adjusting them to account for drift. The clusters and average waveforms were than examined to be sure that they contained data from the same unit. The waveforms were also compared using Pearson correlation across sessions (matching time and tetrode wire) to quantify the extent to which they showed similar waveforms. No difference was observed between SC and DC waveform correlations ($T[97]=1.01;p=.32$), and the average values were similar to those previously reported for putatively stable neurons (see, for example: Feingold et al., 2012). All waveforms showed a r values > 0.92.



Supplemental Figure 9. Example Epoch Trial Classification. An example trial classification result to illustrate the epochs used to train and test the classifier. Epochs were flanked by 5 temporal events: 1s before the trial started, the trial start (when the rat crossed an imaginary line just beyond the divider location), the arrival at the first cup (when the rat's nose crossed the rim of the cup), a fixed 400ms period following the cup arrival, and a period extending to 400ms following the arrival at the second cup (for those trials where the rat visited both cups). Data were divided into 100ms bins, and classified using linear discriminant analysis. The periods correspond to the named epochs on the bottom of the figure.



Supplemental Figure 10. Running Speed across Sessions. The average running speed for each rat on each session is plotted. No systematic differences were seen between average running speed in SC or DC rats.

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