THE ROLE OF HIPPOCAMPAL LONG-TERM DEPRESSION IN NOVEL SPATIAL EXPLORATION

by

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Abstract

The exploration and encoding of a novel environment is a fundamental learning process that occurs on a short time scale, and is a useful model for studying how the hippocampus encodes and represents complex and arbitrary associations, as is required for episodic memory. Novelty exploration has been demonstrated to promote long-term depression (LTD) induction in area CA1 of the hippocampus, but it is unclear what role this LTD plays as the novel space becomes familiarized and encoded in the hippocampus.

In order to determine whether de novo LTD occurs during novelty exploration, we utilized multi-array electrophysiological recording in freely moving rats and demonstrated an AMPA receptor endocytosis-dependent LTD in CA1 in the absence of a paired LTD induction protocol. To determine what role this LTD played in forming spatial representations, we recorded the spiking activity of multiple single units in hippocampal CA1 using multi-tetrode electrophysiological recordings to observe the development of place field firing in a novel environment, and the effect of LTD blockade using an inhibitor of AMPAR endocytosis. Place fields formed in the presence of LTD blockade, however the maintenance of place field firing location between the novel environmental exposure and re-exposure one day later was impaired by inhibition of LTD.

To investigate the dynamics of place field formation over the first several minutes of exposure, hippocampal neurons were recorded during exposure to a novel linear environment. While fields developed and stabilized over several minutes in control rats, we found that LTD blockade produced a rapid establishment of stable place fields after a single lap, suggesting the dynamics of field formation are altered with LTD blockade.

To test the role of this LTD in novel spatial learning, the effect of LTD blockade on contextual fear was assessed using a modification of inhibitory avoidance training that separated the acquisition of contextual information from the pairing with an aversive stimulus. This demonstrated that LTD is required on the first exposure to a novel context.

These results place the activity dependent weakening of synapses as a central process in the rapid acquisition of novel spatial information in the hippocampus.

Lay Summary

The memory of personal experiences is called episodic memory. This type of memory, which involves specific events and places, depends on an area of the brain called the hippocampus. The connections between brain cells allows the brain to processes information, and these connections can be strengthened or weakened, which changes the way information is processed and might lead to memory. In rodents, the brain cells that make up the hippocampus are known to signal to other cells when in a specific location within an environment. We found that many connections quickly weaken the first time a rat explores a new environment, and found that blocking this weakening changes the location that cells signal the next time a rat enters the same environment. We suggest that synaptic weakening is part of the process that allows reliable spatial memory to form and persist, and therefor might also allow episodic memories to form and persist.

Preface

The studies in this dissertation were designed by me and my supervisor, Dr. Yu Tian Wang. I conducted all experiments, including surgeries, electrophysiological recordings, behavioral studies, and data analysis. Methods and approaches for data analysis were suggested and guided by Dr. Yu Tian Wang, Dr. Jeremy Seamans, and Dr. Anthony Phillips. Some recording probes used for unit recording were constructed by Jamie Grewal (Seamans Lab).

The experiments using animals were carried out in accordance with the Canadian Council of Animal Care, and with the approval of the Animal Care Committee at the University of British Columbia (A12-0157;A16-0118).

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AMPA-R/GluA-R	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
APV/AP5	amino-5-phosphonopentanoic acid
CA1	Cornu Ammonis Area 1
CA3	Cornu Ammonis Area 3
CAMKII	Calcium/calmodulin-dependent protein kinase II
CFC	Contextual Fear Conditioning
CPP	(+/-)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid
D1/5-R	Dopamine d1/5 receptor
DG	Dentate gyrus
EC	Entorhinal cortex
EEG	Electroencephalography
fEPSP	field Excitatory Post-synaptic Potential
GFP	Green Fluorescent Protein
GluA1/2/3/4	AMPA receptor subunit 1/2/3/4
GPCR	G-protein coupled receptor
ICV	Intracerebroventricular
IV	Intravenous
KO	Knock-out
LEA/LEC	Lateral entorhinal area/cortex
LFP	Local field potential
LTD	Long-term depression
LTP	Long-term potentiation
MEA/MEC	Medial entorhinal area/cortex
MTL	Medial Temporal Lobe
NAcc	Nucleus Accumbens
NiCr	Nickel Chromium
NMDAR/GluNR	N-methyl-D-aspartate receptor
NR1/2A/B/C/D	NMDA receptor subunit 1/2A/B/C/D
PFC	Prefrontal Cortex
PKA	Protein Kinase A
PP	Perforant Path
SPM	Synaptic Plasticity and Memory
SRF	Serum Response Factor
VTA	Ventral Tegmental Area

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This work was completed with the patient supervision of Dr. Yu Tian Wang, and supported by my committee members Dr. Anthony Phillips, Dr. Jeremy Seamans, and Dr. Stan Floresco, all of whom engaged enthusiastically with every graph and scrap of data, despite some truly long and meandering presentations.

I would like to acknowledge all the members of the Wang Lab, past and present, along with members of the Seamans Lab and Phillips Lab, many who I have known for a long time and count among my closest friends.

I am grateful to my father David and my sister Elizabeth. They have supported me through difficult times, have learned to ignore my temperament, and will have to continue to ignore it. I would also like to thank my father-in-law and mother-in-law Ian and Colleen for their support and good humor.

Finally I am most of all indebted to my wife Janel, with whom I am lucky to share my life.

This dissertation is dedicated to the memory of my mother Jill McIntyre, who died a few weeks after I started this work, and has been missed every day since then.

Chapter 1 Introduction

Memory allows adaptive interaction with the physical world. It is what allows you to navigate home and open the door when you arrive. Memory lets you ride a bike and remember a tune, and somehow memory lets you remember what you had for breakfast along with what you didn't get for your birthday at eleven years old. Although there is no immediate life-dependent need for the brain to store and access memories, the prevalence of mnemonic mechanisms throughout higher vertebrate species points to the importance that such an ability can have to the success of a species and the individuals therein.

The study of memory in the mammalian brain over the past century has yielded a model in which distinct anatomical regions of the brain subserve different memory systems that act in parallel and interact in numerous ways to produce adaptive behavior. In different circumstances different memory systems will dominate the behavioral response, but most experiences will drive storage in multiple memory systems, with the nature of the memory stored from the same experience differing depending on the system.

This functional segregation of memory was one in a set of early observations regarding the segregation of cognitive processes that formed the basis of neuroscience. Although basic sensory and motor function segregation, along with language facility were convincingly demonstrated to be anatomically localized in the brain, early studies suggested that no such localization existed for memory, and memory was best thought of not as a specific function of the brain but an emergent property of sensory processing and thought.

The seminal work with HM and other medial temporal lobe (MTL) resectioned patients (Scoville & Milner, 1957) demonstrated that memory can be specifically impaired without

impairing general intellectual or sensorimotor function, and further dissociated types of memory: MTL resectioning produced retrograde amnesia, impairing the conscious recall of facts and experiences, and impaired the ability to remember new experiences (anterograde amnesia). This deficit in declarative memory contrasted with relatively intact non-declarative memory, including the procedural learning of motor and perceptual skills, along with short-term sustained attention and simple associative learning. The memory impairment spanned sensory modalities and was seemingly not restricted to any knowledge domains, suggesting that this declarative memory system represented a truly distinct cognitive function. Although generally impaired in amnesics, the declarative memory domain was theoretically separated in psychology into episodic and semantic memory (Tulving, 1972). This separated the conscious recall of personal experiences, which had an autobiographical index and temporal context, from the recollection of facts that lacked temporal context or an autobiographical context.

In general, the segregation of multiple memory systems anatomically suggests that cognitive processes fundamentally emerge because of the anatomical structure of particular brain areas and the functional activity of the cells within the structure. This thesis is concerned with understanding the structural and functional basis of hippocampal memory. In particular it will examine three functional properties in one area of the hippocampus and how they might relate together to produce memory; the role of hippocampal area CA1 in the acquisition of novel information, the place specific firing properties of pyramidal cells in area CA1, and the description of activity dependent modification of synaptic strength in area CA1 as a mechanism for memory.

1.1 The Hippocampus as the Locus of Episodic Memory

1.1.1 The features of hippocampal memory in humans and animals

The study of early amensiacs consistently implicated the medial temporal lobe as the locus of a particular type of memory impairment that would gradually become defined as declarative memory. However the lesions in most patients encompassed a broad and varying set of anatomical structures, making it unclear whether a subset of structures contributed predominately to the memory impairments, and even to what degree the memory impairments were a unitary phenomenon. HM, for example, had a resectioning that included not only the hippocampus bilaterally, but the amygdala and a significant amount of cortex adjacent to the hippocampus (Scoville & Milner, 1957). Although careful study of the amnesic profile of other patients contributed evidence suggesting the central role of the hippocampus (Zola-Morgan, Squire, & Amaral, 1986), experimental lesions in animals were necessary to fully explore the cognitive functions of medial temporal lobe. Although the primary challenge in these non-human experiments is to identify homologous memory tasks that can be performed by animals, it is also necessary to consider the comparative neuroanatomy between humans and the two animal models used most extensively in memory research, non-human primates and rodents.

Comparative anatomy of the medial temporal lobe in humans and animals

The hippocampus is centered around the trisynaptic loop (Cajal, 1893), a canonically one directional excitatory circuit that begins with perforant path inputs from layer 2 of entorhinal cortex (EC) to granule cells of the dentate gyrus (DG). Mossy fibres from dentate gyrus granule cells input to CA3 pyramidal cells, and schaffer collateral projections from CA3 to CA1 pyramidal cells (Amaral & Lavenex, 2009). CA1 completes the loop by projecting to entorhinal cortex both directly and via the subiculum (Fig 1.1).

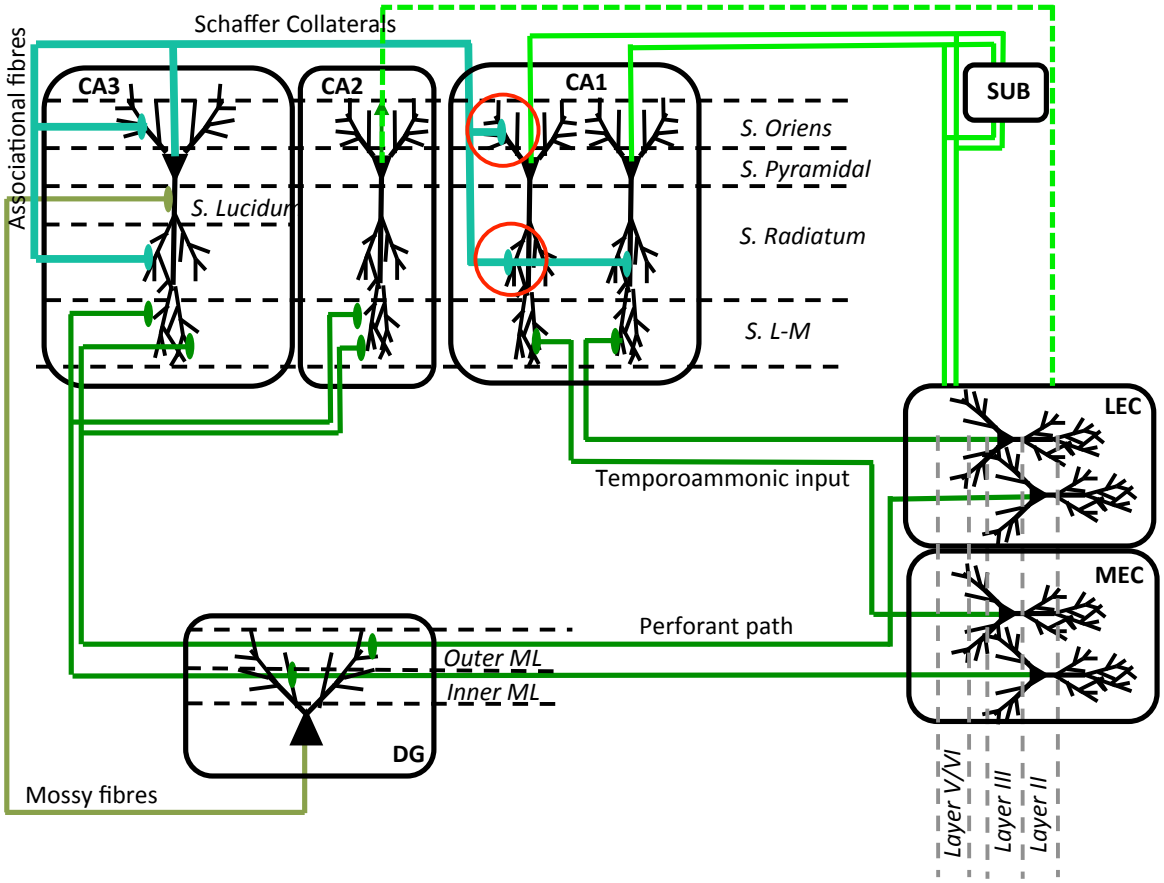

Figure 1.1 Schematic of major excitatory hippocampal circuitry.

Major cortical input orginates from the entorhinal cortex (EC), with segregation both between medial (MEC) and lateral (LEC) and between layer 2 and layer 3. The dentate gyrus granule cells (DG-GCs) recieves a single major excitatory input from Layer 2 entorhinal cortex, with LEC and MEC inputs segregated to the outer and inner molecular layer (ML), respectively. CA3 pyramidal cells receive three major excitatory inputs. Mossy fibre inputs from DG-GCs synapse close to the cell body of CA3 pyramidal neurons in stratum lucidum. Other CA3 cells input via associational-commisural fibres that synapse on apical dendrites in stratum radiatum and basal dendrites in stratum oriens. Layer 2 EC cells synapse on distal apical dendrites in stratum lacunosum-moleculare (L-M), with segregation of LEC and MEC along the dendritic arbor. CA1 pyramidal cells receive two major excitatory inputs. CA3 neurons input to CA1 via schaffer collateral fibres on basal dendrites in stratum oriens and apical dendrites in stratum radiatum and are the focus of the current research, circled in red. Layer 3 EC inputs via the temporoammonic direct path to apical dendrites in stratum lacunosum-moleculare. LEC and MEC are topographically organized, with LEC inputs targeting distal CA1 close to the subiculum and MEC inputs

targeting proximal CA1 close to CA2. CA1 outputs return to the entorhinal cortex both directly and via subiculum, in addition to non-cortical outputs via the fornix. CA2 is a functionally distinct region from CA3, receiving less mossy fibre input and limited CA3 input, with major input from MEC. In addition to inputs to CA1, CA2 has a proposed direct input to EC (dashed line).

There are several other major anatomical connections thought to mediate information processing. In addition to mossy fibres from DG, CA3 neurons contain two further major excitatory inputs. Cortical inputs directly from Layer 2 EC cells target apical dendrites in stratum lacunosum-moleculare. Association-commisural fibres from numerous other CA3 pyramidal cells synapse in stratum radiatum and stratum oriens, forming a recurrent network within CA3. Area CA1 receives a second major excitatory input, a direct cortical input from layer 3 EC via the temporoammonic pathway. (Fig 1.1) Although more detailed anatomical connectivity diverges between species, this core hippocampal formation network is remarkably preserved in most mammals (Insausti, 1993).

More varied is the organization of cortical inputs, which were commonly removed in early medial temporal lobe surgeries. In humans and non-human primates that have cortical gyrification, these cortical areas are mostly contained within the parahippocampal gyrus, containing both the entorhinal cortex and the parahippocampal areas TF and TH along with the anteriorly positioned perirhinal cortex (Insausti, Amaral, & Cowan, 1987).

Numerous macaque and rodent lesion studies carried out following the description of human amnesia produced conflicting results (Horel, 1978): Hippocampal lesions in Rhesus Macaques produced deficits that were not nearly as severe as those observed in humans, and rodent hippocampal lesions produced a variety of behavioral deficits that did not map clearly onto the deficits observed in human patients. However a series of experiments beginning in the late 1970s (Mishkin, 1978) identified a severe and specific deficit on the delayed non-match to sample task in macaques with large hippocampal and amygdala lesions that most credibly

replicated the most severely amnesic patients. A central conclusion of these studies is that the full amnestic profile originally described as dependent on the hippocampus is only observed when a hippocampal lesion is extended from the hippocampus proper to include the parahippocampal areas, the entorhinal cortex, and the perirhinal cortex (Zola-Morgan et al., 1989). The role of cortical inputs to the hippocampus is not restricted to a highly processed, polymodal sensory input, but includes independent mnemonic functions that likely interact with the mnemonic functions of the hippocampus to produce human declarative memory. Any animal model of human memory can therefor only be expected to model certain features, depending on the task parameters.

How do the memory effects observed in humans and non-human primates translate to the function of the rodent hippocamapal memory system? On a connectivity level, some differences between rodent and primate cortical inputs bear mentioning. Although both the perirhinal cortex and parahippocampal areas have analogs in the rodent (the perirhinal cortex is identifiable in both species, while the postrhinal cortex is homologous for the primate parahippocampal areas), there are more significant additional sensory inputs to the entorhinal cortex in the rat than in primates (Suzuki & Amaral, 1994; Burwell & Amaral, 1998; Insausti et al., 2002). These input differences may produce notable difference in memory performance between species, but anatomical studies in both species indicate a common organizing principle: Spatial information converging on the postrhinal cortex (areas TF and TH in macaques) projects preferentially to the medial entrohinal area (MEA), while non-spatial information converges on the perirhinal cortex and projects preferentially to the lateral entorhinal area (LEA). This functional segregation is carried into the hippocampus, with lateral and medial inputs targeting different layers of DG and CA3, with lateral EC inputs superficial to medial EC inputs on dendrites of granule cells and

pyramidal cells. In contrast, this segregation is maintained topographically in the temporoammonic input to CA1, with distal CA1 neurons (near the subiculum border) receiving LEA inputs, while more proximal CA1 receives MEA inputs (Van Strien et al., 2009, Fig 1.1).

The common organizing principles observed between species suggests homologous functions in the rodent as in the human and non-human primate hippocampus. However even more so than in primates, valid characterization of behavioral tasks suitable for rodents that model key aspects of human medial temporal lobe memory processes has been a challenging endeavor.

The focus of the researched summarized above and below is on the dorsal horn of the hippocampus, however it is widely understood that functional divisions exist along the dorsal-ventral extent of the hippocampus. These can be observed with respect to both the topography of cortical inputs and particularly with regards to the output targets of CA1 and subiculum (Strange et al., 2014). While dorsal hippocampus outputs to many cognitive cortical targets, the ventral hippocampus has privileged output to areas of the brain known to mediate emotion, particularly the hypothalamus and amygdala (Strange et al., 2014). CA1 and CA3 pyramidal cells shows more spatially specific place cells in dorsal versus ventral hippocampus, and the most marked behavioral impairments on spatial memory tasks are observed with dorsal hippocampal lesions. Based on these observations, it has been suggested that there is a putative functional gradient from cognitive to emotional processing from dorsal to ventral hippocampus (Moser & Moser, 1998). It is increasingly clear that gene expression differences correspond to this gradient, and further indicate that more fine-grained functional gradients may exist (Faneslow, 2010; van Strien et al., 2009; Strange et al., 2014; Insausti, 2017).

1.1.2 Rodent spatial memory as a model of episodic-like memory

A critical question concerns the description of specific features of declarative memory that may be identifiable and measurable in rodents. As constructs, semantic and episodic memory have many features that are closely tied to distinctly human attributes (Tulving & Murray, 1985; Tulving, 2002). Semantic memory concerns facts or concepts about the world acquired through experience but not tied to a particular experience. In humans, it is closely tied to the communication of that knowledge, making it difficult to conceive of assessments that address an analogous process in rodents, where measurement is constrained to behavioral expression. Episodic memory refers to the acquisition and recall of specific experiences that are associated with spatial and/or temporal context and are autobiographical in nature. There are attributes of episodic memory that are difficult to infer in animal models, particularly autonoetic consciousness, which concerns the mental time travel and imaginative aspects of episodic memory. However animal models are not required to be complete to be useful, and there are many aspects of episodic memory that are amenable to modeling in rodents. Behavior modified by a single unique experience, associated with a particular spatial and temporal context, incorporates many cardinal features of episodic memory, and has been the primary focus of cross-species memory research (McGaugh, 2000; LeDoux, 2000; Eichenbaum, 2000; R. G. Morris et al., 2003).

As a second issue, the choice of measure can itself affect what memory system is being utilized to direct behavior: As considerable research has established, typical MTL amnesiacs show normal skill and procedural learning in the absence of explicit recognition of the task or parameters (Cohen & Squire, 1980), suggesting that analogous tasks in rodents could presumably be performed without hippocampal memory system contributions. Rodent memory is inferred by

measuring a behavioral output, which can lead to both false negatives and false positive: A real memory deficit could be obscured by behavioral performance being served by an alternative mnemonic system, or (more commonly) a behavioral deficit due to an experimental intervention that is ascribed to memory impairment could in fact be due to some deficit in the performance of the behavior unrelated to memory. Training effects are often a confounding factor in this way, as training animals to baseline levels of performance on a specific behavioral task raises the potential for behavioral strategies that are not mediated by the hippocampus to predominate.

Working memory and spatial correlates of hippocampal lesions

Early research using hippocampal lesions in rodents revealed a variety of behavioral deficits that did not map clearly onto the declarative memory deficits observed in human patients, including elevated activity levels (Kimble, 1963), passive avoidance (Isaacson & Wickelgren, 1962), and reversal learning (Kimble & Kimble, 1965). The development of the radial arm maze (Olton & Samuelson, 1976) and the specific impairments on random foraging behavior observed with hippocampal lesions (Olton & Werz, 1978; Olton, 1979) led to the briefly influential idea that in rats the hippocampus subserved working memory (Olton, 1979). This working memory – reference memory framework was applied to explain how rodents with hippocampal lesions were impaired on a version of the radial maze that rewarded foraging behavior (win-shift strategies) but not on a version of the maze that consistently paired reward with specific locations (win-stay strategy).

The discovery of place specific firing in hippocampal cells presented an alternative interpretation, that working memory impairments on the radial arm maze were in fact driven by a spatial memory impairment (O'Keefe & Dostrovsky, 1971; O'Keefe et al., 1975). A framework for reinterpreting the behavioral deficits initially presented by Olton (1979) was provided by

Packard and colleagues (1989), who demonstrated that win-shift and win-stay performance are dissociable based on lesion location: hippocampal lesions impaired win-shift performance but not win-stay performance, whereas caudate lesions impaired win-stay performance but not win-shift performance. In this new framework, it was assumed that hippocampal lesions affected behavior in cases where successful task performance depended on navigation within space using sets of external sensory cues. The development of the water maze and the specific impairment observed in the hidden platform version in rats with hippocampal lesions (Morris et al., 1982) supported the spatial map interpretation.

Contextual Memory

Contextual fear conditioning (CFC) is a learning paradigm wherein animals exhibit a conditioned emotional response (typically measured with freezing behavior) to a context in which they experienced an aversive stimulus. CFC is a simple associative learning process, but similar to spatial navigation tasks, good task performance requires the binding of external sensory cues into a context that can drive behavior (Fanselow, 1980). Intriguingly, dorsal hippocampal lesions that impair spatial navigation were shown to also specifically impair contextual freezing after fear conditioning, without affecting auditory cue fear conditioning (Phillips & LeDoux, 1992; Kim & Fanselow, 1992). A similar result was seen in recognition of spatial novelty, in which a natural exploratory preference is seen not only for novel objects within an environment (Ennaceur & Delacour, 1988), but also for familiar objects in novel locations within an environment. Whereas hippocampal lesions only mildly affect recognition for novel objects, the same lesions cause significant impairment when rats are given the opportunity to re-explore objects in new locations or configurations (Save et al., 1992; Ennaceur, Neave, & Aggleton, 1997). Changes in exploration of novel environments are also observed in

hippocampal lesioned animals who show increased motor activity, although these effects are not specific to novelty and are observed in reaction to most environments and stimuli (Gray & McNaughton, 1983; Whishaw et al., 1994).

Non-spatial descriptions of hippocampal memory

These observations supported the hypothesis that the hippocampus is crucial in representing spatial environments. The more broad range of mnemonic functions encompassed by human episodic memory suggests, however, that a purely spatial description of hippocampal memory processes is incomplete. Indeed even in rodents, considerable work has established a role for the hippocampus in learning and behavior that is decidedly non-spatial, particularly when tasks involve temporal relationships (Chiba, Kesner, & Reynolds, 1994; Kim, Clark, & Thompson, 1995), or complex associations between cues (Bunsey & Eichenbaum, 1996). In this view, the hippocampus is not dedicated to representing space, but is better described as a system for representing and rapidly storing associations between sets of stimuli in different domains, of which spatial relationships is just one prominent example (Eichenbaum, 1996; Eichenbaum & Cohen, 2014). This may be a more accurate and complete description of the range of possible functions, especially considering that the information processing occurring in the hippocampus will change depending on the sensory information it receives, which differs between species even when the architecture of the hippocampus itself is quite similar (Insausti, 1993; Manns & Eichenbaum, 2006). However even human episodic memory is strongly influence by context (Maguire & Mullally, 2013), so examining the processes that contribute to developing a coherent representation of a spatial context should have relevance to understanding the general mechanisms responsible for episodic memory formation.

Subregional specialization of mnemonic function.

The primary hippocampal subregions have very different connectivity, and their respective contributions to overall information processing within the hippocampus is an area of both theoretical and experiment focus. As information flow in the hippocampus is primarily one directional and the majority of outputs are routed through CA1, subregion specific lesions will affect both computations that are mediated by the target region as well as any processes by non-target regions whose output or input depends on the target region. In combination with assessment at the behavioral level, where compensatory mechanisms (Lee & Kesner, 2003) and other behavioral strategies may mask deficits, only the most broadly independent functions may be discernible with subregion specific lesions. Consequently, novelty detection (Lee, Hunsaker & Kesner, 2005) and contextual fear conditioning tasks (Lee & Kesner, 2004) are impaired by lesions of CA1, CA3, or DG, although a slightly reduced effect of CA3 lesions on contextual fear conditioning is reported. Pattern separation and pattern completion computations are strongly linked to DG and CA3, respectively (Rolls, 2013; Neuneubel & Knierem, 2014), however behavioral assessments may be unable to distinguish these computational roles.

1.1.3 Spatial novelty as a model of hippocampal memory formation

Exposure to a novel environment represents an attractive model with which to study the formation of an episodic-like memory. It requires only a single exposure to produce long lasting memory and does not depend on pairing with appetitive or aversive stimuli. Although more sophisticated behavioral paradigms have been developed that may recapitulate more features of human episodic memory (Clayton & Dickson, 1998; Day & Morris, 2003), encoding a novel environment is a necessary precursor to performance on these tasks. As detailed above, the encoding and retrieval of a novel environment memory depends on the hippocampus (Gray &

McNaughton, 1983; Save et al., 1992; Whishaw et al., 1994). Rats introduced to a novel environment or a novel spatial arrangement of cues (Poucet et al., 1986) exhibit increased locomotion (Renner, 1990) and rearing (Lever, Burton, & O'Keefe, 2006), along with attentive scanning (Golani, Benjamini, & Eilam, 1993; Whishaw et al., 1994; Drai et al., 2001) for a transient initial period. This exploration is necessary for successful navigation (Whishaw & Brooks, 1999), latent learning (Tolman, 1948; Keith & McVety, 1988) and association of an environmental context with salient stimuli (Fanselow, 1986; Fanselow, 1990). Although simple, this behavioral reaction requires the recognition of the environment as novel (Brown & Aggleton, 2001), engagement of exploratory behaviors driven by novelty recognition (Mogenson & Yang, 1991), and then disengagement of those same behaviors, presumable after some representation of the environment has been established. This representation would then need to be maintained, such that a re-exposure to the environment would not trigger the same novelty response on future exposures. Various aspects of the physiological basis for this process have been intensively studied, and several well-developed theoretical ideas have also been articulated (Vinogradova, 2001; Lisman & Otmakhova, 2001; Lisman & Grace, 2005; Rolls, 2010; Otmakhova et al., 2013). In general, these theories position the hippocampus as a novelty or mismatch detector, where an unexpected or unfamiliar context provokes signaling from the hippocampus to the Nucleus Accumbens (NAcc). This activation in the NAcc in turn enhances VTA dopaminergic cell activity, increasing dopamine efflux in the NAcc, along with other targets of the VTA, including the hippocampus and PFC (Ihalainen, Riekkinen Jr, & Feenstra, 1999). Dopamine in the NAcc serves to promote exploratory behavior by its action on the Accumbens output to the mesecephalic motor region (Mogenson & Yang, 1991; Salamone, 1994; Wise, 2004; Salamone & Correa, 2012). Furthermore, the specific nature and direction of

the exploratory behavior is directly influenced by inputs to the NAcc, particularly the hippocampus, PFC, and amygdala. Simultaneously, dopamine efflux in the hippocampus is proposed to facilitate plasticity events that subserve learning (Lisman & Grace, 2005). This learning process would then negatively regulate the mismatch signal feeding forward from the subiculum to the NAcc, reducing the overall exploratory drive.

1.1.4 Outstanding questions

There continues to be numerous points that require clarification in such a model, including how mismatch detection is actually implemented in the architecture of the hippocampus and how a novelty signal is communicated from the hippocampus to promote exploration. The present thesis is primarily concerned with the role of synaptic plasticity in encoding an initially novel environment into memory. The behavioral consequences of novelty serve in part the creation of an enduring spatial representation that can subsequently be used to guide navigation and other adaptive behaviors. Regardless of whether spatial memory is a product of a more general mnemonic function (Eichenbaum & Cohen, 2014), or whether spatial representations are the organizing structure for episodic memory that recruits non-spatial components (O'keefe & Nadel, 1978), understanding the mechanisms by which environments and contexts are represented in the hippocampus should inform our knowledge of how the hippocampus serves general episodic memory.

1.2 Spatial Coding in the Hippocampus

To understand how the hippocampus might support memory functions, it is necessary to understand how information is encoded. Prior to the discovery of place-specific firing, hippocampal activity was understood to have various behavioral correlates, and a considerable amount of research focused on relating hippocampal eeg patterns to behavior (Vanderwolf, 1969; Issacson & Pribram, 1975). Additionally, single unit recording had shown hippocampal complex cells to have behavioral correlates including arousal/orienting responses (Vinogradova, 1975) and approach (Ranck, 1975). Although place firing was initially described as a new behavioral correlate seen in a subset of recorded hippocampal complex cells (O'Keefe & Dostrovsky, 1971; O'Keefe, 1976), it is understood today that the majority of pyramidal cells in CA3 and CA1 that fire in a given environment encode place information, although this does not preclude them from also encoding non-spatial information (Wood, Dudchenko, & Eichenbaum, 1999). The described firing pattern was stark: When a rat was within a spatial area a cell would have a high firing rate but be virtually silent outside of that area.

1.2.1 General features of place cells

The general features of place-specific firing were described by O'Keefe and other in the proceeding years, with a particular focus on examining their activity in relation to the cognitive map described by Tolman (1948)and expanded on by O'Keefe and Nadel (1978). As originally described by Tolman, the cognitive map enabled allocentric decision making: Reinforcing a set or sequence of behaviors implies that a reward location is defined relative to the idiocentric state/location of the animal, however real navigational strategies observed in rats suggested that information about the relative locations of landmarks, regardless of perspective, was being used to guide behavior. This implied an allocentric 'cognitive map' of these relations. Place cells, in

this conceptualization, provided an active read-out of a rat's current position within this representation.

Place cells were found to respond to multiple distal landmarks within an environment: Most fields were resistant to the removal of a single cue, though removing multiple cues would trigger a change in the location of the field (O'keefe & Conway, 1978; O'Keefe & Speakman, 1987)The same cells recorded in multiple environments would show fields with no discernable relation, even if different environments shared some similar external cues (Muller & Kubie, 1987)Place cells established in an environment continued to fire in a stable location when visual input was removed (Mcnaughton, Leonard, & Chen, 1989; Quirk, Muller, & Kubie, 1990), however if the rat was removed from an environment and replaced after the visual input was removed, a majority of firing fields change location. This set of observations supported the idea that hippocampal place cells were encoding place by using sensory cues generally, but without dependence on any single cue within an environment.

Sensory independence of place fields

Despite the clear evidence that place cells were responding in a manner more consistent with allocentric space coding than with high level sensory processing, a number of observations were inconsistent with a purely allocentric space coding account of place fields. Place fields recorded in two-dimensional environments did not generally show directional preference, firing regardless of the direction from which the rat entered the field. However when running a linear maze, many cells showed place firing exclusively in one direction of travel (McNaughton, Barnes, & O'keefe, 1983), contrary the predicted behavior in an allocentric account. In a study examining the source of this directionality, Markus and colleagues (1995) found that the same cell could exhibit different place fields within the same environment depending on the task

parameters: A rat randomly foraging within a circular arena showed generally non-directional place field, however when the task was changed to one in which the same rat sequentially visited four locations within the arena for food reward, which required stereotyped clockwise or counter-clockwise routes, place fields not only changed location, but also acquired directionality. Although the environment was consistent, the change in the path taken by the rat and the parameters of the behavioral task were sufficient to change place field locations. Although there are several interpretations of these results it strongly precludes the idea that place cells are purely coding for allocentric space using some combination of sensory inputs.

Path integration

Contemporaneously, head direction responsive cells were recorded in the presubiculum (Taube, Muller, & Ranck, 1990), and subsequently in other hippocampal input areas (Chen et al., 1994; Taube, 2007). These observations led to various influential computational accounts of place cells that focused around 'path integration' (Zhang, 1996; Touretzky & Redish, 1996; McNaughton et al., 1996). Path integration was originally a concept that accounted for the common behavioral observation that rodents could return to a home cage location with a direct path after an exploratory outbound journey that was often highly indirect (Mittelstaedt & Mittelstaedt, 1980). The explanation for this behavior was that rodents could keep an updated representation of their location relative to a reference point by integrating their linear and angular self-motion. Several observations supported the general idea that self-motion contributed to place field location: When allocentric cues and self motion cues were put in conflict, for example by altering the location of a start/end box during a trial, some cells showed fields indicative of self-motion coding, while others showed fields indicative of allocentric coding (Gothard et al., 1996; Redish et al., 2000). Similarly, altering the typical 1:1 relationship between self-motion and

visual cue flow in a virtual environment produces an intermediate shift in the location of place fields suggesting that place cells are using both inputs to compute place (Chen et al., 2013). Overall, although place fields code for allocentric space in typical environments, this space coding is highly dependent on self-motion through the environment, and may use sensory cues and landmarks to anchor place fields to a consistent location.

1.2.2 Do place fields show memory-like effects?

If hippocampal place coding is involved in memory, some features of the hippocampal code for space should be consistent in cases where spatial memory is maintained. This has typically been taken to mean that place fields should fire in the same location upon re-exploration of the same environment. The logic of this is relatively straightforward: A place field in one environment does not predict the location of firing from the same cell in a different environment (Muller, Kubie, & Ranck, 1987; Muller & Kubie, 1987), whereas place cells fire in consistent locations with repeated exposure to the same environment. In addition, environmental changes that trigger the remapping of place fields to different locations also trigger behavioral changes associated with novelty response, suggesting that since changing place field locations is associated with the detection of an environmental change, a consistent environment should also be associated with consistent place field locations (Lenck-Santini et al., 2005; Wells et al., 2009). The place field location for the same cell recorded across days tends to be consistent (Thompson & Best, 1990; Lever et al., 2002; Cacucci et al., 2007). There may be some bias in these observations, since identifying the same cell across days using traditional electrophysiology requires that the cell fire enough in each session to be sorted. Recent work using two-photon endoscopic recoding of calcium transients suggest that cells with a place field frequently drop in and out of the ensemble code for space in repeated exposures, though tend to fire in a consistent

location, such that place information is maintained at the ensemble level, if not on a cell-by-cell basis (Ziv et al., 2013). Although broadly consistent, these effects are different enough from electrophysiology recordings in rats that it remains to be determined whether place fields in rats would show the same variability between exposures: This variability may reflect poorer memory function in mice as compared to rats, who show more enduring spatial memory performance behaviorally.

Perhaps most importantly, considerable evidence suggests that field location is used for spatial navigation. Experimental manipulations that cause a coherent shift of place fields, typically by removing or rotating major cues produced behavioral deficits that corresponded to the shift of place fields (O'Keefe & Speakman, 1987; Markus et al., 1994; Lenck-Santini, Save, & Poucet, 2001). This suggests that place field firing constitutes a map that is guiding navigation.

1.2.3 What are the inputs to area CA1 that support hippocampal place fields?

Place field firing must be a consequence of the excitatory and inhibitory inputs, along with the excitation-spike coupling of these inputs, to the recorded cell. While the general sensory features that control place field firing have been established, how these computations are actually instantiated in the hippocampus and associated cortex is still actively being researched.

Major Anatomical Connections

Fundamentally, the anatomical connectivity of the hippocampus must support place field firing either intrinsically or in combination with synaptic plastic changes. Although the trisynaptic loop is understood as the backbone of hippocampal information flow, several other inputs and levels of organization greatly affect the nature of the information that is passed through the hippocampus. As in most neuroanatomy, there is nearly limitless complexity as these

circuits are studied in greater detail (Van Strien, Cappaert, & Witter, 2009), however the standard model of hippocampal connectivity represents both the major connections that drive place firing in pyramidal cells as well as the most thoroughly described connections on a functional level. It is important to note that this standard model of connectivity typically focuses on the dorsal hippocampal, ignoring the septo-temporal organization of connectivity. The functional segregation of the dorsal and ventral hippocampus is well understood and is seen notably in the extrinsic anatomical connectivity differences (Jay & Witter, 1991; Eichenbaum, 2017), place coding differences (Strange et al., 2014), and behavioral effects (Fanselow & Dong, 2010).

Area CA1 originates the majority of hippocampal outputs, which can broadly be separated into two routes: Long range projections via the fornix include targets to the nucleus accumbens, medial septum, and thalamus, while intrinsic return projections target the entorhinal cortex and other parahippocampal regions (Delatour & Witter, 2002; Van Strien et al., 2009). These connections are excitatory, and most include both direct CA1 projections along with projections via the subiculum. Backprojections from the targets of these efferents, from the entorhinal cortex to the perirhinal and postrhinal cortex, and from those regions to higher neocortical regions subserve the second major route by which hippocampal information is output (Witter et al., 2000; Lavenex & Amaral, 2000). The inputs to area CA1 are dominated by two pathways, the schaffer collateral pathway from CA3 and the direct input from entorhinal cortex, variously called the direct cortical input, the temporoammonic (TA) pathway, or the perforant path input to CA1 (Lorente de N 'o, Rafael, 1934; Remondes & Schuman, 2002). This input is distinguished from the traditional perforant pathway in that it originates predominately from layer 3 cells of the entorhinal cortex, rather than the layer 2 cells that predominate the perforant path input to dentate

gyrus and CA3 (Van Strien et al., 2009). In contrast to the schaffer collateral pathway that synapses onto apical dendrites in stratum radiatum and basal dendrites in stratum oriens, the TA input synapses deeper in the dendritic arbour of CA1 pyramidal cells in stratum lacunosum-molecular (Van Strien et al., 2009). The interaction between these two pathways in driving cellular activity in CA1 cells is an ongoing area of research (Kamondi, Acsady, & Buzsaki, 1998; Jarsky et al., 2005; Bittner et al., 2015).

Lesion effects on CA1 Place fields

Lesion studies have given considerable insight into the relative contribution of these two pathways, both towards place field firing and behavior that may depend on hippocampal coding. Early studies (Mizumori et al., 1989; McNaughton et al., 1989; Shapiro et al., 1989) found that while lesions to the fimbria-fornix output of the hippocampus affected the stability of local cues, impairing the information transferred to CA1 through the canonical trisynaptic path had no strong effect on CA1 place field firing, despite spatial navigation being impaired. The stability of CA1 place firing was confirmed in an experiment in which schaffer collateral inputs were surgically severed. A modest decrease in sparseness was the only observable change (Brun et al., 2002), suggesting that place field firing could be supported by direct input from the entorhinal cortex.

Lesion studies targeting the entorhinal cortex have broadly supported this conclusion, though there are several general difficulties in interpreting results from such experiments. First, the volume of tissue sending convergent cortical input to the hippocampus is considerable, meaning even large lesions could conceivably leave some inputs intact. Studies have typically focused on lesioning the dorsal medial entorhinal cortex, where the most spatially selective grid cells are found (Fyhn et al., 2004). In these studies, additional EC inputs that might preserve

spatial firing would be left intact. A second issue relates to the selectivity of a lesion of the direct input to CA1. To accomplish this without affecting the main EC input via the trisynaptic loop, the preferential input from layer 3 of entorhinal cortex directly to CA1 is targeted. However there is enough heterogeneity in these input pathways that some direct inputs would be preserved and some trisynaptic inputs would be affected. Nonetheless, this selective lesion of layer 3 medial entorhinal cortical neurons significantly affected place field firing in CA1, with larger place field sizes and less information density, although a subset of CA1 place fields were unaffected (Brun et al., 2008). A larger, non-selective lesion produced a similar place field size expansion and reduction in information density, along with an overall decrease in the number of CA1 cells that were active in an environment (Hales et al., 2014). A similar expansion of place fields was observed with reversible inactivation of different regions of MEC (Ormond & McNaughton, 2015). In all studies, although place information was degraded, it was not completely abolished, suggesting that residual information inputs could drive some degree of spatial firing.

If the schaffer collateral input from CA3 is not necessary for place field firing in CA1, what is the function of these thoroughly studied synapses on place cell activity? A genetically targeted blockade of schaffer collateral synaptic output onto CA1 demonstrated a specific impairment in the formation of place fields in a novel environment, with spatial information and field size approaching that of controls with subsequent exposures (Nakashiba et al., 2008). With more extended exposure to an environment as is typical for most place field studies, it is presumed that place representations in CA1 cells without CA3 input will become as specific and information rich as control fields (Brun et al., 2002). However the rapid acquisition of spatial information seems to be uniquely dependent on the schaffer collateral input to CA1.

In sum, considerable research has established that entorhinal cortex input is necessary and sufficient for accurate CA1 place field firing, provided that the represented environment is familiar. However in novel environments, the rapid formation of accurate place fields in CA1 is dependent on inputs from CA3. In the absence of these inputs, place fields gain information content and specificity over days.

1.2.4 Dynamics of place field formation in a novel environment

The mechanisms through which stable place fields rapidly form in a novel environment are still not fully understood. Although early research suggested that place fields were formed immediately the first time an animal entered the specified area (Hill, 1978), a more thorough examination showed that place fields form and stabilize gradually over the first several minutes of exploration of a new environment (Wilson & McNaughton, 1993; Tanila et al., 1997). Using a reconfigurable T-maze, a finer temporal resolution showed a variety of field formation dynamics that occurred primarily over the first 5-6 minutes of experience. In many cases cells would be initially silent throughout the maze and then rapidly form a place field that would stabilize over several more laps (Frank, Stanley, & Brown, 2004). Although not the only dynamic observed, this rapid formation dynamic has been associated with location specific rearing or scanning behavior that immediately precedes the appearance of a new place field (Monaco et al., 2014). New experimental techniques that make use of head fixed mice exploring virtual environments (Harvey et al., 2009) have observed similar dynamics (Epsztein, Brecht, & Lee, 2011; Lee, Lin, & Lee, 2012) and implicated dendritic plateau potentials and other nonlinearities in the rapid recruitment of new place fields (Bittner et al., 2015). Importantly, these reports support the idea that an active synaptic plasticity process is required even over the short time span in which this

occurs, as the subthreshold voltages recorded before, during, and after recruitment change dramatically.

The possible importance of synaptic plasticity to place field activity has of course been postulated virtually since their initial descriptions. The strongest evidence has typically come from using known inhibitors of synaptic plasticity, either pharmacologically or transgenically. The first major evidence came from a series of studies using mice with transgenic knockout of the NR1 subunit of the NMDA receptor in CA1 pyramidal cells (Tsien, Huerta, & Tonegawa, 1996; McHugh et al., 1996). This alteration produced spatial memory impairments and produced larger place fields, although spatial coding was still present and field location was consistent. Transgenic alteration of CAMKII that affects theta-burst LTP and LTD in the hippocampus (Mayford et al., 1995) similarly affected place fields, although an additional effect was instability in the location of place fields between trials (Rotenberg et al., 1996; Cho et al., 1998). These initial studies used mice, which tend to have less stable place fields than rats in control conditions (Hok et al., 2016), and did not look specifically at place fields in a novel environment, where fields may be most affected by plasticity impairments. A study addressing both of these shortcomings found that systemically administered CPP, a pharmacological blocker of NMDA receptors, impaired the maintenance of place field location across days, but did not affect the size of fields (Kentros et al., 1998). In some studies place field stability in a novel environment was affected by NMDA blockade on a shorter time scale between exposure and re-exposure, with 30 minutes sufficient to observed dissimilar maps between trials (see Shapiro & Eichenbaum, 1999). Similar results on the long-term stability of place fields in a novel environment were observed with a reduced PKA transgenic mouse (Rotenberg et al., 2000) and anisomycin administration

(Agnihotri et al., 2004), both manipulations implicating the maintenance of long-term synaptic plasticity.

1.2.5 Outstanding questions

There are two issues that bear mentioning in considering these results. Although they convincingly demonstrate that newly formed place fields depend on NMDA receptor dependent synaptic plasticity to maintain stability across days, it is not clear whether strengthening and/or weakening of synapses mediates this plasticity. Both experimentally induced LTP and LTD tend to depend on NMDA receptors for induction, and are supported by common downstream mediators such as CAMKII and protein synthesis. Additionally, the dynamics of place field formation suggest that an active learning process occurs during the acute exposure to a novel environment, however limited effects of plasticity blockers have been observed in the acute exposure to novelty: Place fields still formed, with no obvious differences compared with controls. It is possible that field formation occurs without plasticity changes (Rolls & Kesner, 2006; de Almeida, Idiart, & Lisman, 2009; Savelli & Knierim, 2010; Monaco & Abbott, 2011), however artificial induction of LTP provokes immediate remapping without affecting the spatial characteristics of the fields (Dragoi, Harris, & Buzsaki, 2003), suggesting that changing synaptic weights is fundamental determinant of active remapping. Considering how well-suited synaptic plasticity is as a mechanism for learning, it has at times been presupposed that synaptic strengthening is occurring in cases where new hippocampal representations are formed. However there is considerable evidence that learning and synaptic plasticity are not equivalent, and much remains to be determined as to the full range of events, both synaptic and non-synaptic, that give rise to spatial learning.

1.3 Synaptic Plasticity during Hippocampal Learning

The discovery of hippocampal LTP (Bliss & Lomo, 1973) fulfilled a number of postulations (Hebb, 1949; Kandel & Spencer, 1968) that had been made to describe how information processing and storage could occur in the brain based on the general functional principles of neurons, which had been elucidated over the first half of the 20th century (Cajal, 1893; Golgi, 1906; Lopez-Munoz & Alamo, 2009). The discovery was of considerable importance considering the context of research at the time: Over the preceding decade, the centrality of the hippocampus to human memory had been fully appreciated, so the capacity of the animal hippocampus to undergo activity dependent increase in synaptic strength reinforced the idea that LTP might be involved in memory.

1.3.1 General mechanisms of LTP and LTD

Bliss and Lomo's initial observations were conducted in the perforant path of the rabbit, but this discovery was rapidly translated to slice electrophysiology and expanded to the other major synaptic pathways in the hippocampus in the rat (Alger & Teyler, 1976; Andersen et al., 1977). A considerably productive line of inquiry established that this LTP showed associativity and cooperativity (McNaughton, Douglas, & Goddard, 1978; Levy & Steward, 1979), that it depended on voltage dependent NMDA receptor activation due to physiological magnesium blockade (Collingridge, Kehl, & McLennan, 1983; Nowak et al., 1984; Mayer, Westbrook, & Guthrie, 1984) and calcium influx through the NMDA receptor (Lynch et al., 1983; MacDermott et al., 1986). Further, the frequency stimulation was found to be effective due to sustained depolarization of the post-synaptic cell, in which case only a concomitant stimulation was required to produce potentiation (Kelso, Ganong, & Brown, 1986; Malinow & Miller, 1986). Although heterosynaptic LTD had been observed in several LTP paradigms (Lynch, Dunwiddie,

& Gribkoff, 1977; Levy & Steward, 1979; Abraham & Goddard, 1983), homosynaptic, activity-dependent LTD was not at first observed. This was considered an impediment to many computational theories of synaptic memory storage, and eventually a set of parameters was discovered that reliably produced de novo LTD in the hippocampus (Dudek & Bear, 1992; Dudek & Bear, 1993; Heynen, Abraham, & Bear, 1996) and the visual cortex (Kirkwood & Bear, 1994). This LTD shared with LTP a dependence on NMDA receptors (Dudek & Bear, 1992) and calcium influx (Mulkey & Malenka, 1992). Despite this symmetrical seeming relation between LTP and LTD, it was clear very early on that LTD was experimentally inducible over a far narrower set of circumstances than LTP (Bashir & Collingridge, 1994; Kerr & Abraham, 1995). In considering the role of LTP and LTD in computational theories of learning and memory, in some cases this incongruency is considered minimally important (Cooper & Bear, 2012), while in other cases it has been taken to indicate fundamentally different functions for LTP and LTD (Tsumoto, 1993; McClelland, McNaughton, & O'reilly, 1995; Kemp & Manahan-Vaughan, 2007b).

1.3.2 Synaptic plasticity and memory

One of the fundamental questions in neuroscience is how synaptic plasticity generates memory. There is no alternative account of mammalian memory that does not depend on changes in synaptic weights, and so in this sense there is little debate that synaptic plasticity produces memory. However the formalization of synaptic plasticity and memory as a general hypothesis has typically made fairly weak predictions, since it does not try to account for the mechanisms by which brain areas function, either individually or collectively (although see Marr, 1971; Rolls, 2006). This has often led to difficulty interpreting signals that might indicate either the presence or absence of memory: Any number of non mnemonic impairments might degrade

behavioral performance, and true mnemonic impairments might be masked by preserved behavioral performance. As well, there are clearly neural dynamics that can subserve forms of memory that do not depend on LTP or LTD-like changes, such as short-term memory using persistent firing of single neurons (Goldman-Rakic, 1995; Wang et al., 2013), attractor states in ensembles of neurons (Rolls, 2010), and adaptation of individual synapses (Kohn, 2007). The theory is thus usually restricted to long-term memory in certain domains, though the learning processes that instantiates long-term memory almost certainly depend on short-term memory dynamics to a certain degree, so divorcing these two categories of memory is difficult in practice. For example, persistent firing in PFC (Wang et al., 2013) and integrative properties of CA3 neurons (Makara & Magee, 2013) depend on NMDA receptors, and would be affected by their pharmacological blockade or genetic deletion. Changing these short-term dynamics might affect subsequent memory storage independently of the direct effect of NMDA-R inhibition on LTP and LTD, but this could not be shown using NMDA receptor based strategies. Regardless, the synaptic plasticity memory (SPM) hypothesis (Morris et al., 2003) has rested mostly on two forms of evidence, anterograde alteration and detectability, with another two criteria (retrograde alteration and mimicry) less established experimentally. Consideration of this evidence and its relevance to this thesis will be restricted to hippocampal memory, which is also the typical focus for the SPM hypothesis. It is somewhat ironic that the clarity of evidence supporting the SPM hypothesis is far stronger in non-hippocampal memory systems. Both amygdala-based fear conditioning (Rogan, Staubli, & LeDoux, 1997; LeDoux, 2000; Nabavi et al., 2014) and visual cortex based neuronal selectivity (Bienenstock, Cooper, & Munro, 1982; Cooper & Bear, 2012) have made and fulfilled strong predictions about the synaptic changes that subserve learning and

memory in those regions, in part because the information processing in cortex and amygdala is more clearly understood.

Blocking Hippocampal Learning with inhibitors of synaptic plasticity

A considerable body of work has investigated whether memory tasks that are impaired by lesions of the hippocampal formation are similarly impaired by inhibitors of synaptic plasticity. The genesis of this work came from experiments in the Morris water maze. Lesion studies demonstrated that the hippocampus was required for successful navigation to a hidden platform, and the effectiveness of the NMDA receptor blocker APV on LTP (Collingridge et al., 1983) predicted that the same drug should impair learning on the watermaze, if LTP mechanisms were important for hippocampal dependent learning. Systemic (Morris et al., 1986; Davis, Butcher, & Morris, 1992) and intrahippocampal (Morris, 1989) administration of APV impaired the acquisition of accurate navigation to a fixed, hidden platform location. A similar impairment on water maze performance was also observed in mice with selective knockout of NMDA receptor subunit NR1 in CA1 (Tsien et al., 1996).

However unlike the dependence on hippocampal lesions, impaired task performance on the water maze could be rescued with certain pre-training paradigms that involved pre-exposure to a different maze, or a watermaze with minimal extra-maze cues (Saucier & Cain, 1995; Bannerman et al., 1995). Although it was argued that these pre-training paradigms protected against non-mnemonic sensorimotor impairments caused by NMDA receptor blockade (Cain et al., 1996), it is more likely that whatever hippocampal process is occurring on the first exposure to a water maze is sufficient for subsequent task performance, even with NMDA receptor blockade.

An alternative transgenic model that leaves NMDA receptor function intact while impairing LTP uses AMPA receptor subunit GluR1 receptor knockout. GluR2/3 containing AMPA receptors continue to maintain basal synaptic transmission, however tetanization induced LTP is abolished. In these animals, navigation to a fixed hidden platform was unaffected (Zamanillo et al., 1999; Reisel et al., 2002). A knockout of the NR2A subunit of the NMDA receptor has produced conflicting results, with either a modest impairment on the water maze (Sakimura et al., 1995) or no effect (Bannerman et al., 2008), despite impaired LTP. Both of these transgenic models have either fully functional NMDA receptors (GluR1 KO) or considerable residual NMDA receptor function via NR1/NR2B/C/D (NR2A KO). These results strongly suggest that, at least on this version of the water maze, an alternative NMDA receptor dependent mechanism is mediating the impairment originally described by Morris and colleagues. Although there are many possible NMDA receptor functions, our laboratory demonstrated that maintenance of memory for a fixed location on the water maze is disrupted by either NR2B antagonism or inhibition of AMPA receptor endocytosis using a membrane permeable peptide inhibitor of the GluR2 c-tail ($GluA2_{3y}$, Ge et al., 2010). Both GluR1 deletion and NR2A deletion impair LTP, but may leave LTD relatively intact (Zhao & Constantine-Paton, 2007). In conjunction with the impairments observed with NR1 deletion or APV, these results are consistent with LTD being necessary for spatial reference memory maintenance on the water maze.

Pre-exposure to task elements prior to training also modifies the effect of APV on other hippocampal dependent tasks. Hippocampal lesions disrupt contextual fear conditioning (Kim & Fanselow, 1992), as does APV systemically (Kim et al., 1991; Fanselow & Kim, 1994) or in the hippocampus (Young, Bohenek, & Fanselow, 1994). However if rats are pre-exposed to the

context one day prior to context-shock pairing, APV during conditioning no longer produces impaired learning (Fanselow, 1990; Roesler et al., 1998; Sanders & Fanselow, 2003). These results strongly suggest that a critical, NMDA dependent process is occurring on the first exposure to an environment, and that process allows subsequent spatial navigation or contextual associations even in the absence of functional plasticity.

Novelty exposure itself can also be examined as a learning process. A rich body of work has described the interaction between novel object exploration and synaptic plasticity in the perirhinal cortex (Griffiths et al., 2008; Ranganath & Ritchey, 2012), however learning in this task is mostly spared with hippocampal lesions. When a novel environment itself is explored, or a novel arrangement of cues is presented within an environment, the exploratory behaviors elicited by the first exploration are markedly reduced upon re-exploration hours or days later, which has been taken as an index of learning (Poucet et al., 1986). This reduction in exploratory behaviors is impaired in hippocampal lesioned animals (Gray & McNaughton, 1983; Save et al., 1992; Whishaw et al., 1994; Ennaceur et al., 1997). NMDA receptor blockade in the hippocampus also impairs novel spatial memory (Izquierdo et al., 1992; Vianna et al., 2000) or object-place memory (Yamada et al., 2017) when given prior to the first exploration. Although no transgenic animals with confirmed LTP impairment have been reported for non-associative novel spatial learning, a serum response factor (SRF) forebrain knockout model which showed a specific impairment in LTD with preserved LTP induction showed a marked impairment in habituation to a novel environment, as well as memory for the novel environment assessed with re-exploration (Etkin et al., 2006). This is consistent with our observations that the $GluA2_{3y}$ peptide impaired memory of a spatial configuration assessed 24 hours after exposure (Dong et al., 2012).

In sum, there is considerable evidence that interventions that block hippocampal synaptic plasticity also affects hippocampus-dependent learning tasks. However the simple fact that a hippocampal lesion impairs performance on a task does not imply that blocking synaptic plasticity will have the same effect. Especially in the case of complex learning tasks that require multiple trials and higher order strategies, it is not easy to pinpoint which aspects of behavior are subserved by the hippocampus, and which processes therein might depend on changes in synaptic strength. The strongest evidence comes from simple, one trial behavioral tasks, and impairments are mostly observed on the initial exposure to the task rather than at subsequent stages. It is interesting to note that although some evidence is inconsistent with a role for LTP during this stage, most of the reported evidence is consistent with a role for LTD.

Detectability of synaptic changes after learning

Another key pillar of the synaptic plasticity memory hypothesis is that synaptic changes should be measurable after learning, if learning is mediated by those changes. This can be measured in a number of different ways, but historically externally evoked field potentials or synaptic protein levels in tissues have been most widely used. Although the hypothesis is sound in abstract terms, the resolution of both of these methods is almost certainly not high enough to record changes in synaptic strength occurring in a small or distributed set of synapses. Therefor the approach has been predicated on the expectation that large-scale changes occur during learning. While its unclear if this is the case, it seems unlikely that such a large-scale change would persist for memory maintenance, where the high mnemonic capacity of the brain is predicated on sparse distributed storage (Brady et al., 2008).

Regardless, changes in hippocampal evoked field excitatory post-synaptic potentials associated with behavior were observed soon after the discovery of LTP. Evoked potential

magnitude was found to modulate based on behavioral state (and accompanying EEG changes) on schaffer collateral inputs to CA1 (Leung, 1980). Longer lasting changers were seen in the evoked population spike in the perforant path input to the dentate gyrus, which was facilitated over several days after environmental exposure in rats (Sharp, McNaughton, & Barnes, 1985), or in other cases immediately during and after exploratory behavior (Sharp, McNaughton, & Barnes, 1989). These changes were dissociated from behavioral state (Green, McNaughton, & Barnes, 1990), suggesting that they related to a mnemonic function. However exploratory activity also provoked transient changes in brain temperature that potently affected evoked potential magnitude, suggesting that the bulk of these changes were driven by non-mnemonic physiological changes that co-occurred with behavior (Moser, Mathiesen, & Andersen, 1993; Moser, Moser, & Andersen, 1994; Moser, 1995). These studies provided some guidance on what methodologies would be required to record synaptic changes. First, a closely matched behavioral state that does not involve memory formation would demonstrate a dissociation from motor and sensory driven influences. Second, a control pathway that does not mediate the mnemonic change would demonstrate the selectivity of the experience-induced change to specific synapses in the brain.

In more recent studies examining memory detectability have focused on the CA1 region of the hippocampus on aversive learning tasks. As discussed above, NMDA receptor inhibition in the hippocampus impairs acquisition of contextual fear conditioning, so synaptic plasticity is predicted to occur. Trace eyeblink conditioning is a similarly hippocampal dependent form of fear conditioning in which the hippocampus facilitates the association between a predictive tone and a footshock delivered after a delay. Recording of schaffer collateral evoked potentials in CA1 found a gradual increase in the magnitude of the evoked potential across days as the

conditioning was acquired. The change in plasticity tracked the change in the conditioned response, and a pseudo-conditioned control group in which both tone and shock were delivered unpaired showed no conditioning and no change in evoked potential magnitude (Gruart, Munoz, & Delgado-Garcia, 2006; Madronal et al., 2010). Interventions that blocked LTP or induced it in an occlusion protocol disrupted conditioning. Although this is a strong link between trace eyeblink conditioning to LTP, it is important to note that the change in evoked potential occurred across a 10-day training protocol, and acquisition of a conditioned response preceded a detectable evoked potential change. Several studies have linked the acquisition of contextual fear conditioning with rapid increases in strength in a subset of CA1 synapses. Whitlock and colleagues (2006) showed inhibitory avoidance training was associated with potentiation in a subset of recording locations in CA1, while other channels show a slight decrease in synaptic strength. This effect was selective for rats trained on the context-shock association, with either contextual exposure alone or unconditioned shock did not provoke changes. A similar result was observed using a transgenic mouse that expressed GFP tagged GluR1 receptors under a c-fos promoter (Matsuo, Reijmers, & Mayford, 2008). This induction was doxycycline regulated, such that gene expression was restricted to a controllable time window. Experience drove GFP-GluR1 expression in a minority of CA1 pyramidal cells. In context-shock trained mice specifically, a higher proportion of spines in active cells showed GFP-GluR1 expression, suggesting that new receptor recruitment was higher in active cells in trained mice. An alternative viral strategy in the rat showed a similar pattern: In a randomly infected subset of hippocampal cells, new GluR1 receptor recruitment was higher specifically in context-shock trained rats. Additionally, blocking this recruitment blocked conditioning, using a specific inhibitor of plasticity that preserved NMDA function (Mitsushima et al., 2011). With both group

controls and within subjects' controls, these studies convincingly show that synaptic potentiation occurs in contextual fear conditioning. However, the results are surprising considering the role of the hippocampus in contextual fear conditioning. The hippocampus mediates contextual representations, and training protocols that separate contextual learning from the association of that context with an aversive stimulus consistently find that the association is unaffected by plasticity blockade in the hippocampus if the context was pre-exposed (Roesler et al., 1998; Anagnostaras, Gale, & Fanselow, 2001; Roesler et al., 2003; Matus-Amat et al., 2004; Malin & McGaugh, 2006). Considering this view, the context exposure control groups in these experiments should have demonstrated similar synaptic changes to the conditioned group. Thus, although synaptic potentiation changes have been observed on some hippocampal learning tasks, they are absent in others. This suggests that either these changes are incidental to the memory process occurring, or that the synaptic mechanisms of hippocampal learning are different in different tasks. It is possible, for example, that a footshock delivered within an established context changes the representation of that context in the hippocampus independently of the process that associates the context with the aversive unconditioned stimulus (Moita et al., 2003; Moita et al., 2004). This would represent a real learning process, but one that is not required for the behavioral expression of the context-shock association, which can use either a modified or unmodified hippocampal representation of the context.

The results of memory detectability experiments are mixed. There are measureable changes in evoked potential magnitude that are unrelated to learning, but there are also replicated reports of provably synaptic-based changes that are at least correlated with learning, and impaired by the same interventions that impair learning. In other memory systems such as the amygdala (Rogan et al., 1997; Rumpel et al., 2005) the perirhinal cortex (Clarke et al., 2010), the

barrel cortex (Makino & Malinow, 2011) and the motor cortex (Xu et al., 2009) there are also measurable changes associated with relevant learning tasks. However in these regions the information schema is generally better understood, and the effect of the observed synaptic changes can be interpreted in that context. In the hippocampus, the proposed role in forming non-associative representations of contexts is so far inconsistent with the observed synaptic changes in context-shock associations.

Behavioral Metaplasticity

Early in the exploration of experimental LTP, a variety of pre-induction manipulations were observed to alter the magnitude, threshold, or duration of induced potentiations, which were broadly termed metaplasticity (Abraham & Tate, 1997). It was immediately apparent that cellular activity based metaplasticity could have relevance for some theories of hebbian learning (Abraham & Bear, 1996). Behavioral metaplasticity has been considered an analog, in which behavioral experience before, during, or after a traditional plasticity induction protocol alters the effect of that induction protocol.

Tail shock stress was first observed to impair LTP induction (Foy et al., 1987), and a similar effect was observed with inescapable, but not escapable footshock (Shors et al., 1989). This dissociated the LTP impairment from being a simple consequence of noxious stimuli, since equal footshocks were administered in both cases. In contrast, platform stress facilitated LTD induction (Kim, Foy, & Thompson, 1996; L. Xu, Anwyl, & Rowan, 1997). Stress is a well-known mediator of cognitive performance, and can facilitate or impair memory performance depending on stress level, cognitive load, stage of memory, and other task demands (McGaugh & Roozendaal, 2002; Robbins & Arnsten, 2009). A well-described circuit for this type of stress involves systemic adrenaline and glucocorticoid release by the adrenal cortex, which acts via the

amygdala to mediate memory consolidation of emotional memories (McGaugh, 2000). Amygdala lesions can block the stress-induced inhibition of LTP in the hippocampus and reverse stress-induced memory impairments (Kim et al., 2001; Kim et al., 2005), although it has not been demonstrated that stress facilitated LTD is similarly affected. Stress can affect plasticity at other hippocampal synapses, and a range of effects have been described particularly in the perforant path input to dentate gyrus. Most commonly a facilitation of LTP has been described (Seidenbecher, Reymann, & Balschun, 1997), however depotentiation of induced LTP has also been observed (Shors & Dryver, 1994). These opposing effects seem to be driven by both the relative timing of exposure to stress versus plasticity induction, along with the duration or possibly magnitude of the stressor. The facilitation of LTP in the dentate gyrus has also been show to be dependent on the amygdala as well as glucocorticoid transmission (Korz & Frey, 2003; Korz & Frey, 2005).

Similar to aversive stress procedures, novelty exposure can depotentiate LTP (Xu et al., 1997) and facilitated LTD induction more dramatically than stress (Manahan-Vaughan & Braunewell, 1999). It also facilitates LTP in the dentate gyrus (Seidenbecher et al., 1997; Straube, Korz, & Frey, 2003), although again can depotentiate LTP depending on the relative timing of novelty exposure and LTP induction (Straube et al., 2003). Certain aversive stress procedures have elements of novel environment exposure, and the reaction to novelty in animals recruits elements of the stress system, however novelty is distinguished from traditional stress responses by virtue of it being non-aversive and eliciting approach versus avoidance behavior (Beerling et al., 2011). Do the plasticity effects of novelty, like aversive stress, depend on amygdala function? This question has not been directly tested, however the behavioral effect of novel context exposure, which includes facilitation of consolidation on certain subsequent

learning tasks (Moncada & Viola, 2007), does depend on amygdala function (Okuda, Roozendaal, & McGaugh, 2004; Roozendaal et al., 2006). As well, amygdala lesions reduce the degree of novelty-induced cfos activity in the hippocampus (Sheth et al., 2008).

Despite the clear facilitation of LTD in response to novelty, paradoxically facilitated LTP has also been reported. Li and colleagues (2003) found that a brief, but not prolonged, exposure to a novel environment facilitated LTP in response to a weak high frequency stimulation, and Kemp and Manahan-Vaughan (2004) found exposure to a changed floor in a familiar context promoted LTP, while the inclusion of objects promoted LTD and depotentiated recent LTP. Other pathways in the hippocampus including the perforant path input to the dentate gyrus (Straube, Korz & Frey, 2003;Wiescholleck & Manahan-Vaughan, 2014; Hansen & Manahan-Vaughan, 2015), the mossy fibre input from dentate gyrus to CA3 (Hagena & Manahan-Vaughan, 2011; Hagena & Manahan-Vaughan, 2012), and the associational commissural inputs between CA3 cells (Hagena & Manahan-Vaughan, 2011) have also reported bidirectional modulation, depending on both the presence and size of objects, as well as their rearrangement. These effects clearly demonstrate that different types of environmental change provoke different plasticity responses in different subregions of the hippocampus. However these diverse effects are observed when some features of the environment are stable and others are changed whether a floor change within and unchanged box, small objects introduced or rearranged, or larger objects introduced or rearranged. It is not clear what the dominant plasticity response is in response to an entirely novel environment.

Unlike other forms of stress, novelty requires a primary information processing stage to detect and signal novelty. This puts a minimum limit on both the exposure time in a novel environment and the degree of contextual novelty. Graded changes in an environment are known

to produce graded behavioral novelty responses (Wells et al., 2009), so it is unclear whether these different plasticity effects are due to qualitative differences in experience or more simply a quantitative difference in degree of novelty. In this interpretation, a mildly novel contextual change might bias plasticity changes towards LTP, while more pronounced contextual change might bias plasticity towards LTD. A third possibility is that non-homogenous metaplastic changes may take place within the schaffer collateral CA1 pathway, depending on local differences in ongoing activity (Whitlock et al., 2006).

Beyond the likely participation of the HPA axis and amygdala on novelty evoked hippocampal metaplasticity, it is clear that monoamines regulate both the plasticity response and novelty recognition memory. Systemic (ICV) dopamine D1/5 antagonists block both the novelty facilitated LTD and memory for a novel context tested 24 hours after exposure (Bevins et al., 2002; Lemon & Manahan-Vaughan, 2006). Systemic (ICV) beta-adrenergic receptor antagonists similarly block novelty facilitated LTD (Kemp & Manahan-Vaughan, 2007a). Agonism of D1/5, or activation of beta-adrenergic receptors via direct agonism or locus coerulus stimulation was sufficient to facilitate LTD induction (Lemon & Manahan-Vaughan, 2006; Kemp & Manahan-Vaughan, 2007a; Lemon et al., 2009). Both dopamine and noradrenaline are elevated in the hippocampus in response to a novel environment (Ihalainen et al., 1999), however the direct action of dopamine and noradrenaline in the hippocampus has not been demonstrated to mediate these effects.

Behavioral metaplastic effects are clearly observable in the hippocampus, and are particularly provoked by novelty. These effects are clearly differentiated by generalized behavioral effects on synaptic potentials, since they are specific to the first contextual exposure. They are also behaviorally relevant, as blocking the hypothesized signaling pathways or directly

blocking AMPA receptor endocytosis in the hippocampus with the $GluA2_{3y}$ peptide blocks the facilitation of inhibitory avoidance by novelty (Dong et al., 2012).

1.3.3 Outstanding questions

There are a number of outstanding questions related to the metaplastic effects of novelty. For example it is unclear to what degree the metaplastic mechanisms that are engaged by novelty overlap with those engaged by stress. Although there are clearly shared mechanisms, the behavioral and learning consequences of novelty versus aversive stress can be different enough that divergent mechanisms might be expected. It is also not entirely clear whether LTP or LTD is the dominant effect in response to novelty, and what fundamental differences drive their selective activation.

More generally, the synaptic plasticity and memory hypothesis is broad enough that, in certain respects it is almost certain to be true. However the detection of any synaptic change associated with learning requires one to consider how this change may contribute to the representation of information in the ensemble activity of the cells innervated by those synapses. Experiments designed to examine synaptic potentiation in response to context-shock associations have often detected no change in response to the context itself, even though hippocampal learning is clearly concerned with contextual representation. As well, common inhibitors of synaptic plasticity can in many cases leave certain forms of learning intact. Impairments of synaptic plasticity produce the most reliable effects when targeted at initial contextual learning, which raises the questions as to which neurobiological features distinguish novel contextual learning from other forms of learning where associations or features are modified within an established context.

1.4 Rationale, Hypotheses and Research Aims

1.4.1 Rationale

The hippocampus is specialized to mediate the rapid acquisition of contextual information, a process that can be modeled by examining place field formation in novel environments. While synaptic plasticity is implicated in the functions of the hippocampus, its role in the crucial processes that mediate the rapid formation of place fields has not been identified. Electrophysiological evidence points to a role for LTD during certain forms of novelty exposure, however there is conflicting evidence as to whether a novel context, in the absence of novel object exposure within that environment, elicits a metaplastic change that promotes LTP or LTD. Whereas experiments utilizing multielectrode arrays suggest that bidirectional de novo plasticity changes occur in the hippocampus in response to learning (Whitlock et al., 2006), these findings were specifically observed when a context was paired with an aversive stimuli. It remains to be determined whether specific de novo plasticity can be induced by unpaired novel environment exposure, a circumstance where place field formation takes place. Novel place field maintenance is dependent on NMDA receptors, however it is unknown whether and how LTD specifically contributes to place field maintenance, and whether the dynamics of initial place field formation can be affected by disruption of synaptic plasticity.

1.4.2 Hypotheses

Exposure to a novel spatial environment will elicit de novo synaptic LTD in hippocampal schaffer-collateral CA1 synapses that play an important role in the formation and/or maintenance of place specific firing of CA1 place fields established in a novel environment. A specific inhibitor of AMPA receptor endocytosis will block this LTD, and thereby impair the formation/maintenance of CA1 place fields.

1.4.3 Research Aims

This dissertation was designed to validate our hypotheses with the following specific research aims:

1. To demonstrate that synaptically mediated, pathway specific long-term depression (LTD) occurs during novel spatial exploration, and that a specific blocker of experimentally induced LTD is effective in blocking this de novo LTD;
2. To determine whether LTD blockade affects contextual learning as indicated by inhibitory avoidance;
3. To determine whether this same blocker of LTD affects the formation and maintenance of place field firing in CA1 pyramidal cells;

Chapter 2 Hippocampal LTD is induced by Novel Spatial Exploration

2.1 Introduction

Previous research (Manahan-Vaughan & Braunewell, 1999; Kemp & Manahan-Vaughan, 2004; Dong et al., 2012) has demonstrated that novelty exploration facilitates the induction of LTD in the schaffer collateral CA1 pathway by low frequency stimulation. Some reports have suggested that some form of decreased evoked fEPSPs is observed even in the absence of a traditional induction protocol (Whitlock et al., 2006; Goh & Manahan-Vaughan, 2012), but other studies have found no change with basal stimulation (Dong et al., 2012). Typically, this electrophysiology is conducted using a single, large diameter (0.75-1.5 μm) metal recording electrode, which records local field potentials (LFPs) from a large area of tissue. Use of a multi-electrode array, composed of a number of smaller (0.08-0.25 μm diameter) electrodes, each recording LFPs from a smaller section of tissue, can record changes in evoked potential strength that are not apparent with larger electrodes (Whitlock et al., 2006). Given a non-homogenous, distributed change in synaptic weights, an electrode array may observe synaptic changes as reported by evoked fEPSP that a single larger diameter electrode would not. Changes in the absence of a traditional induction protocol may not be mediated by synaptic changes, and many observations of putatively learning driven changes in evoked potentials have indeed been shown to have non-synaptic origins (Hargreaves, Cain, & Vanderwolf, 1990; E. I. Moser, 1995). It is unknown if a de novo decrease in evoked potential in the CA1 pathway would be impaired by blockers of synaptic plasticity.

In addition to the observed effects on LTD, facilitated LTP induction after certain forms of novelty exposure have also been reported (Kemp & Manahan-Vaughan, 2004; Li et al., 2003). The switch from facilitated LTP to facilitated LTD is speculated to depend on perhaps the

duration of exposure (Li et al., 2003) or the presence of objects within an environment (Kemp & Manahan-Vaughan, 2004). This has led to speculation that LTP and LTD are encoding fundamentally different aspects of an environment, and that the presence of objects is necessary to observe facilitated LTD (Kemp & Manahan-Vaughan, 2007; Kemp & Manahan-Vaughan, 2008; Hoang, Aliane, & Manahan-Vaughan, 2018). However the effect of an object-free novel environmental change that includes geometric changes to the environment (i.e. changing the size or shape of the environment) has not been tested.

In recording changes unprovoked by an inductive stimulation, there is a need for carefully considered controls, as previous work on memory detectability makes clear (see introduction). The magnitude of an evoked local field potential, when recorded in a freely moving animal, can be subject to a variety of external factors unrelated to a putative learning process, particularly motor activity and brain temperature. An internal control pathway can address many of these issues, however in a situation where the localization of distributed changes is unknown a priori, the path must be separated from the set of synapses expected to undergo alteration. Here we take advantage of the fact that CA1 pyramidal cells have parallel schaffer collateral inputs to their basal and apical dendritic arbors. The inputs to the apical dendrites are activated by stimulation in the stratum radiatum, and are the subject of the previously described experience dependent metaplastic effects discussed above. The inputs to the basal dendrites are activated by stimulation above the cell layer in stratum oriens. These synapses are excitatory and can undergo synaptic plasticity (Leung & Shen, 1995), but are functionally compartmentalized from activity at apical dendrites (Sajikumar, Navakkode, & Frey, 2007).

Our lab has previously shown that the $GluA2_{3y}$ peptide is effective in blocking novel object configuration recognition when given prior to the first context exposure (Dong et al.,

2012), however no mnemonic role for LTD has been tested in an object-free context. Furthermore, we have previously demonstrated that the $GluA2_{3y}$ peptide or GluN2B subtype NMDA receptor antagonism does not impair contextual fear conditioning, suggesting that LTD does not play a necessary role in representing contexts (Dalton et al., 2008). However contextual fear conditioning is greatly affected by prior habituation to the training context an effect sometimes termed the contextual pre-exposure facilitation effect (CPFE; Rudy, Huff, & Matus-Amat, 2004). It has been shown that NMDA receptor antagonism in the hippocampus is less effective in blocking inhibitory avoidance in rats subject to context pre-exposure (Roesler et al., 1998; Roesler et al., 2003), so we sought to address whether blocking LTD during contextual pre-exposure affected the subsequent expression of inhibitory avoidance.

This experiment was designed to address several questions: First, does exposure to a highly novel environment elicit induction pattern independent LTD that can be blocked by an inhibitor of AMPA receptor endocytosis, and are any *de novo* changes in synaptically evoked field potentials localized to specific recording electrodes within an array, or coincident with evidence of *de novo* synaptic potentiation? Second, does blocking LTD lead to a behavioral impairment in a standard contextual fear task when the LTD blockade is targeted to the initial context exposure period?

2.2 Methods

2.2.1 Subjects

All experiments were approved by the University of British Columbia Animal Care Committee in accordance with the policies of the Canadian Council on Animal Care. Male sprague-dawley rats (Charles River) weighing 300-450 grams were housed in pairs on a 12hr:12hr standard light cycle, or individually for freely moving electrophysiology experiments. Rats were provided food and water access *ad libitum*.

2.2.2 Drugs

The interference peptide Tat-GluA2$_{3y}$ (YGRKKRRQRRR-$_{869}$YKEGYNVYG$_{877}$) and scrambled control Tat-Scramble (YGRKKRRQRRR-VYKYGGYNE) were synthesized in house and dissolved in saline (2.25 μmol/kg) for intravenous bolus injection.

2.2.3 Surgery

Jugular vein catheterization

Rats were anesthetized with isoflurane (5% induction, 2% maintenance) and an incision made in the upper right quadrant of the thorax to expose the right jugular vein. An indwelling silastic catheter (Dow Corning Corp, USA) was inserted into the jugular vein, and the distal end was run subcutaneously to a port on the dorsal surface of the rat between the scapulae. In the case of rats undergoing electrophysiology recordings, the port was integrated into the headcap.

Evoked Potential Electrophysiology

For electrophysiological experiments, following jugular vein catheterization, anesthetized rats were placed in a stereotaxic frame, and a 3 by 2.5mm cranial window (-1.75 to 4.75mm AP,

0.5 to 3.0mm ML) was drilled over the right dorsal hippocampus. Dura was excised to allow implant of a probe containing 8 bundles of 4 electrodes (25μm tungsten, California Fine Wire) arranged in a 4 by 2 pattern (Fig 2.1A). Within each bundle, electrode tips were cut at 300μm spacing to span the strata of CA1, and bundles were separated by 635μm. Lateral to the implanted probe, two individually moveable stimulation electrodes (bipolar 50μm stainless steel, AM systems) were implanted into the stratum radiatum and stratum oriens respectively, using standard electrophysiological responses to confirm placement (Fig 2.1B). Electrodes were fixed in place to the skull by dental cement and implant skull screws, which also served as ground and reference points.

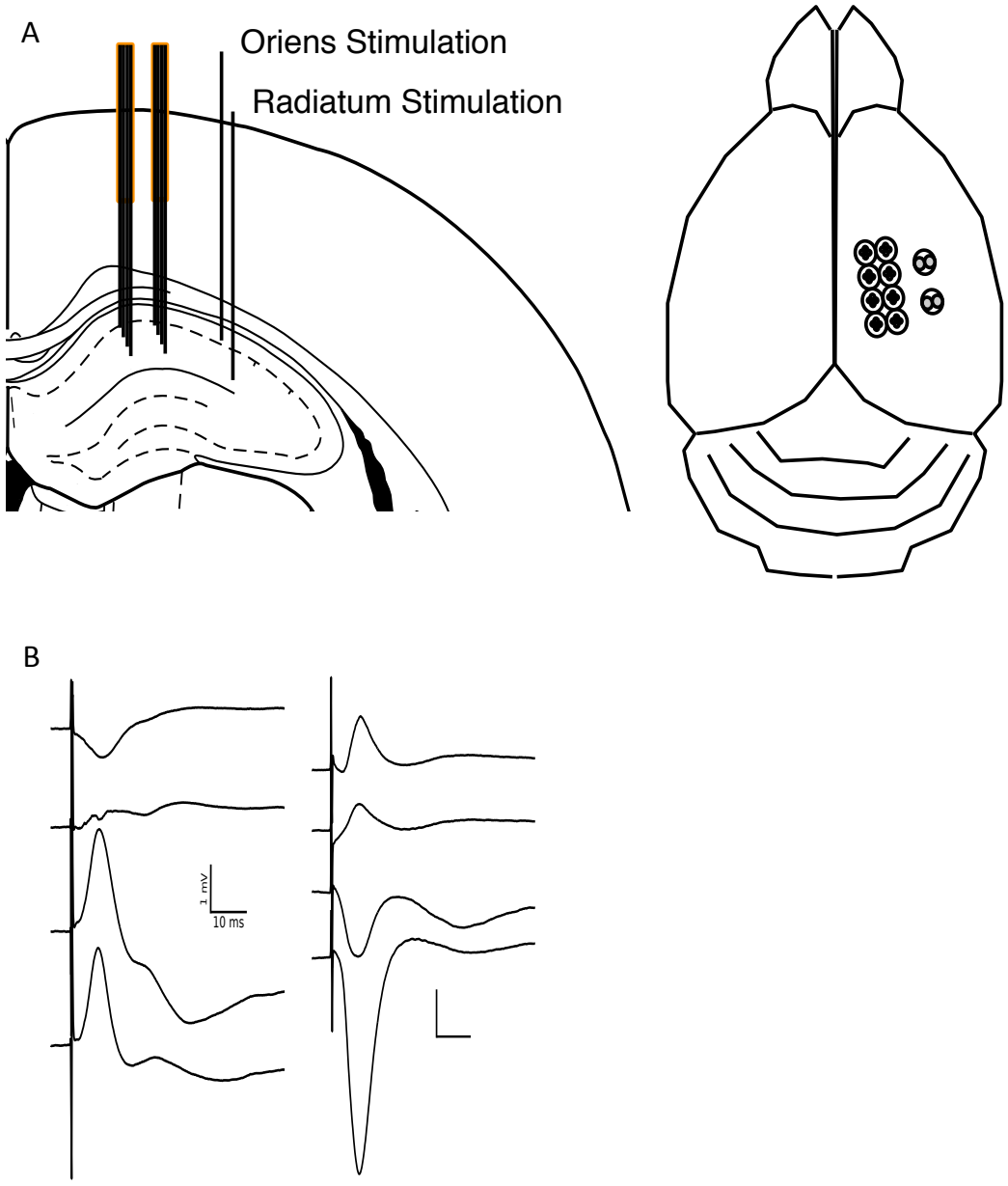

Figure 2.1 Multi-electrode array recording in dorsal CA1.

(A) Bundles of four electrodes spaced by 300 μm recorded through the laminar extent of CA1. Basal dendritic fEPSPs were evoked with a stimulation electrode in stratum oriens, and Apical dendritic fEPSPs were evoked with a stimulation electrode in stratum radiatum. Electrode bundles were spaced in two rows, stimulation electrodes were inserted lateral to the recording array. (B) Example waveforms from oreins (left) and radiatum (right) stimulation. Scale bar is 1mV and 10ms.

2.2.4 Behavioral assays

Evoked field recordings in novelty exposure

Rats were acclimated to a recording chamber (40x40x60cm) for several days after recovery from surgery. The baseline recording chamber was composed of four uniform dark walls with a removable blue corrugated plastic floor. A single white vertical strip polarized the box for orientation. Extramaze cues were available on all four walls in the recording room.

Baseline evoked responses were then tested from 20-200μA stimulation intensity (0.2ms biphasic stimulation), and a stimulation magnitude evoking 50% of the maximal response was used for the remaining recordings. Baseline recordings were made for a minimum of three days (alternating oriens and radiatum test stimulations, 0.016 Hz per site), and the day prior to testing baseline responses were recorded for 1 hour, with brief handling at 30 minutes. On the test day, drugs (saline, tat-GluA2$_{3y}$/tat-Scramble 2.25 μmol/kg) were administered 45 minutes prior to recording. After 30 minutes in the recording chamber, rats were transferred to a novel environment (60x60x60cm polarized box). The novel environment was composed of three black walls and one white wall, with a removable black painted corrugated plastic floor. It was positioned adjacent to the baseline recording box in the same room. Extramaze cues within the room were unchanged. Rats were recorded for 30 minutes in the novel environment, with test stimulation continuing as before, and then transferred back to the baseline recording chamber for 30 minutes.

On the following day, rats were re-exposed to the novel environment using the same testing protocol as above, with the exception that no drugs were administered prior to testing.

Inhibitory Avoidance

The inhibitory avoidance apparatus consisted of a light and dark chamber separated by a removable door. Each chamber was 35x30x35cm, and the stainless steel flooring of the dark compartment was connected to a programmable scrambled shock generator (Colbourne Instruments, USA). Inhibitory avoidance was assessed over a three-day protocol designed to isolate the novel exposure to the recording chamber from the chamber-shock association learning (Fig 2.9A; Liang, 1999; Malin & McGaugh, 2006). On day one, rats received a contextual exposure to the chamber, in which they were placed in the light side of the box with the door open and allowed to freely explore both sides of box for 8 minutes. Prior to exploration, rats received bolus IV injection of saline, scrambled peptide, or $GluA2_{3y}$ peptide (tat-$GluA2_{3y}$/tat-Scramble 2.25 μmol/kg). On day two, 24 hours after contextual exposure, rats were placed directly in the dark chamber with the door closed, and received two brief footshocks 2 seconds after placement in the chamber (0.4mA footshock, 0.5 second duration). Five seconds after termination of the footshock rats were removed from the chamber and returned to their homecage. No drugs were administered in the training session. On day three, 24 hours after training, rats were placed in the light compartment facing the far wall, and after 5 seconds the door to the dark chamber was opened. Latency to cross entirely into the dark compartment (all four paws within the compartment) was taken as a measure of inhibitory avoidance learning. A control group of rats was administered saline on day one and trained as above, with the exception that no contextual exposure was performed on day one.

2.2.5 Data acquisition and analysis

Local and evoked field potentials were recorded using a 32-channel electrophysiology recording system with integrated headstage preamplifier (Digital Lynx 32, HS-36, Neuralynx, AZ). A native sampling rate of 32kHz was broad-band filtered between 0.1 and 1000 Hz and downsampled to 6.4kHz for analysis. Stimulation (0.2 ms biphasic pulse) was delivered via AM 2100 stimulus isolators, controlled via computer generated TTL pulses. Data was analyzed offline using custom MATLAB analysis code. The amplitude of evoked field potentials was taken as a measure of synaptic strength. As evoked potentials within a recording tract were highly correlated and likely reflected the same population of evoked synapses, only the channel with the highest magnitude potential was used. This also avoided the analysis of complex waveforms recorded from channels intermediate between stratum oriens and stratum radiatum.

2.3 Results

Consistent with previous work and the known architecture of area CA1 of the hippocampus, stimulation of stratum radiatum produced a characteristic negative field excitatory post-synaptic potential (fEPSP) in the more ventral channels of a bundle, with a positive fEPSP in the more dorsal channels. Conversely, stimulation in the stratum oriens produced a negative potential in the more dorsal channels, while a positive fEPSP was observed in the more ventral channels (Fig 2.1B). Due to the non-uniform topology of the hippocampal lamina, the depth at which the polarity shifted occasionally varied, but was typically between channels 2 and 3 within a bundle. As fEPSP magnitude within session was highly correlated within each bundle of four channels, only the channel with the highest magnitude evoked potential was used for analysis.

2.3.1 Stable synaptic strength in a familiar environment

Evoked potentials in rats allowed to explore the familiar recording chamber for a 1 hour session the day prior to testing showed no significant difference between groups, with a slight increase across the session, in response to stratum oriens (103.3%±1.3 SEM) and stratum radiatum (105.7%± 4.4) stimulation (Fig 2.2A,B, 2-way group by epoch ANOVA, $F(1,74)=5.29$, $p=.02$ main effect of epoch, $p>.05$ group effect/interaction).

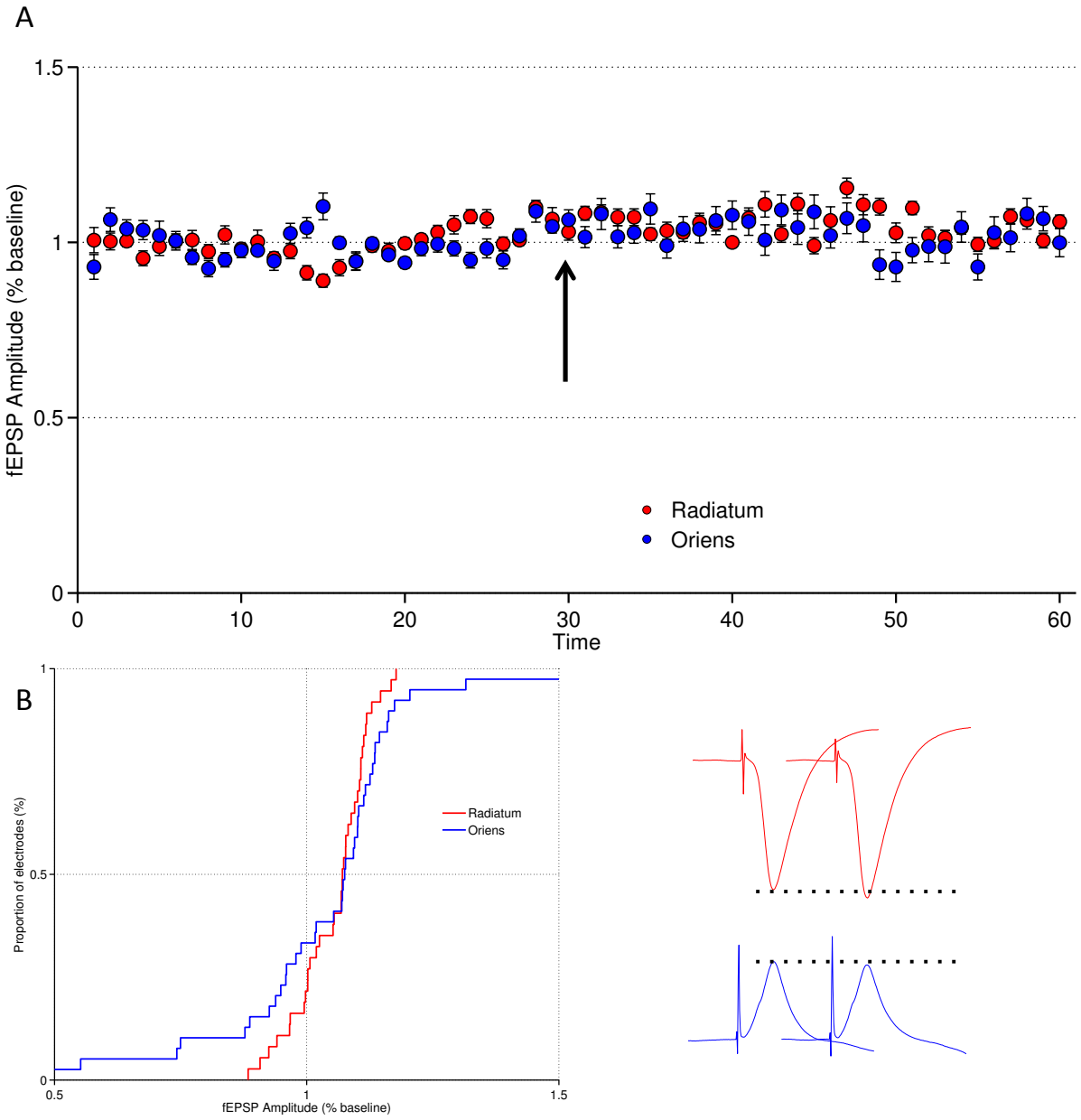

Figure 2.2 Baseline evoked fEPSP amplitude in familiar recording chamber.

(A) Evoked potentials from radiatum (red) and oriens (blue) stimulation (0.016 Hz) were stable over a 1 hour recording period in the familiar recording chamber. Arrow indicates brief handling at 30 minutes. (B) Cumulative distribution of amplitude change in the second 30 minutes vs first 30 minutes. Radiatum vs Oriens $p>.05$.

2.3.2 Input specific changes during novel environment exploration

In rats administered saline prior to the recording session, fEPSPs were evoked from stimulation of the stratum oriens (n=37 channels, from 5 rats) and stratum radiatum (n=39 channels, from 5 rats) throughout a 30-minute baseline period in the familiar recording chamber, a subsequent 30-minute period in a novel environment, or a final 30-minute period in the familiar environment (Fig 2.3A,B). Significant effects of epoch ($F(2,148)=41.51, p<.001$, group ($F(1,74)=22.13, p<.001$), and interaction ($F(2,148)=3.06, p<.001$) were observed. A follow-up comparison between radiatum and oriens evoked potentials during each epoch showed that radiatum evoked fEPSPs exhibited a pronounced decrease in magnitude relative to oriens stimulation after exposure to the novel environment (84.1%±2.1) , a depression that was maintained during the final 30 minutes in the familiar recording chamber (76.2%±3.0) (Fig 2.3A,B, $t(74)=4.26, p<.001$ novel environment, $t(74)=4.73$ $p<.001$ familiar environment, *vs oriens stimulation*). Nearly every channel (38/39 channels) showed a small decrease in amplitude (Fig 2.3B) over the duration of the recording session. Oriens evoked potentials showed a modest, non-significant decrease during novelty exposure (96.9%±2.1) and on return to the familiar environment (95.1%±2.5). These results demonstrate that novel environment induces *de novo* LTD at the radiatum inputs to the hippocampal CA1 neurons in a pathway specific manner.

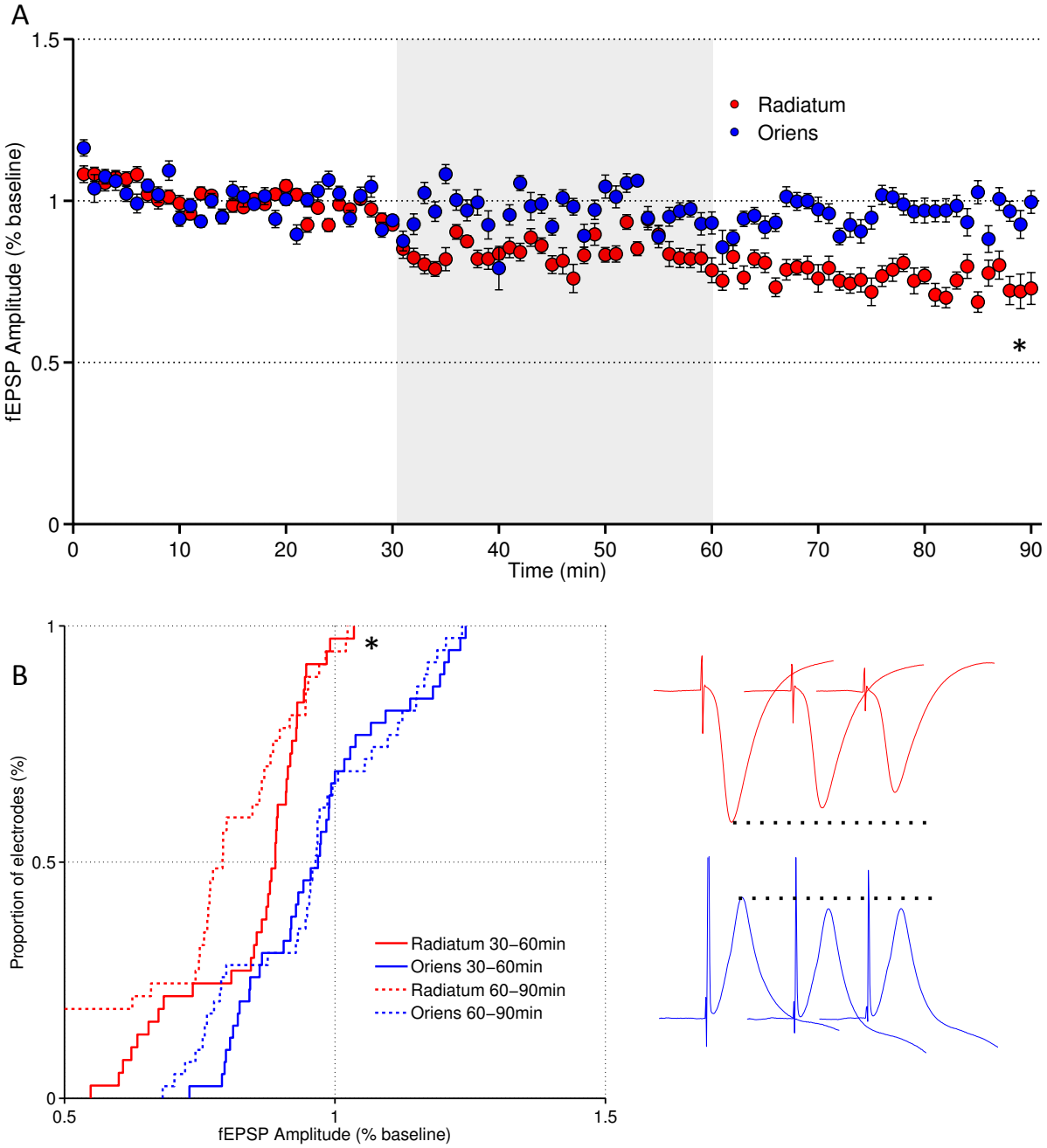

Figure 2.3 Exposure to a novel environment elicits a decrease in fEPSP specific to stratum radiatum stimulation.

*(A) A 30-minute baseline recording was followed by 30 minutes in a novel environment (grey shading), followed again by 30 minutes in the familiar recording chamber. A specific decrease in radiatum (red) stimulation was observed with no change in oriens stimulation (blue). (B) Cumulative distribution of amplitude change relative to baseline period for radiatum (red) and oriens (blue) stimulation during novelty (solid) and after returning to the familiar chamber (dashed). * indicates p<.05, follow-up comparison between group for epochs, ttest for average fEPSP change.*

2.3.3 Peptide mediated inhibition of LTD blocks novel environment induced fEPSP depression

To test whether the observed fEPSP depression was mediated by a facilitation of endocytosis of postsynaptic AMPA receptors at the synapse, the $GluA2_{3y}$ interference peptide or a scrambled control was administered IV prior to the recording session on the novelty exposure day. Stratum oriens evoked potentials were recorded from five rats administered the $GluA2_{3y}$ peptide (n=26 channels) and five rats administered the scrambled control peptide (n=31). Stratum radiatum evoked potentials were also recorded from $GluA2_{3y}$ (n=34 channels) and scrambled treated rats (n=29). Consistent with the results from saline treated rats, fEPSP magnitude was relatively stable during exploration of a familiar environment in the baseline day (Fig 2.4A,B). A slight, non-significant increase was observed across epoch in oriens (102.5%±2.0 $GluA2_{3y}$, 103.2%±1.9 scrambled, epoch main effect $F(1,55)=4.17, p=.04,$ other *ps>.05)* and radiatum *(*106.9%±2.1 $GluA2_{3y}$ $F(1,61)=4.59,p=.03$), with a slight decrease specifically in the scrambled-radiatum group (98.6%±1.9, $F(1,61)=8.41, p<.01$ interaction).

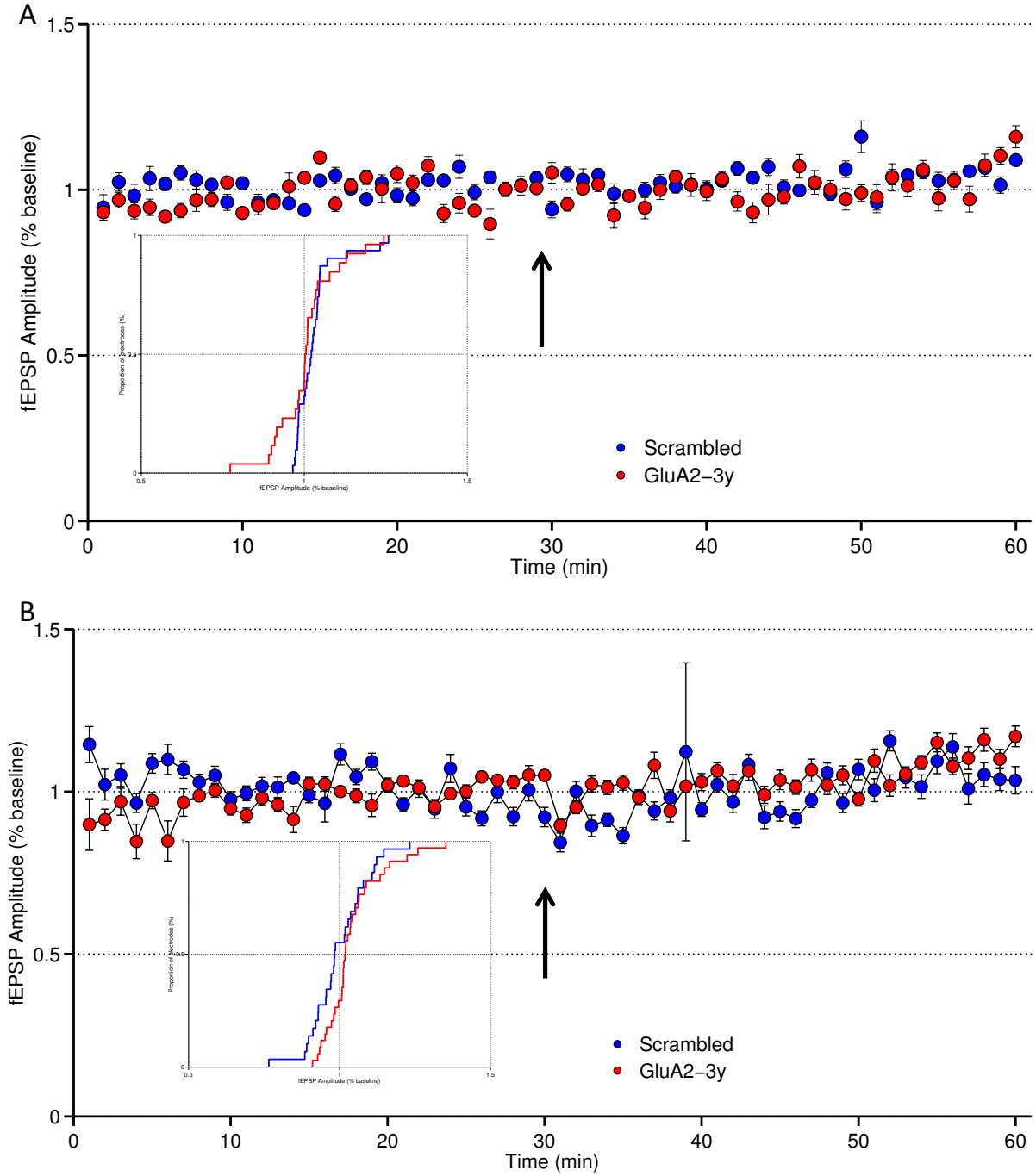

Figure 2.4 Baseline day evoked fEPSP amplitude in familiar recording chamber.

(A) Evoked potentials from radiatum stimulation in scrambled (blue) or GluA2$_{3y}$ (red) treatment groups. (B) Evoked potentials from oriens stimulation in scrambled (blue) or GluA2$_{3y}$ (red) treatment groups. Arrow indicates brief handling at 30 minutes. Insets show cumulative distribution of amplitude changes relative to first 30 minutes. No drugs were administered during the baseline day, and no changes were

observed. A modest difference in average change in the last 30 min was observed between scrambled (98.6%±1.9) and GluA2$_{3y}$ (106.9%±2.1).

Oriens evoked potentials in response to novelty exploration were unaffected by drug treatment (Fig 2.5A,B), with a modest decrease observed during the novel environment in both scrambled (95.5%±1.7) and GluA2$_{3y}$ (98.3%±2.9), and no change on return to the novel environment (101.3%±1.5 scrambled, 98.8%±1.1 GluA2$_{3y}$, *F(2,110)=3.09, p=.049* main effect of epoch, group and interaction *ps>.05*).

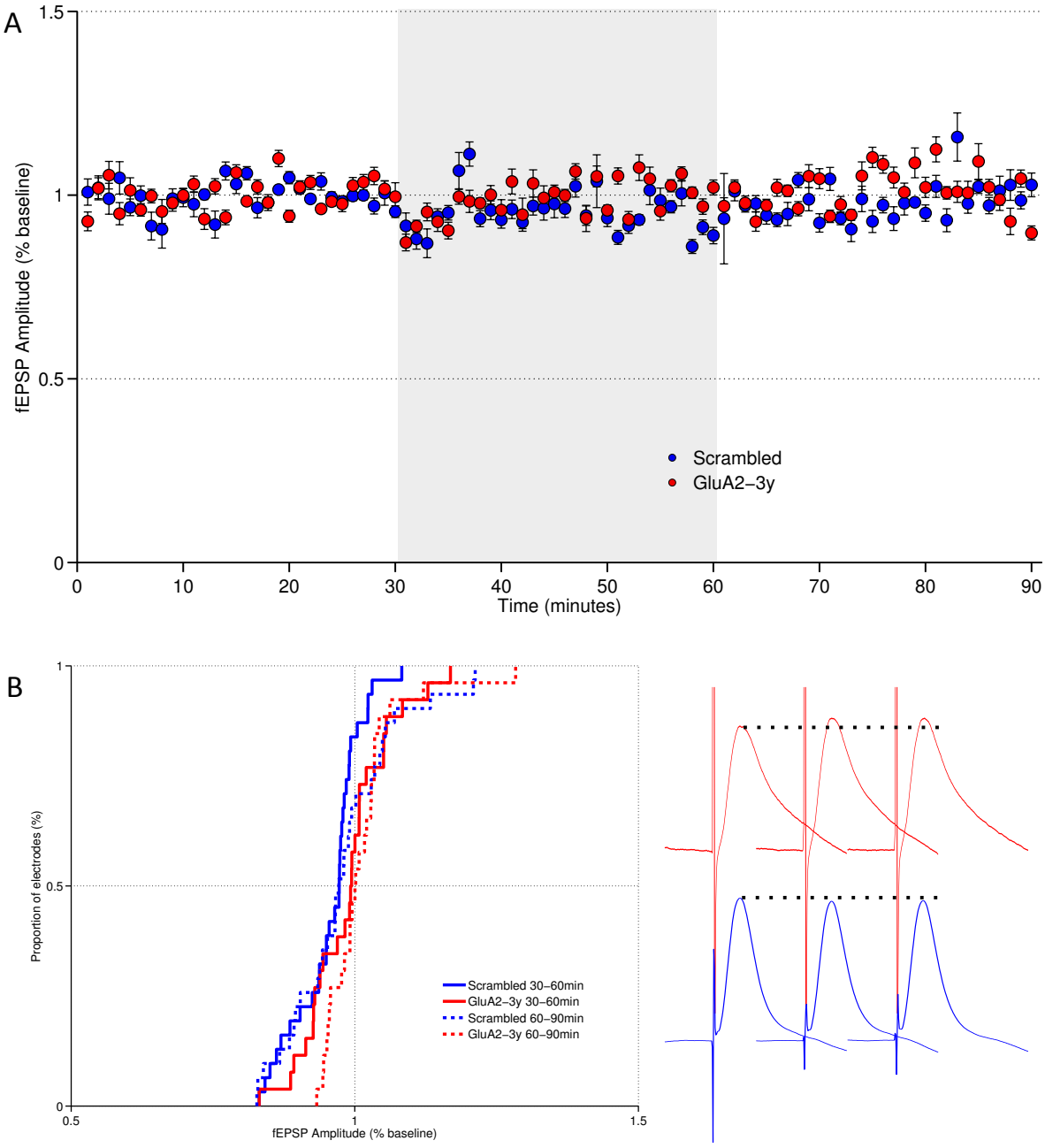

Figure 2.5 Exposure to a novel environment does not affect oriens evoked fEPSPs.

$GluA2_{3y}$ peptide (red) or scrambled control (blue;2.25 µmol/kg in saline) administered 30 minutes prior to baseline. (A) No change in evoked potential amplitude was observed in oriens stimulation for either group in response to a novel environment (grey shading). (B) No change in the distribution of changes relative to baseline were observed during novelty (solid) and after returning to the familiar chamber (dashed). Scrambled vs $GluA2_{3y}$ $p>.05$.

A decrease in evoked potential magnitude was observed in response to radiatum stimulation specifically in scrambled peptide treated rats both during novel environment exploration (88.8%±1.3) and on return to the familiar chamber (83.1%±3.7), consistent with the effect observed with saline treatment (Fig 2.6A,B). In contrast, no significant change in fEPSP magnitude was seen in rats treated with the active interference peptide either during novel environment exploration (99.8%±1.5) or on return to the familiar chamber (104.8%±1.6, Fig 2.6A,B, $F(2,122)=7.41$, $p<.001$ epoch, $F(1,61)=35.99$, $p<.001$ group, $F(2,122)=23.91$ $p<.001$ interaction, follow-up comparisons during the novel environment $t(61)=5.40$, $p<.001$, and the familiar environment $t(61)=5.49$ $p<.001$, $GluA2_{3y}$ relative to scrambled treatment). Together these results suggest that *de novo* LTD in the stratum radiatum pathway in CA1 in response to novel environment exposure is mediated by activity dependent AMPA receptor endocytosis.

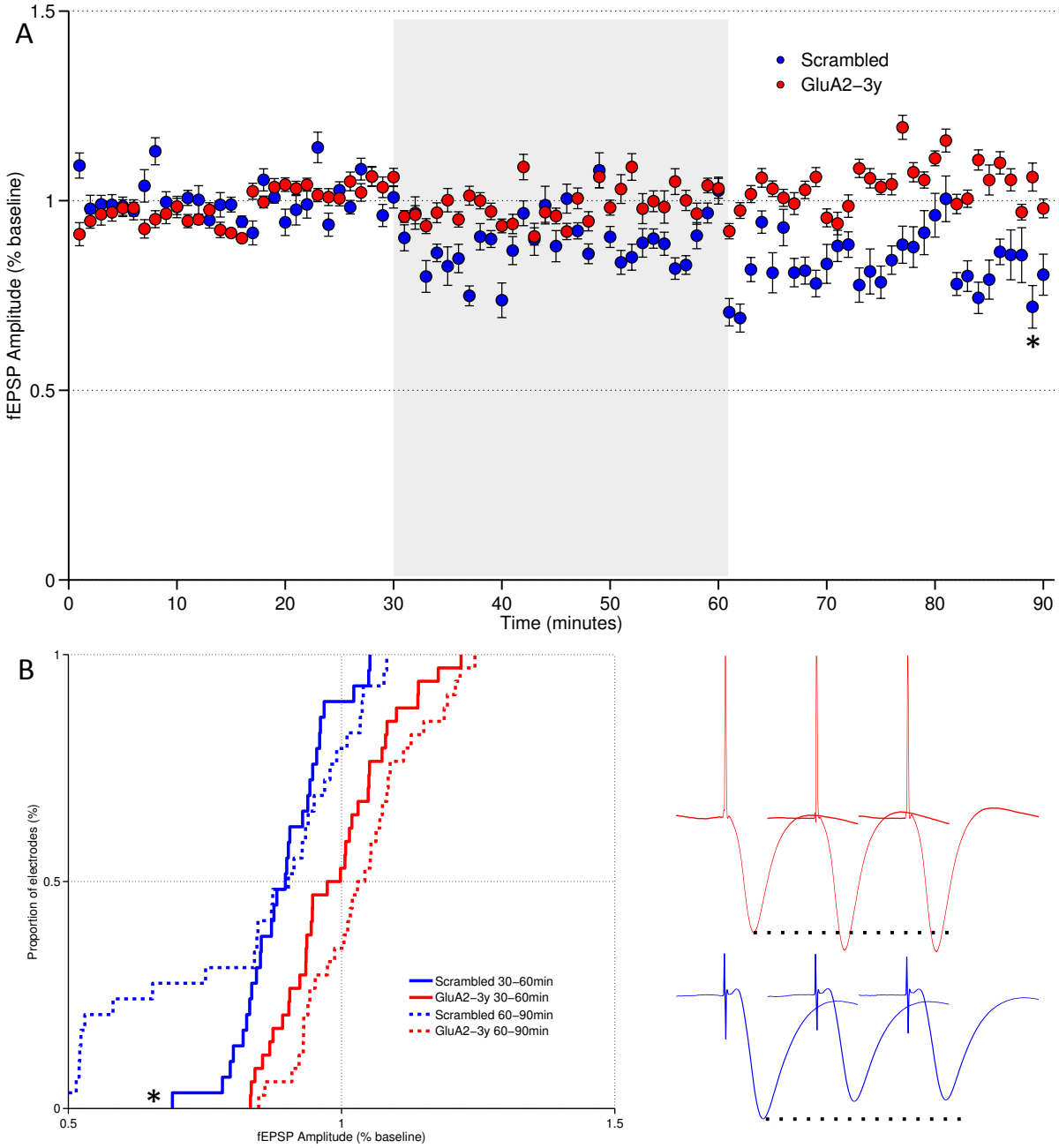

Figure 2.6 Exposure to a novel environment elicits a decrease in radiatum evoked potentials that is blocked by LTD inhibition.

$GluA2_{3y}$ peptide (red) or scrambled control (blue;2.25 μmol/kg in saline) administered 30 minutes prior to baseline. (A) A 30-minute baseline recording was followed by 30 minutes in a novel environment (grey shading), followed again by 30 minutes in the familiar recording chamber. A specific decrease in radiatum stimulation was observed in scrambled peptide treated rats, with no change in $GluA2_{3y}$ peptide treated rats. (B) Cumulative distribution of amplitude change relative to baseline showed a broad decrease in the novel environment (solid) that was maintained on return to the familiar environment

*(dashed) in control rats that was blocked by GluA2$_{3y}$ peptide. * indicates p<.05, follow-up comparison between group for epochs, ttest for average fEPSP change.*

2.3.4 Re-exploration of a novel environment does not provoke synaptic change

Exposure to the previously novel environment produced no pronounced difference on stratum radiatum or stratum oriens evoked potentials between rats previously treated with either GluA2$_{3y}$ or the scrambled control peptide (Fig 2.7A,B oriens;Fig 2.8A,B radiatum). A modest decrease in radiatum evoked potentials was observed during re-exploration in scrambled (92.8%±1.6) that reverted to baseline in the familiar environment (103.0%±1.5), while GluA2$_{3y}$ treated rats showed no change during re-exploration (99.8%±2.0) with a modest decrease following return to the familiar environment (97.5%±2.5, *F(2,118)=4.04, p=.02* main effect of epoch*, F(2,118)=10.78, p<.001* interaction*, p>.05* group effect, follow-up *t(59)=2.54, p=.01* during novelty*, t(59)=1.71, p=.09* in familiar chamber). A similar modest decrease in the magnitude of oriens evoked fEPSPs was observed in both groups, with re-exploration producing a modest decrease in scrambled (94.0%±1.3) and GluA2$_{3y}$ (96.9%±1.3) treated rats that was partially maintained after return to the familiar recording chamber (96.6%±1.5 scrambled, 97.6%±2.3 GluA2$_{3y}$, *F(2,114)=8.33, p<.001* main effect of epoch, other *ps>.05*). Thus the re-exploration of a previously novel environment does not trigger a similar long-lasting, novelty-like response in this synaptic pathway, even when that synaptic change was previously blocked pharmacologically. It might be expected that in rats treated with an inhibitor of LTD, subsequent re-exposure to the same novel environment in the absence of the blocker will induce depression of evoked potentials as if it were novel, however we did not observe this effect.

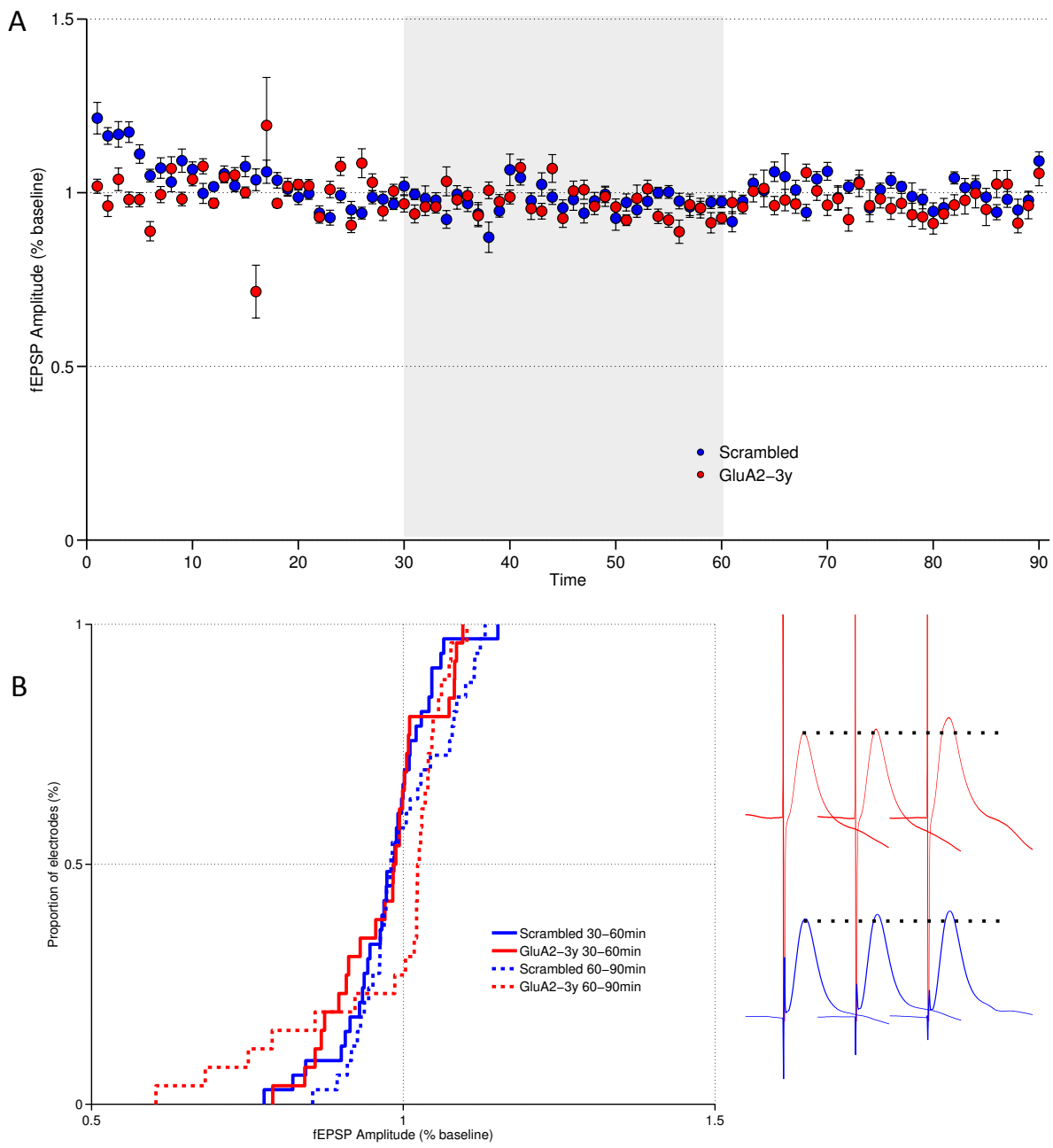

Figure 2.7. Oriens evoked fEPSPs during re-exposure to a novel environment.

No difference in oriens evoked fEPSPs in rats previously treated with scrambled peptide (blue) or GluA2$_{3y}$ (red). (A) A modest decrease in evoked potential amplitude was observed in oriens stimulation for both groups in response to a novel environment (grey shading). (B) No difference in the distribution of changes relative to baseline were observed during novelty (solid) and after returning to the familiar chamber (dashed). Scrambled vs GluA2$_{3y}$ p>.05.

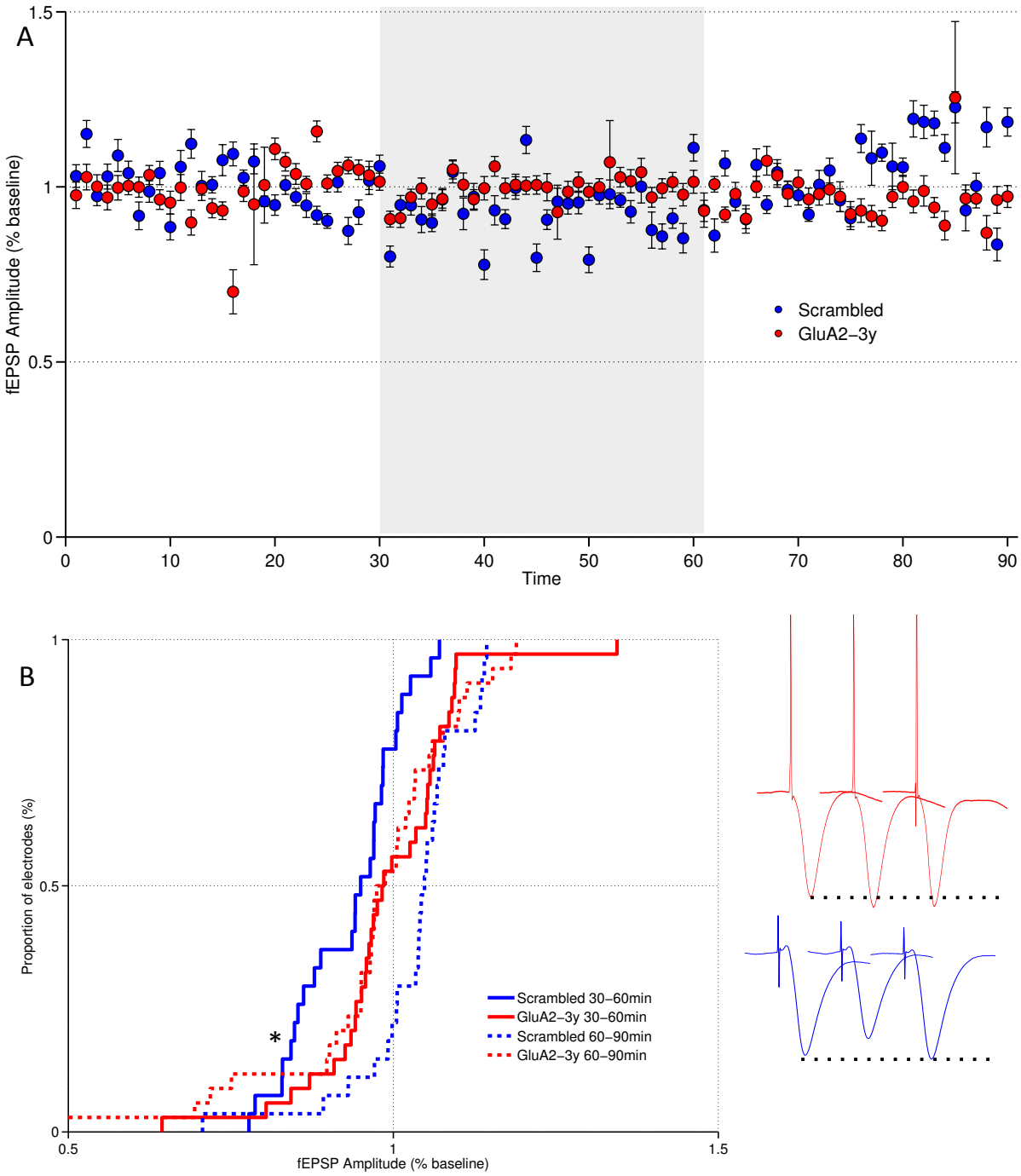

Figure 2.8. Radiatum evoked fEPSPs during re-exposure to a novel environment.

*Re-exposure to the novel environment does not produce a sustained decrease in radiatum evoked fEPSPs in rats previously treated with scrambled peptide (blue) or GluA2$_{3y}$ (red). (A) A modest decrease in evoked potential amplitude was observed with radiatum stimulation that reverted after return to a familiar environment. (B) A transient change in the distribution relative to baseline was observed during novelty (solid) in the scrambled group. * indicates decrease in scrambled relative to GluA2$_{3y}$, specific to the novelty period $p<.05$.*

2.3.5 LTD blockade impairs contextual learning on inhibitory avoidance

We next applied inhibitory avoidance tests to address if this AMPA receptor endocytosis-induced De Nova LTD plays a critical role in contextual learning. All rats exposed to the inhibitory avoidance chamber on the contextual exposure day readily explored both sides of the apparatus. Consistent with previous work showing the importance of contextual pre-exposure on inhibitory avoidance learning (Roesler et al., 1998;Matus-Amat et al., 2004), rats that did not receive contextual exposure a day prior to context-shock training (n=8) showed impaired inhibitory avoidance, crossing to the dark chamber within an average of 63 seconds ±46s SEM relative to saline treated rats with contextual pre-exposure (n=8), who showed robust inhibitory avoidance (238±58 sec, follow-up $t(14)=2.27, p=.039$ vs habituated saline, after 1-way ANOVA between all groups $F(3,41)=3.57, p=.022$). Scrambled peptide treated rats showed robust inhibitory avoidance (n=14, 188±48 sec), while $GluA2_{3y}$ treated rats were markedly impaired in inhibitory avoidance (n=15, 68±21 sec, $t(27)=2.25, p=.033$ vs scrambled). Thus, LTD blockade during contextual pre-exposure is sufficient to impair the subsequent context-shock association in the absence of drugs (Fig 2.9B), supporting a critical role of the novel environment-induced LTD in contextual learning.

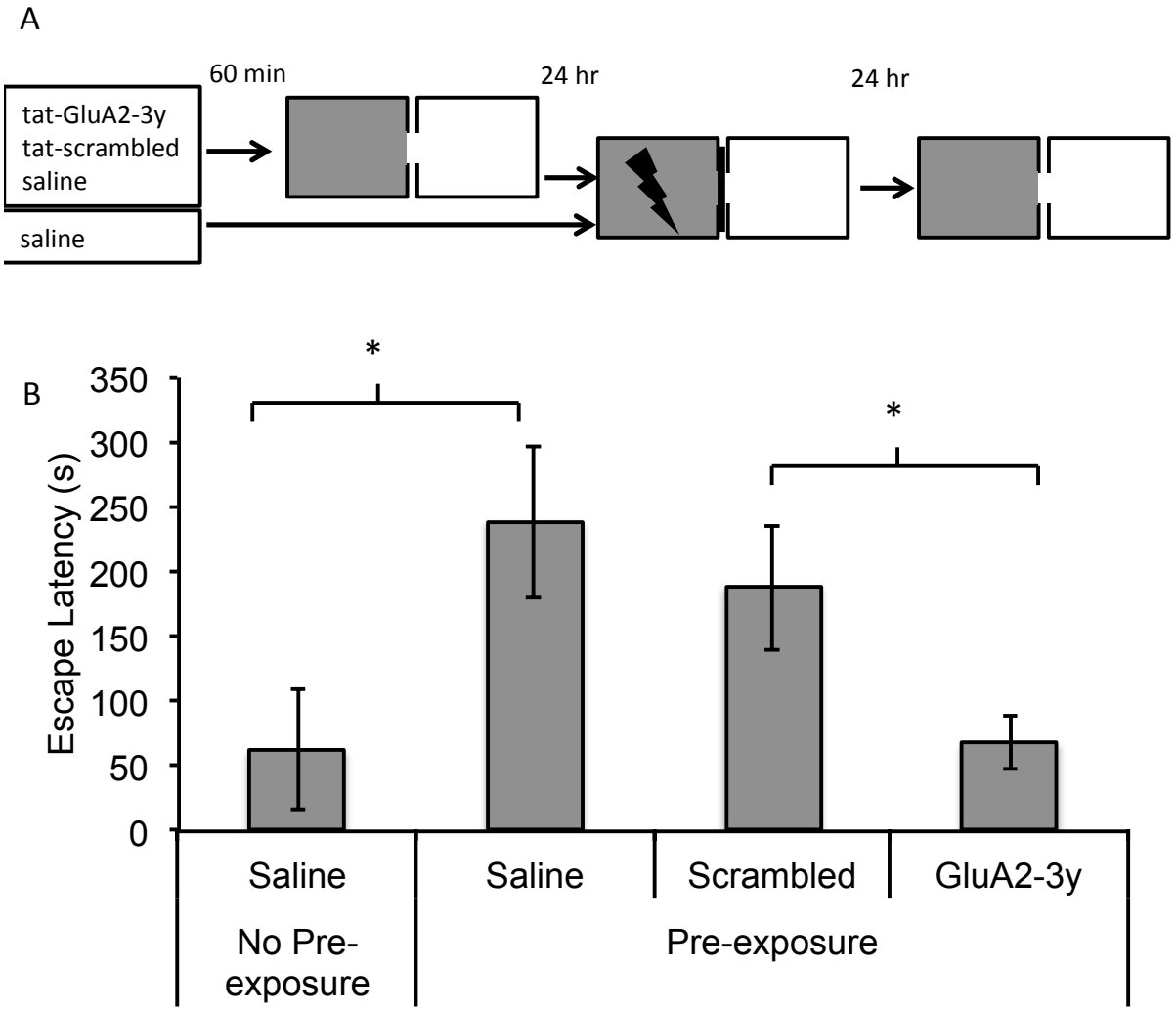

Figure 2.9 LTD inhibition prior to contextual pre-exposure impairs subsequent inhibitory avoidance learning in the context.

*(A) 60-minutes prior to contextual pre-exposure (8-minutes free exploration of both chambers), rats were administered $GluA2_{3y}$ peptide, scrambled control, or saline vehicle (2.25 μmol/kg IV). 24 hours later rats were conditioned by direct placement into the dark compartment and immediate shock (2 x 0.4mA, 0.5s shocks) and removal. 24 hours after training, rats were place in the light compartment and escape latency to the dark compartment was measured. (B) Rats with no contextual pre-exposure show poor memory relative to pre-exposed rats. $GluA2_{3y}$ during pre-exposure impairs inhibitory avoidance conditioning relative to a scrambled control. * indicates $p<.05$ pairwise comparison following ANOVA.*

2.4 Discussion

This work demonstrates a central role for LTD during exploration of a novel environment. Rats implanted with a recording array and dual stimulation electrodes in the stratum oriens and stratum radiatum showed a pathway specific decrease in evoked potential in response to stratum radiatum stimulation, which depolarizes apical dendritic synapses on CA1 pyramidal cells. The stable response profile of oriens evoked field potentials precludes many non-synaptic interpretations of this change: temperature (Moser et al., 1993) or behavioral state (Leung, 1980) based changes in evoked potential would affect synapses uniformly, in contrast to the specific change observed here. Considerable previous work has clearly established LTD as a mechanism facilitated by novelty, however this novelty typically includes conjunctive object-space novelty, and clear differences have been observed in the absence of objects (Kemp & Manahan-Vaughan, 2004). Spatial and non-spatial information are segregated within the entorhinal cortex (Knierim, Lee, & Hargreaves, 2006), and these inputs may be processed differentially within the hippocampus (Ito & Schuman, 2012), however we suggest that a sufficiently novel context will engage the same mechanisms as conjunctive object-context novelty. Novelty-driven synaptic plasticity has been demonstrated to depend on multiple receptors beyond NMDA receptors, including dopamine (Lemon & Manahan-Vaughan, 2006;Hansen & Manhan-Vaughan, 2014), noradrenaline (Kemp & Manahan-Vaughan, 2008;Hagena et al., 2016), and metabotropic glutamate receptors (Popkirov & Manahan-Vaughan, 2011). It is likely that these pathways are relevant for the *de novo* LTD observed in this study.

Our observation of LTD induced without a patterned induction stimulation is consistent with several reports. Depotentiation of established LTP was reversed by novelty exploration (Qi, Hu, & Rowan, 2013). Walk-though of a conditioning chamber was observed to induce decreased

evoked potentials in most channels of an array (Whitlock et al., 2006) and holeboard exposure with test pulse stimulation was sufficient to produce a short-term depression (Manahan-Vaughan & Braunwell, 1999). However these effects were not tested with inhibitors of synaptic plasticity, or show to be pathway specific. Here we show that this *de novo* decrease in evoked potential is blocked by inhibition of AMPA receptor endocytosis, and is specific to the apical dendritic inputs to CA1.

Using a multielectrode array, we were able to assay whether bidirectional changes in synaptic strength were observable. Our results suggest a broad based, though not necessarily homogenous LTD: No potentiation was observed on any electrode in the radiatum pathway after novelty exposure, however the long tail of the cumulative distribution function in both saline treated and scrambled treated rats might indicate a heterogenous distribution of changed synaptic weights. However caution should be taken in weighing this result, as the evoked potentials recorded here still represent the contribution of potentially thousands of individual synapses.

If LTD is required for acquisition of contextual information, it should be impaired in standard assays of contextual learning, particularly inhibitory avoidance. The hippocampus is thought to provide contextual cue inputs to the amygdala for association with an aversive unconditioned stimulus in contextual fear paradigms (Phillips & Ledoux, 1992; Kim & Fanselow, 1992). Previous work demonstrated that systemic $GluA2_{3y}$ peptide did not interfere with acquisition of contextual or cued fear conditioning in a paradigm in which there is prior habituation exposure to the context (Dalton et al., 2008). This demonstrates that LTD is not involved in acquisition of the context-shock association, however it is untested whether the formation of the contextual representation is itself vulnerable to LTD impairment. $GluA2_{3y}$ peptide given prior to the context pre-exposure marked impaired subsequent inhibitory

avoidance performance, with similar escape latencies to saline treated rats with no experience in the avoidance chamber prior to footshock conditioning.

This evidence in combination with the observation of *de novo* LTD during novel environment exploration strongly implicates LTD as a necessary mechanism for the acquisition of contextual information in the hippocampus.

2.4.1 Implications

We show here that novel spatial exposure elicits a broad de novo LTD, and that a specific inhibitor of AMPA receptor endocytosis blocks this LTD. Although previous work has established that novel object and object configurations facilitate LTD induction, we show that even in the absence of objects, a novel environment not only facilitates LTD induction, but produces de novo LTD with only test pulse stimulation.

Place field formation occurs in a novel environment, and these novel environment place fields are specifically affected by NMDA receptor blockade (Kentros et al., 1998). However NMDA receptor blockade would affect both LTP and LTD. The question naturally arises whether a specific blockade of LTD would similarly affect place fields in a novel environment, and whether other effects specific to LTD blockade might occur. Therefor we designed experiments to specifically address these important questions in the following Chapter.

Chapter 3 Development and Maintenance of Place Field Firing in a Novel Environment

3.1 Introduction

Place specific firing is the dominant coding mechanism for pyramidal cells in the dorsal hippocampal CA1 area (O'Keefe & Speakman, 1987). The apparent stability of the place coding of individual cells upon repeated exposures to the same environment (Thompson & Best, 1990) suggests that this feature of pyramidal cell firing is a mechanism for memory. This idea is supported by studies showing that interventions that would be expected to interfere with hippocampal memory also affect the location stability of place cells (Rotenberg et al., 1996; Cho et al., 1998; Kentros et al., 1998; Rotenberg et al., 2000; Agnihotri et al., 2004), and that induction of LTP induces place field remapping (Dragoi et al., 2003). Administration of CPP prior to exploration of novel environment led to typical place field firing in the novel environment, however the location of this firing within the environment was not maintained upon re-exposure to the same novel environment on the following day (Kentros et al., 1998). This effect was specific for a novel environment, as the firing location for cells within a familiar environment was unperturbed with CPP. While these effects imply that LTP is required for place field maintenance, the interventions used, whether NMDA blockade, CAMKII genetic deletion, or protein synthesis inhibition, would also block LTD based on their known signaling pathways. Given the consistent evidence of LTD facilitation in novelty exploration, we asked whether specifically blocking LTD during the same protocol that elicited LTD of the schaffer collateral CA1 pathway would affect place fields in the novel environment.

As the plasticity events observed during novelty exposure occur rapidly in the early portion of the trial, a question exists as to whether this plasticity is involved in the initial development of place related firing. Observing these dynamics is difficult, since a cell's place field can only be accurately assessed over the time period required for the rat to explore the entire maze. In a standard size two-dimensional environment with pseudorandom exploration, this time period is variable and takes at least several minutes, which may be too slow to observe place fields as they are forming. This confound may have contributed to the early belief that place fields were immediately formed on first exposure to an environment (Hill, 1978). However it is clear that there is an early labile period in which place fields are either unstable in their location or not firing at a high rate (Wilson & McNaughton, 1993; Tanila et al., 1997). More recently, recordings in one-dimensional mazes (linear or circular running tracks) have shown that fields emerge over several initial laps of a novel environment (Frank et al., 2004; Monaco et al., 2014). As well, experiments that recording field firing in mice exploring virtual environments (Harvey et al., 2009) have observed similar dynamics (Epsztein, Brecht, & Lee, 2011; Lee, Lin, & Lee, 2012) and implicated dendritic plateau potentials and other nonlinearities in the rapid recruitment of new place fields (Bittner et al., 2015). Importantly, these reports support the idea that an active synaptic plasticity process is required even over the short time span in which this occurs, as the subthreshold voltages recorded before, during, and after recruitment change dramatically.

In order to address whether blockade of LTD affected the formation dynamics of place fields in a novel environment, a reconfigurable linear maze was used to present novel configurations of intramaze navigation paths and extramaze cues. Both the formation dynamics and the maintenance of place field location across days was assessed in this second place field recording task.

3.2 Methods

3.2.1 Subjects

All experiments were approved by the University of British Columbia Animal Care Committee in accordance with the policies of the Canadian Council on Animal Care. Male sprague-dawley rats (Charles River) weighing 300-450 grams were housed in pairs on a 12hr:12hr standard light cycle, or individually for freely moving electrophysiology experiments. Rats used in place cell experiments were food restricted to 90% of free feeding weight, with water access *ad libitum*.

3.2.2 Drugs

The interference peptide Tat-GluA2$_{3y}$ (YGRKKRRQRRR-$_{869}$YKEGYNVYG$_{877}$) and scrambled control Tat-Scramble (YGRKKRRQRRR-VYKYGGYNE) were synthesized in house and dissolved in saline (2.25 μmol/kg) for intravenous bolus injection.

3.2.3 Microdrive construction

Microdrives with four to eight channels were constructed in order to independently adjust the depth of recording tetrodes. A single bundle of 30-gauge stainless steel guide tubes spaced the tetrodes by 300μm. Tetrodes were made of four braided strands of 15μm NiCr wire (California Fine Wire, USA) and sheathed in polyimide tubing (75μm ID). Each actuator of the microdrive consisted of a 12.7mm brass 00-90 hexagonal head machine screw (JI Morris, USA) coupled to a 23-gauge stainless steel drive tube. Tetrodes were threaded through the 30-gauge guide tubes and 23-gauge drive tubes, and individual wires were pinned to a 36 channel electronic interface board (EIB-36N, Neuralynx, USA). Prior to surgery, tetrode tips were

precision cut and gold plated with a gold potassium cyanide solution to improve the recording of biopotentials by reducing electrical impedance and tissue reactivity.

3.2.4 Surgery

Jugular vein catheterization

Rats were anesthetized with isoflurane (5% induction, 2% maintenance) and an incision made in the upper right quadrant of the thorax to expose the right jugular vein. An indwelling silastic catheter (Dow Corning Corp, USA) was inserted into the jugular vein, and the distal end was run subcutaneously to a port on the dorsal surface of the rat between the scapulae. In the case of rats undergoing electrophysiology recordings, the port was integrated into the headcap.

CA1 Pyramidal Spiking Electrophysiology

Following jugular vein catheterization, anesthetized rats were placed in a stereotaxic frame, and a 2.0 by 2.0mm cranial window (-2.3 to 4.3mm AP, 1.0 to 3.0mm ML) was drilled over the right dorsal hippocampus. Dura was excised to allow placement of the tip of the microdrive bundle above the cortex overlying the hippocampus. The microdrive was fixed to the skull by dental cement and implant skull screws, which also served as ground and reference points.

3.2.5 Behavioral protocol

In order to record from populations of hippocampal neurons, tetrodes were lowered slowly through the overlying cortex over several weeks of daily sessions while filtered LFP activity (1-475 Hz) and high frequency unit activity (600-6000 Hz) were monitored. The hippocampal pyramidal layer was identified by the characteristic appearance of ripple activity (150-200 Hz) occurring irregularly during immobility, grooming, and sleep. Tetrodes were slowly lowered

further until unit activity was observed during ripples, and subsequent tuning took place to maximize stable, separable units.

Place field recording – novel environment

During spike tuning, rats were food restricted and trained to forage for sucrose pellets in a recording chamber (40x40x60cm). Baseline recordings were collected during 15-minute foraging sessions to confirm the appearance of place specific firing. On the test day, rats received IV infusions (tat-GluA2$_{3y}$/tat-Scramble 2.25 μmol/kg in 1 ml/kg saline) 45 minutes prior to recording. Rats first ran a 15-minute foraging session in the familiar configuration, then were removed and placed in a novel environment (60x60x60cm polarized box) to run a 30-minute foraging session. Rats were then returned to the baseline recording chamber for an 15-minute session.

Place field recording – linear maze

Rats were food restricted and trained to shuttle for sucrose pellets delivered to both ends of a linear maze consisting of four 40cm segments (width 12cm, wall height 10cm) linked by turns of 45°, 90°, and 135°. Rats were trained until a minimum of 8 laps were completed within an 8-minute session.

On the first exposure day, rats received IV infusions (tat-GluA2$_{3y}$/tat-Scramble 2.25 μmol/kg in 1 ml/kg saline) 45 minutes prior to recording. Rats first ran an 8-minute shuttling session in the familiar maze configuration, then were removed and placed in a novel configuration to run a 16-minute shuttling session. Rats were then returned to the baseline configuration for an 8-minute recording session.

On the subsequent re-exposure day, rats ran an 8-minute shuttling session in the familiar maze configuration, then were removed and placed in the same, previously novel configuration to run a 16-minute shuttling session. Rats were then returned to the baseline configuration for an 8-minute recording session.

Multiple configurations were possible by adjusting the position of the maze in space and the sequence and direction of turns from one end of the maze to the other.

3.2.6 Data acquisition and analysis

General place cell processing

Spiking and LFPs were recorded using a 32-channel electrophysiology recording system with integrated headstage preamplifier (Digital Lynx 32, HS-36, Neuralynx, AZ), with positional information recorded by tracking a colored LED mounted on the headstage preamplifier with an overhead camera (Neuralynx, AZ). The CA1 pyramidal cell layer was identified using a combination of stereotaxic depth and electrophysiological characteristics. Ripple activity was identified in the LFP channel during quiescence and feeding periods, and tetrode depth was adjusted to maximize multi-unit activity during ripple activity. Spike sorting was conducted offline using manual spike sorting software (Offline Sorter, Plexon). Putative spikes were pre-processed to remove noise and clusters were identified using 3d projections of spike characteristics. Only well-isolated clusters were selected for analysis. Entire recording sessions, whether containing a single or multiple environmental exposures were sorted collapsed over time.

Sorted spike data and LFPs were analyzed using an open source MATLAB toolkit (FMAtoolbox) and custom MATLAB analysis code. For each recording session, positional information was first filtered to remove target points occurring outside the arena. Tracking jitters

were removed by setting a threshold movement speed. Missing points were interpolated from nearby samples and all position samples were smoothed over a ~2 second Gaussian window. Spikes were velocity filtered to remove firing during immobility, defined as movement below 2.5 cm/s over a smoothed 5-second window.

Linearize maze processing

For linear track data, two dimensional position samples were linearized to one dimension by collapsing along a single axis defined by a set of vertices corresponding to the end points and three interior corners of the linear maze. Laps were then identified using local extrema on a 25 second smoothed plot of the linearized data. Incomplete laps were removed and subsequent analysis separated inward and outward going laps. Spatial firing rate maps were generated for each isolated unit using a grid of 50x50 bins for 2 dimensional box data, while 1 dimensional linearized data used 50 bins.

Identification of cells across days

The method was based on Tolias et al., (2007)and Powell & Redish (2014). For each cell on each day, the average waveform was calculated, and highly correlated waveforms (r>.97, taken as a 128x1 array of voltage samples, with 32 samples for each tetrode sequentially) recorded from the same tetrode across days in the same rat were taken as a training set of putative matched pairs. Two measures of waveform shape similarity (tolias distance) were calculated for each pair of cells from the same rat, with waveforms expressed as a 32x4 array of voltage samples, each column representing each channel of the tetrode. The first (D1) was a measure of shape similarity of waveforms normalized to minimize the sum of squares difference between the two waveforms on each channel (Fig 3.2A). These scaling factors were used to calculate a normalized Euclidean distance between the waveforms on each channel (Fig 3.2A),

summing across channels to produce a single D1 value. A separate D2 value, meant to capture both differences between waveform sizes overall and differences between the scaling factor on each channel was also calculated (Fig 3.2A). Plotted on two dimensions, the D1 and D2 values are both small for matching cell pairs, and larger for non-matching pairs. Using the training set, a Bayesian classifier was used to classify all cell pairs as matching or non-matching. A small minority of cells were positively classified to multiple cell on other days, in this case a linearized tolias distance was used to select only the most closely matched cell pair. Firing rate maps for pairs of cells were correlated as two linear arrays using pearson's product-moment correlation.

3.3 Results

3.3.1 Place field characteristics with acute GluA2 receptor endocytosis blockade

Over three recording days, a total of 218 separable cells were recorded from area CA1 in eight rats. Most recorded cells showed characteristic spatially restricted firing patterns in at least one environment. Cells were categorized as active place cells if they showed spatially modulated firing (>1 Hz mean in field firing rate and <20 Hz mean overall rate). In one baseline recording session from putative place cells, the average in-field firing rates (Fig 3.1B, $t(45)=1.19, p>.05$), field size (Fig 3.1A, $t(45)=0.51, p>.05$), and specificity (Fig 3.1C, $t(41)=0.61, p>.05$) were comparable between the control and drug assigned rats.

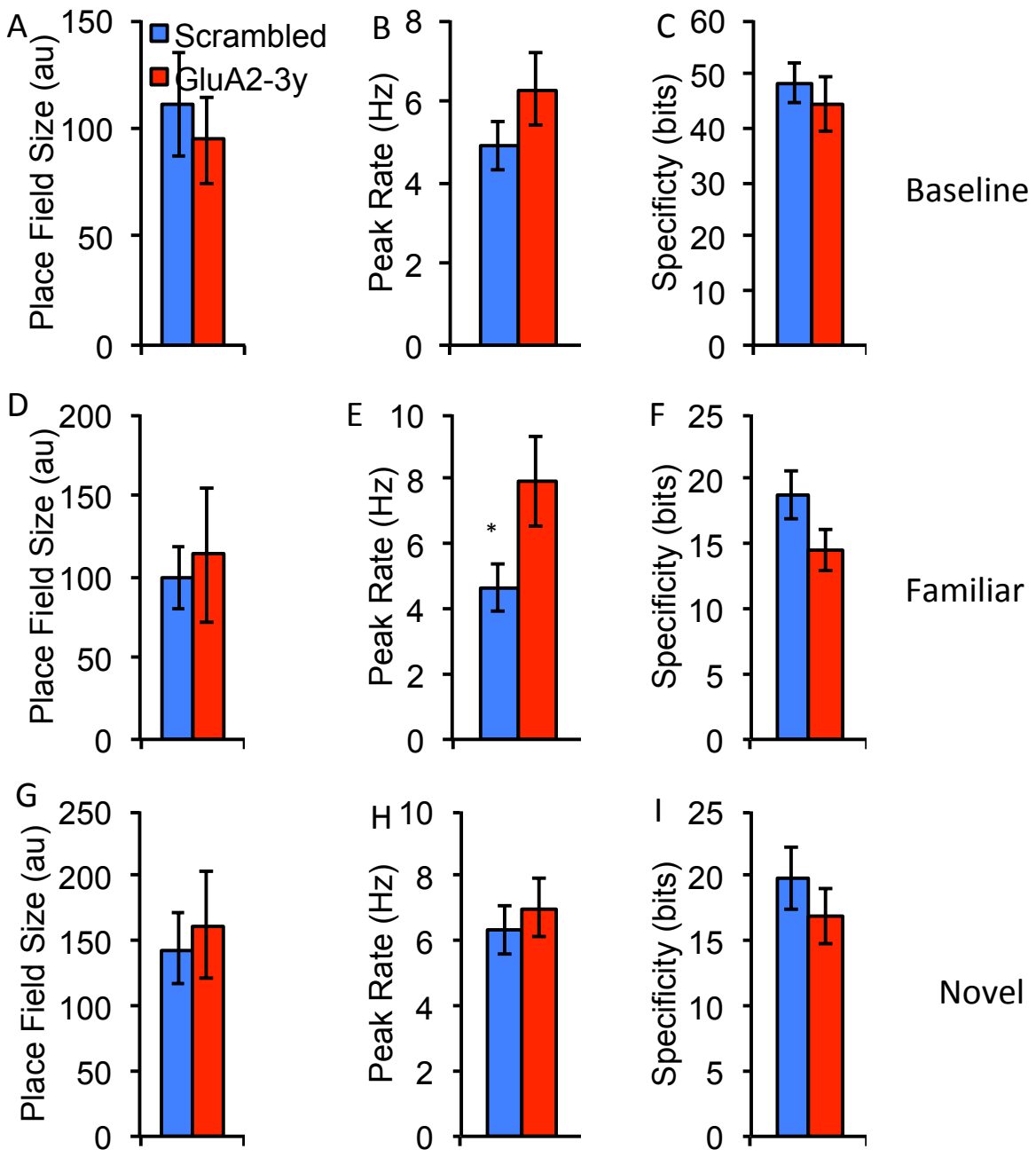

Figure 3.1 General place field parameters.

In a familiar environment on baseline day, place field parameters are similar between group (A-C). Field parameters in familiar recording chamber are normal in $GluA2_{3y}$ *and scramble treatment groups. No drugs were administered on the baseline day.On an exposure day* $GluA2_{3y}$ *peptide (red) or scrambled control (blue) treatment was administered prior to exposure to both environments (2.25 μmol/kg IV). Place field size and specificity were unaffected by drug treatment in both familiar (D,F) and novel (G,I) environments. (E) Peak in-field firing rate was elevated in* $GluA2_{3y}$ *treated rats, specifically in the familiar environment. * indicates p<.05 peptide vs control t-test in familiar environment. All other p>.05.*

Blockade of GluA2 receptor endocytosis does not affect spatial selectivity of place fields

The following day four rats received bolus IV injection of the $GluA2_{3y}$ peptide and four rats given a scrambled variant of the peptide 45 minutes prior to exploration of the familiar environment. Field size and specificity in the familiar environment were comparable between $GluA2_{3y}$ peptide and scrambled treated rats (Fig 3.1D,F, *t(43)=0.42, p>.05* field size, *t(39)=1.62, p>.05*, specificity), however an elevated in-field firing rate was observed in cells from 3y peptide treated rats (Fig 3.1E, *t(42)=2.21, p=.032*).

Upon exposure to a novel environment, spatially modulated firing fields were observable in cells from both $GluA2_{3y}$ and scrambled peptide treated rats. Of these cells, there were no differences in average in-field firing rate, average field size, or average specificity between the two groups (Fig 3.1G,H,I, *t(67)=0.38, p>.05 field size, t(67)=0.60, p>.05 peak rate, t(53)=0.88, p>.05 specificity*).

3.3.2 Maintenance of place field activity across days in a two-dimensional environment

When recording conditions permit identification, hippocampal place cells tend to firing in the same location within an environment across days (Thompson & Best, 1990), and interventions that affect behavioral expression of contextual memory seem to affect the maintenance of firing location (Kentros et al., 1998). Cells were identified across days using waveform characteristics in order to test whether blockade of LTD affected the location specific firing (Fig 3.2A,B;Tolias et al., 2007; Powell & Redish, 2014).

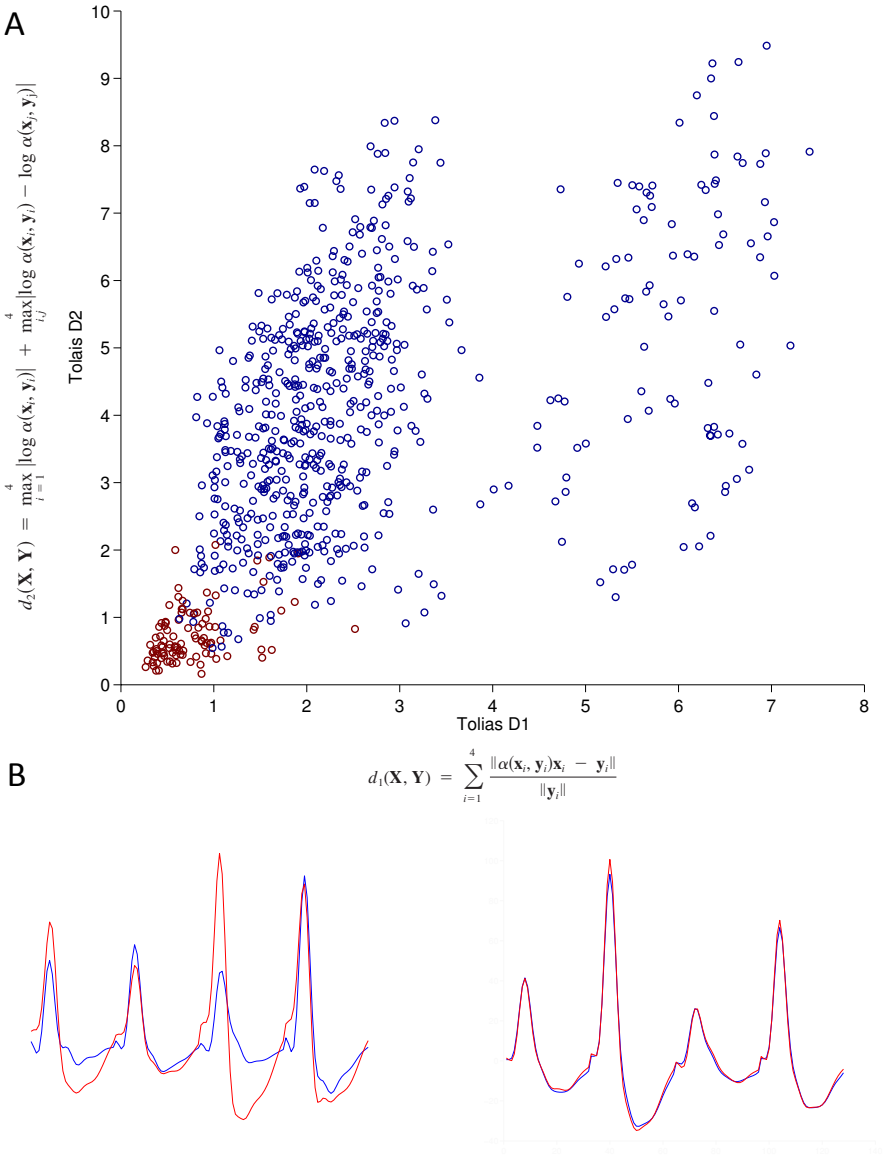

Figure 3.2 Identification of cells across days.

Average waveforms for each sorted cell were compared to average waveforms on subsequent days, recorded from the same tetrode. (A) Projection of computed tolias distance value D1 (x-axis) and D2 (y-axis) indicated a subset of highly similar waveforms (red), classified with a 2-dimensional Bayesian classifier trained on a subset of highly correlated waveforms (p>.97). (B) Example tetrode waveforms from non-matched (left) and matched (right) pairs of cells. Each peak represents the average waveform recorded on each of four tetrode channels.

Place-specific firing is conserved across baseline days

A total of 92 cells from eight rats were identifiable across two days. Within this set, cells were analyzed for correlated place activity across days if they showed a place field within the analyzed environment (peak firing rate exceeding 1 Hz) in at least one recording session. Firing rate map correlations between the baseline exploration of the familiar maze and the familiar maze exploration prior to novel environment exploration were similar in both groups (Fig 3.3, *t(28)=0.24, p>.05*).

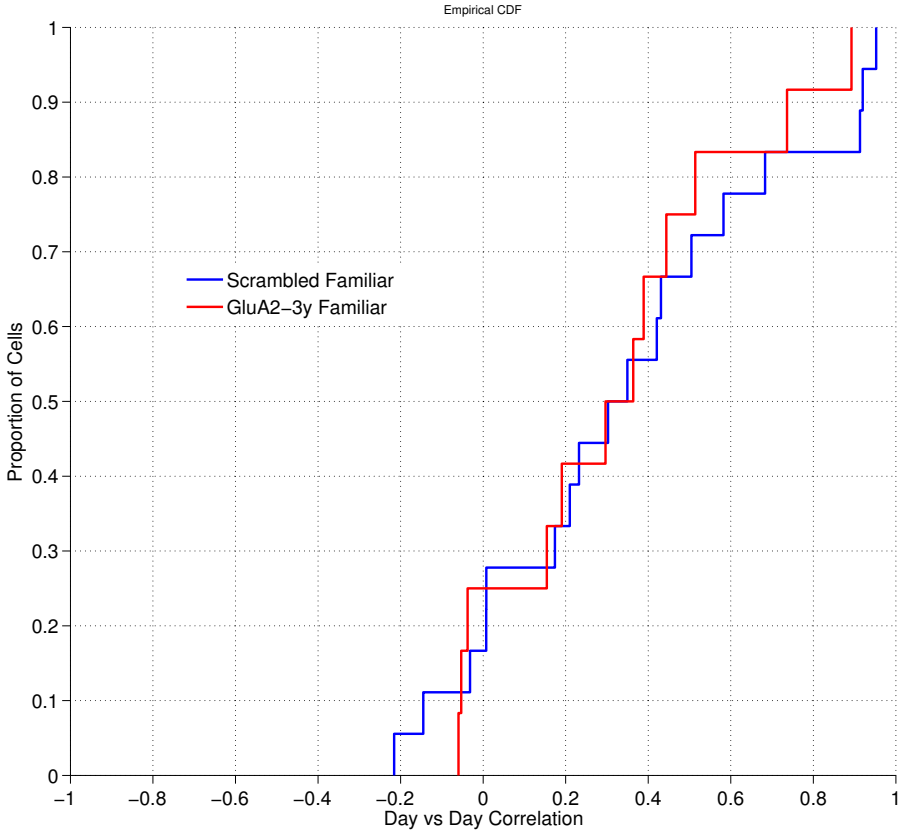

Figure 3.3 Place field maintenance in a familiar environment.

Day over day correlation between baseline day and exposure day, in the familiar environment. Most cells showed a modest positive correlation, no difference was observed between treatment groups Scrambled (blue) and GluA2$_{3y}$ (red). Drug (2.25 μmol/kg IV) was administered prior to recording on the exposure day. p>.05 between scrambled and GluA2$_{3y}$.

Place-specific firing is conserved after exposure to a novel environment in control, but not $GluA2_{3y}$ treated rats

Place fields from four rats administered a scrambled control peptide prior to exposure to a novel environment were compared with fields from the same cells that formed upon re-exploration of the same environment the subsequent day. The median correlation in this group of cells indicated relatively conserved field location in the novel environment (median r=0.58). In contrast, place fields from four rats administered the $GluA2_{3y}$ peptide prior to exposure to a novel environment showed significantly less conservation when compared with fields from the same cells that formed upon re-exploration, although place field conservation was not entirely absent (median r=0.36; $t(43)=2.19, p=.034$; Fig 3.4).

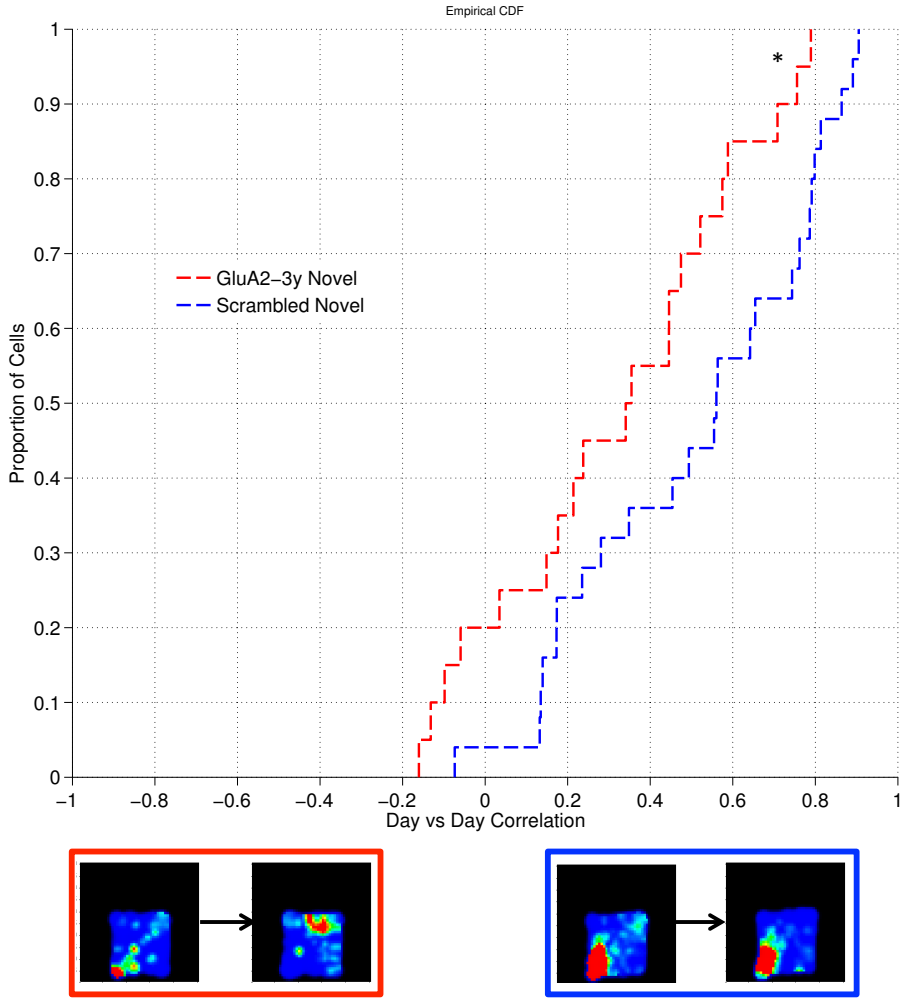

Figure 3.4 Place field maintenance in a novel environment.

*Day over day correlation between exposure day and re-exposure day in the novel environment. Firing fields for cells recorded from scrambled treated rats (blue) were more highly correlated across days as compared with firing fields from cells recorded from GluA2$_{3y}$ (red) treated rats. Drug (2.25 µmol/kg IV) was administered prior to recording only on exposure day. * indicates p<.05 ttest.*

To test whether novelty exposure affects place fields representing familiar environments, cells with fields in the familiar environment were compared with fields upon re-exploration of the familiar environment on the re-exposure day. Treatment with GluA2$_{3y}$ peptide produced no significant impairment in place field location conservation as compared with scramble treated controls (scrambled median r=0.56 vs GluA2$_{3y}$ median r=0.55; *t(35)=0.95, p>.05*;Fig 3.5). This

indicated that the impaired field location conservation was specific to place fields formed in the novel environment.

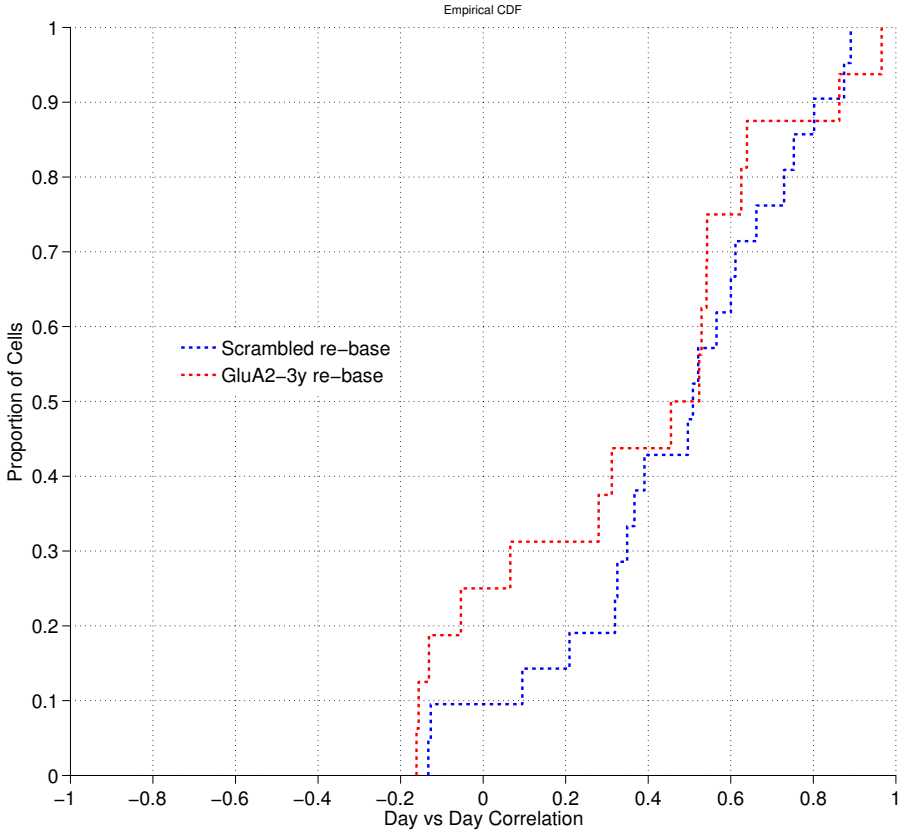

Figure 3.5 Place field maintenance in a familiar environment after novelty exposure.
Day over day correlation between exposure day and re-exposure day in the familiar recording chamber. Firing fields for cells recorded from scrambled treated rats (blue) and from cells recorded from $GluA2_{3y}$ (red) treated rats were similarly correlated in the familiar environment. Drug (2.25 μmol/kg IV) was administered prior to recording only on exposure day. p>.05.

3.3.3 Maintenance of place field activity across days in a novel linear environment

To observe place field conservation and dynamics in an alternative novel environmental exposure task, rats were exposed to multiple novel configurations of a linear maze with rearrangable segments (Fig 3.6A).

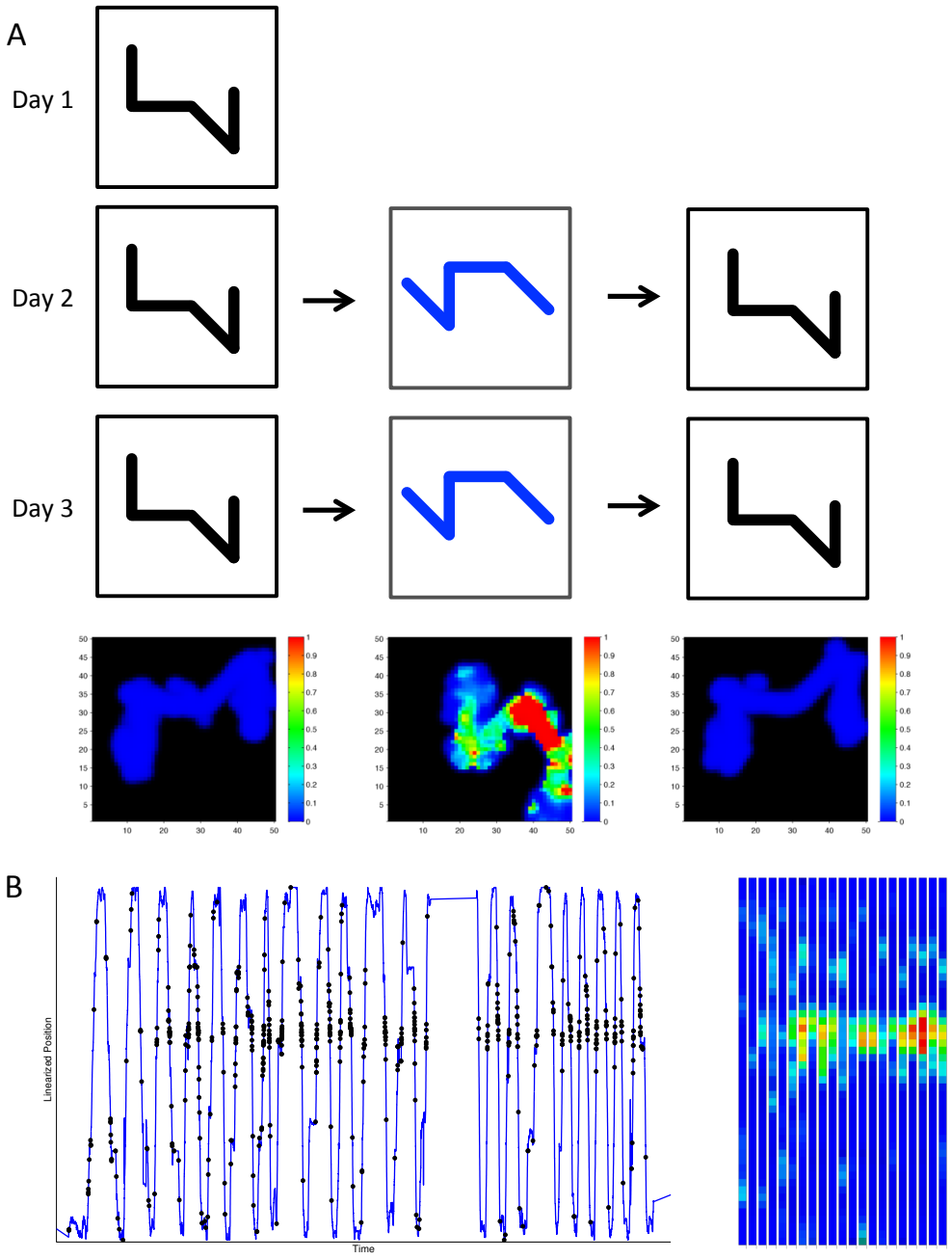

Figure 3.6 Place field recording on a reconfigurable linear maze.

(A) A familiar configuration of four maze sections was recorded on a baseline day. A novel reconfiguration of the maze and external landmarks was presented first on an exposure day, and the same reconfiguration wa presented on a re-exposure day. Example cell that fired specifically in the reconfigured maze. (B) Spatial location was linearized to compare place field firing on multiple laps. Linearized position and spike locations for one cell (left) and the lap-by-lap firing field for the same cell as in A.

A total of N=458 cells from seven implanted rats were recorded over the course of the experiment. Rats were run twice through a three-day protocol with one baseline day, one exposure day on which drug was administered and a novel configuration was presented, and one re-exposure day. The familiar configuration remained constant throughout, and two different novel maze configurations were used. Drug administration was counterbalanced such that three rats received the $GluA2_{3y}$ peptide first and four rats received the scrambled peptide first. Two rats, one from each group, did not complete enough laps during novelty exposure to allow for analysis during the second protocol. This resulted in complete data from six scrambled peptide treated rats and six $GluA2_{3y}$ treated rats.

During baseline exploration of the familiar maze, the majority of recorded cells in both groups (49 of 64 cells scrambled, 47 of 64 cells $GluA2_{3y}$) showed significant firing in at least one location within the maze (peak rate greater than 1 Hz), the balance showing either limited overall firing or consistently elevated firing rates characteristic of interneurons. Of putative place cells, the average in-field firing rate, average field size, and average specificity was comparable between groups (Fig 3.7A-C, *ps*>*.05*).

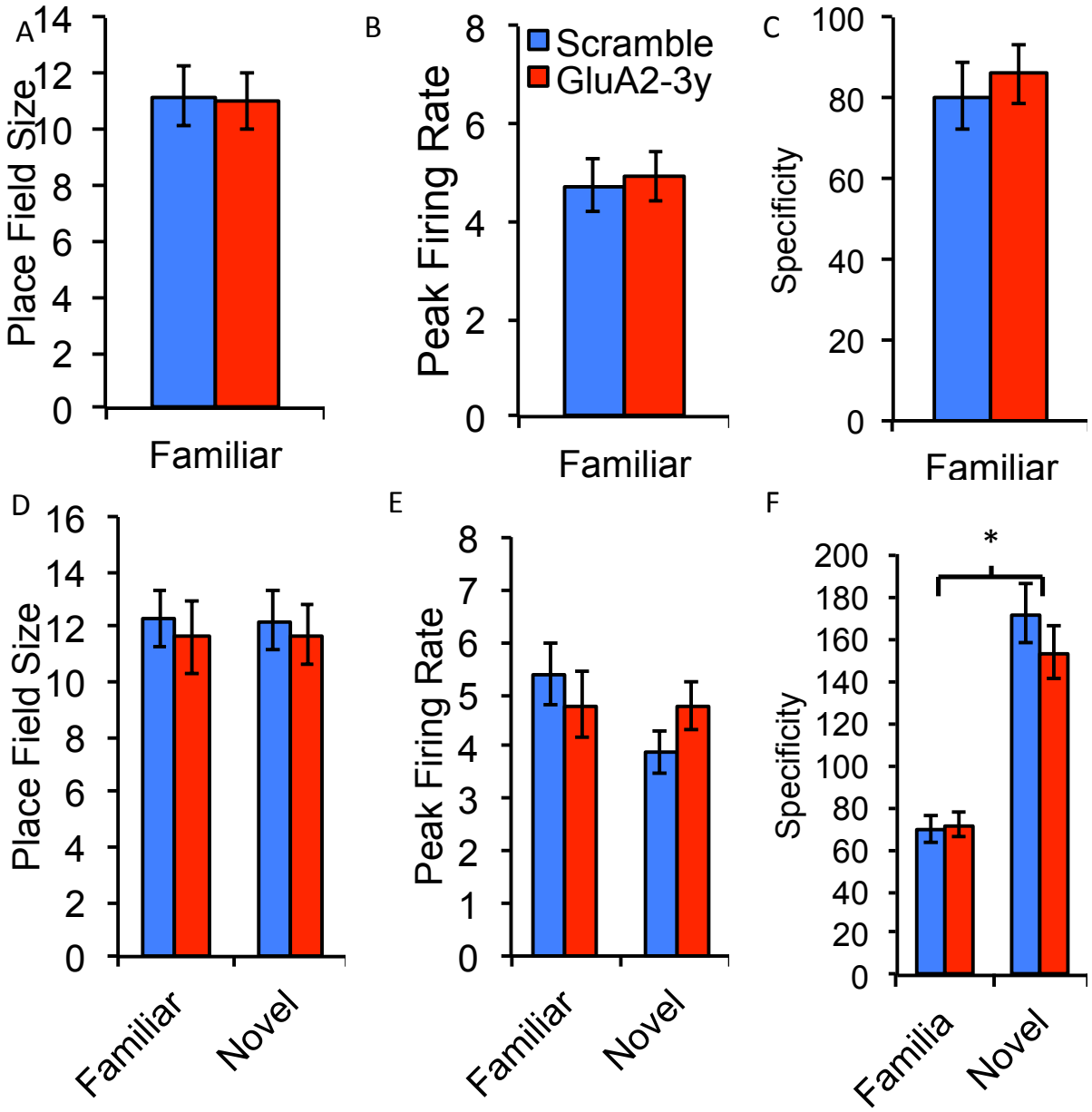

Figure 3.7 Place field parameters are similar between groups.

*On baseline day, place field size (A), peak firing rate (B), and specificity (C) were similar between $GluA2_{3y}$ (red) and scrambled (blue) peptide treatment groups. No drugs were administered on the baseline day. On exposure day, place field size was similar in all conditions (D), peak firing rate was slightly lower in control rats in the novel environment (E), and specificity was elevated in both treatment groups in the novel environment relative to the familiar configuration (F). * indicates $p<.05$ main effect of environment.*

GluA2 receptor endocytosis inhibition does not affect characteristics of place fields in a familiar environment

On the exposure day, rats received either $GluA2_{3y}$ peptide or a scrambled control IV prior to recording. In the familiar configuration, place field characteristics for recorded cells active during the session (48 of 88 recorded cells scrambled, 40 of 72 recorded cells $GluA2_{3y}$) were similar between groups (Fig 3.7D-F, *ps*>*.05*).

GluA2 receptor endocytosis inhibition does not affect characteristics of place fields in a novel environment

As in the exploration of a novel box environment, upon exposure to a novel maze configuration, place firing fields developed in cells from both $GluA2_{3y}$ and scrambled peptide treated rats. Of 72 recorded cells, 41 were active in the novel configuration from $GluA2_{3y}$, while 50 of 88 total cells recorded from scrambled treated rats became active. Place field size and in-field firing rate were similar between groups and similar to field size in the familiar configuration (Fig 3.7D,E, *ps*>*.05*). Specificity was elevated in the novel configuration in both groups (Fig 3.7F, 2-way ANOVA $F(1,284)=67.72$, $p<.001$ for environment, *ps*>*.05* for group and interaction).

Place-specific firing is conserved across baseline days

As previously, cells were identified across days using waveform characteristics in order to test whether blockade of GluA2 receptor endocytosis affected the location specific firing. Cells were analyzed if they showed a place field (peak firing >1 Hz) in at least one recording session. Correlations between firing fields in the familiar maze during the baseline recording and the

familiar maze explored immediately prior to novel environment exploration were high in both groups (Fig 3.8). There was no significant difference between $GluA2_{3y}$ treated rats and scramble controls, although there was a non-significant trend for a reduced correlation in the $GluA2_{3y}$ peptide treated group ($GluA2_{3y}$ r=0.44;scramble r=0.60, t(73)=1.34, *p>.05*).

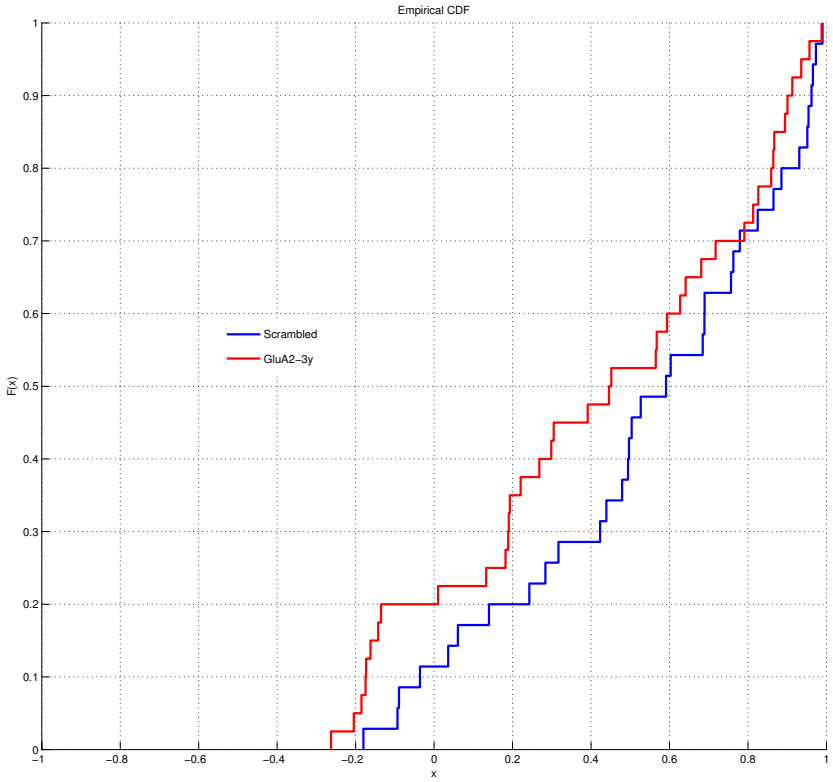

Figure 3.8 Correlation between baseline day and exposure day in the familiar configuration. *Firing fields for cells recorded from scrambled treated rats (blue) and from cells recorded from $GluA2_{3y}$ (red) treated rats were similarly correlated in the familiar environment. A non-significant trend towards lower correlation in the $GluA2_{3y}$ group was observed. Control vs $GluA2_{3y}$ p>.05.*

Place-specific firing is conserved after exposure to a novel environment in control, but not $GluA2_{3y}$ treated rats

Place fields in the novel configuration from rats administered a scrambled control peptide $GluA2_{3y}$ prior to exposure were correlated with fields from the same cells that formed upon re-exploration of the same environment the subsequent day (median r=0.63, Fig 3.9, dotted lines).

In contrast, place fields from rats administered the $GluA2_{3y}$ peptide prior to exposure to a novel environment showed significantly less conservation when compared with fields from the same cells that formed upon re-exploration, although place field conservation was not entirely absent (median r=0.20, Fig 3.9, dotted lines, *t(125)=2.87, p=.008*).

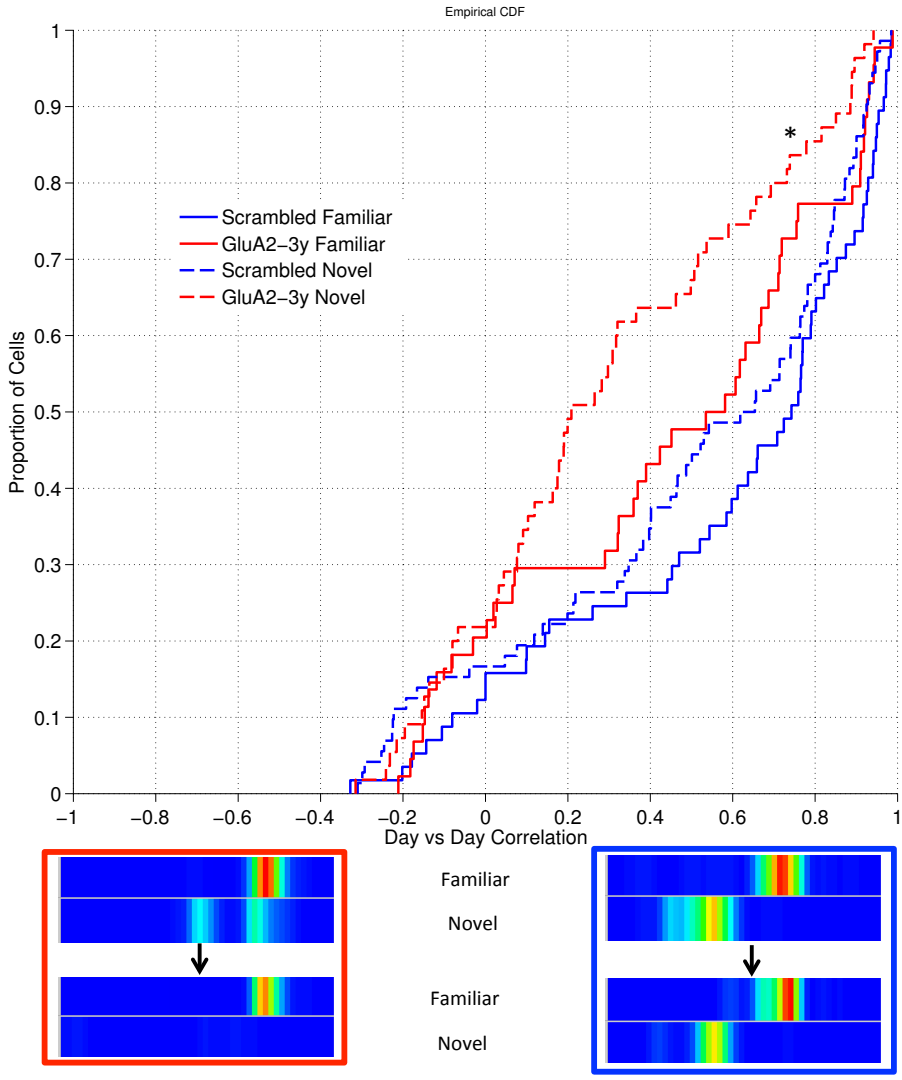

Figure 3.9 Correlation between exposure day and re-exposure day in the familiar and novel configurations.

*Firing fields for cells recorded from scrambled treated rats (blue) and from cells recorded from $GluA2_{3y}$ treated rats (red) were highly correlated in the familiar configuration (solid lines), with a trend towards less correlation in the $GluA2_{3y}$ treatment group. In the novel configuration, $GluA2_{3y}$ treated marked reduced day over day correlation as compared with the scrambled control group (dashed lines). * indicates p<.05 vs control in the novel configuration.*

To compare whether this disruption was specific to fields formed in the novel environment, familiar configuration fields were compared between exposure day and re-exploration of the familiar environment on the re-exposure day. The proportion of field conservation in scrambled peptide treated rats (median r=0.73, Fig 3.9, solid lines) and from rats given the $GluA2_{3y}$ peptide (median r=0.54,Fig 3.9, solid lines, $t(97)=1.96, p>.05$) was not significantly different, although there was a trend for field conservation to be lower in peptide treated rats relative to controls. From this we conclude that blockade of LTD produced a specific impairment on the maintenance of place field location in the novel environment.

3.3.4 The effect of GluA2 receptor endocytosis inhibition on acute formation of place fields

As rats covered the entire maze more than once a minute, the linearized maze design permitted an analysis of firing field stability within a session. Firing maps for each cell were generated for each individual lap (Fig 3.6B). Place cells can show direction specificity on linear mazes so inward and outward going laps were analyzed independently. For each rat, a correlation matrix was calculated for each cell, and the median correlation of each lap to each other lap was taken as a similarity index. As rats completed varying numbers of laps within the fixed time recording session, the first eight laps were averaged for familiar configuration sessions (8-minute sessions) and the first 16 laps were averaged for the novel configuration sessions (16-minute sessions).

On the baseline day shuttling in the familiar configuration, place fields were highly correlated across laps in both groups (Fig 3.10). Place fields remained highly correlated in the familiar configuration on the exposure day, after $GluA2_{3y}$ administration or a scrambled control peptide (Fig 3.11A).

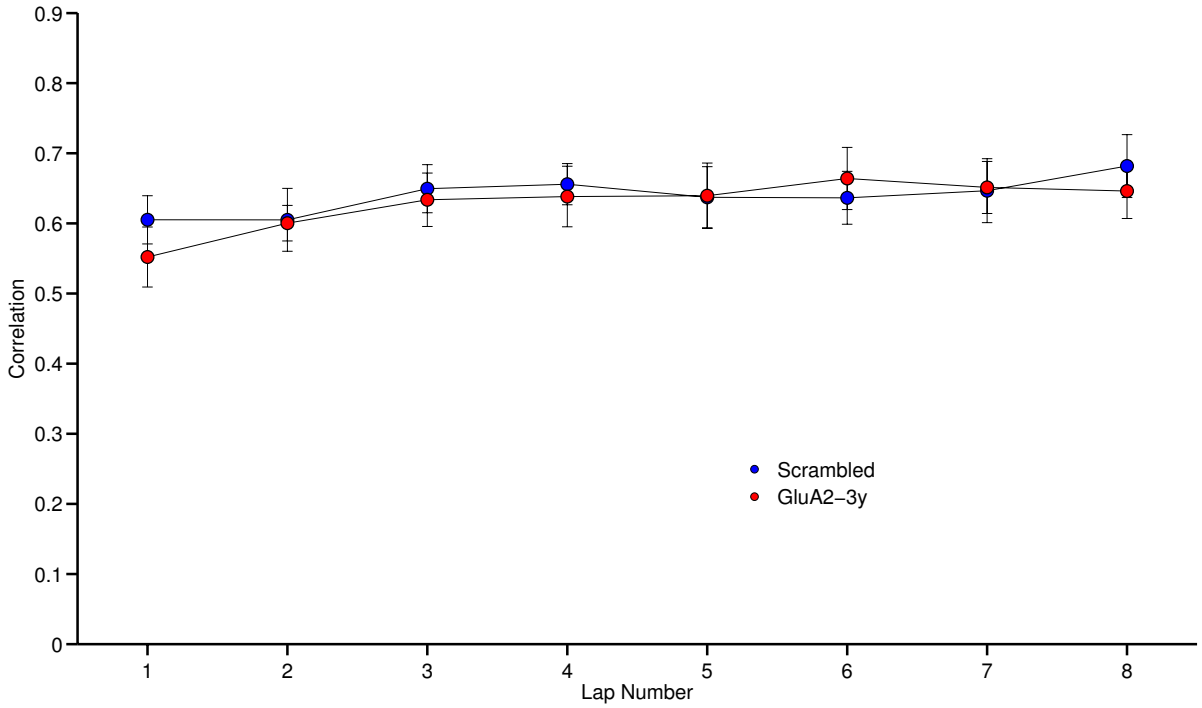

Figure 3.10. Lap by lap correlations in a familiar environment.
Place fields are consistently highly correlated in the familiar environment on baseline days. Place cells from scrambled (red) and GluA2$_{3y}$ (blue) groups showed similar correlation over the first 8 laps in a familiar configuration.

Place fields develop over several laps upon first exposure to a novel environment

In rats administered the scrambled control, the average place cell firing map increased correlation over the first several laps, with significantly lower correlations vs the final lap until the fourth lap (Fig 3.11A,B). An alteration in these dynamics was evident in rats administered the GluA2$_{3y}$ peptide, where highly correlated place fields were established by the second lap (Fig 3.11A,B). There was a significant lap by group interaction ($p<.05$), however a follow-up comparison showed a difference in correlation values on only the second lap. A comparison of the distribution of correlations on specifically the first four laps demonstrated a rapid, single lap shift to a high correlation distribution in GluA2$_{3y}$ treated rats (ks-test vs lap 4: lap 1 $ks=.37$

p<.001, all other laps p>.05), whereas a cumulative shift over four laps was evident in control rats (Fig 3.11B, ks-test vs lap 4: lap 1 *ks=.52, p<.001,* lap 2 *ks=0.29, p=.0017,* lap 3 *ks=.22 p=.042*). Thus LTD blockade did not diminish the lap to lap stability of place fields, instead seeming to accelerate the formation of a stable place field during novelty exposure, producing a measurable change in correlation only over the first several laps of the exposure.

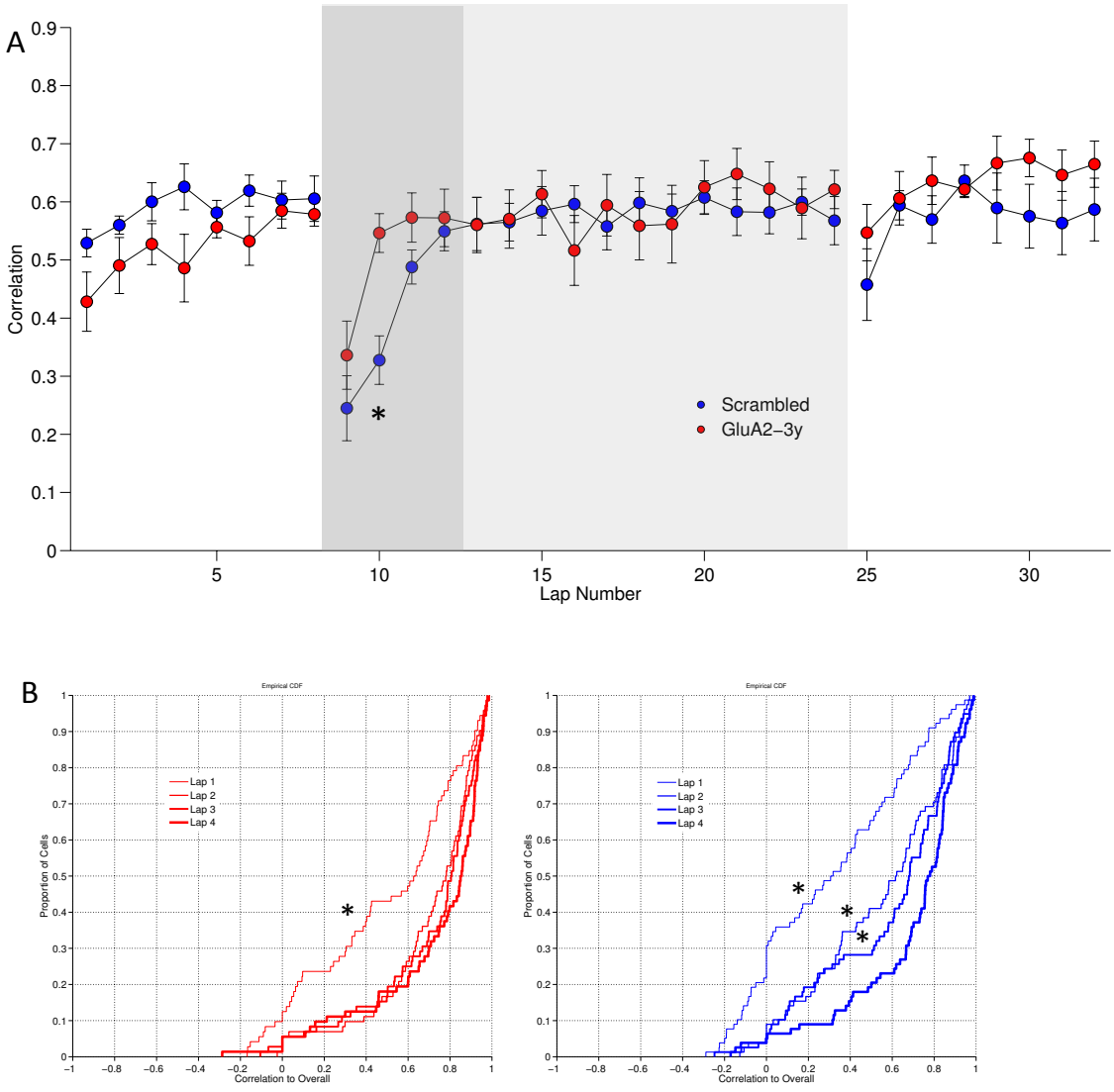

Figure 3.11 Lap by lap correlations develop over several laps in a novel environment.

Rats were administered GluA2$_{3y}$ or scrambled peptide (2.25 µmol/kg IV) prior to a baseline exploration of the familiar configuration, followed by exploration of a novel configuration (grey box). (A) Place fields were highly correlated and similar between groups in the familiar configuration, but were lower in the

*first several laps of a novel configuration (grey shading). (B) The cumulative distribution of correlations on the first four laps (darker shading in A) were examined. Correlations developed progressively over four trials in scrambled treated rats (blue, right), however highly correlated fields were established after a single lap in GluA2$_{3y}$ peptide treated rats (red, left). * indicates p<.05 pairwise comparison only on lap 2 after significant lap by group interaction (A) and p<.05 ks-test vs lap 4 (B).*

Prior LTD blockade impairs maintenance of correlated firing across days

The day following novel configuration exposure and drug administration, rats were re-exposed to both the familiar and the novel maze configurations. In scrambled peptide treated rats, lap to lap correlations were consistently high throughout the trial both in the familiar configuration and the novel configuration (Fig 3.12). Lap to lap correlations for place cells in the familiar configuration were similarly high throughout the session in GluA2$_{3y}$ treated rats. In contrast, upon re-exposure to the novel configuration, initial place field correlations were modest, and increased over time (Fig 3.12). This increase in lapwise correlations was reminiscent of control correlation on first exposure to the environment, suggesting that while control rats showed evidence of memory maintenance upon re-exposure to a novel environment, rats treated with GluA2$_{3y}$ showed evidence that the maintenance effect was disrupted.

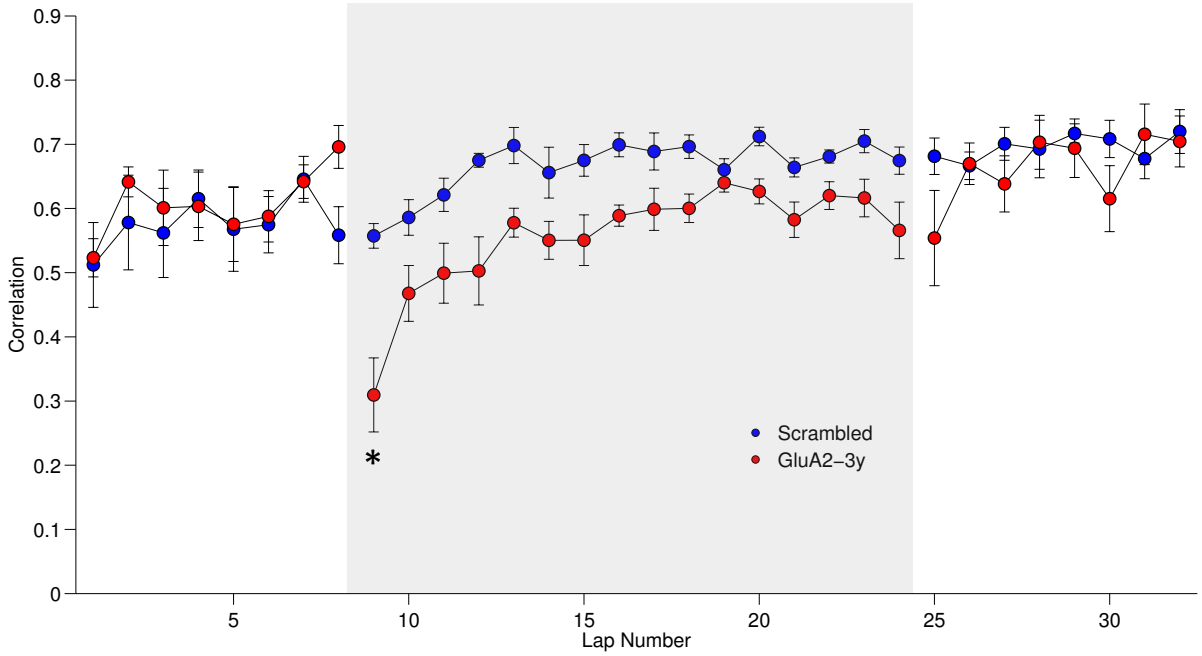

Figure 3.12 Lap by lap correlations during re-exposure to a novel environment.

*Rats previously administered $GluA2_{3y}$ peptide (blue) or scrambled peptide (red) were re-exposed to the novel environment after baseline exploration of the familiar environment. Lap by lap correlations were high in the familiar configuration in both groups, however only rats previously treated with scrambled peptide showed highly correlated fields in the early laps on the previously novel environment (grey shading). In contrast, correlations developed over several laps in rats treated with $GluA2_{3y}$ peptide, reminiscent of the initial exposure to the environment in control rats. * indicates $p<.05$ pairwise comparison after significant group by lap interaction.*

3.4 Discussion

The primary finding in this study is that specific blockade of LTD with the $GluA2_{3y}$ peptide impairs the location stability of place fields established in a novel environment, when assessed 24 hours later. This result was observed in two different maze configurations, a traditional two-dimensional box environment and a one-dimensional linear track. In a two-dimensional box environment, place cells from control treated rats had a moderate median correlation value between exposure and re-exposure, consistent with previous work. $GluA2_{3y}$ treated rats had reduced median correlation, and this difference was specific for the novel environment, as similar moderate place correlation was found in the familiar environment presented concurrently on the exposure and re-exposure days. In a reconfiguration of a linear maze, field location was generally more stable in control treated rats as compared with the two-dimensional maze. However peptide treated rats place conservation was still potently impaired, with a median correlation of approximately r=0.2.

This effect has been observed in rats treated with CPP, a non-competitive antagonist of the NMDA receptor (Kentros et al., 1998). In this case, CPP treated rats showed virtually no correlation in place firing between exposure and re-exposure (0.47 saline to 0.03 CPP), suggesting that CPP entirely abolished the place representation. In our case, some residual correlation was clearly evident in both mazes. As the $GluA2_{3y}$ peptide is a potent inhibitor of LTD and without effect on LTP, the additional effect of CPP is presumably due to either inhibition of LTP or another effect of NMDA receptor blockade. Several alternative methods for inhibiting synaptic plasticity that keep NMDA function intact report impaired place field stability, including CAMKII knockouts, PKA impaired mice, and protein synthesis inhibition (Rotenberg et al., 1996; Rotenberg et al., 2000; Agnihotri et al., 2004). In these cases, place fields from

treated animals showed some residual correlation, as with our $GluA2_{3y}$ peptide treatment. The importance of these small differences is unclear, however it is possible that NMDA receptor activation can promote some low level of place field stability through alternative pathways from the canonical plasticity maintenance pathways.

Our results suggest that impairing the expression mechanism of LTD is sufficient to mediate most of the effects of non-specific plasticity impairment on place field stability. This places LTD as a central mediator for the development of a new contextual representation in area CA1 of the hippocampus. As this treatment was systemic, it is possible that this effect is due to inhibition of LTD at synapses other than the schaffer collateral input to CA1. This pathway is considered a prime candidate due to the considerable evidence that LTD is specifically occurring at this synapse during novelty exposure. However in the alternative case, blocking plasticity would produce a maintenance impairment that is transferred downstream to CA1 from elsewhere, and the inhibition of LTD at the schaffer collateral pathway is simply incidental. The most likely alternative mediators for this effect would be perforant path inputs to any of dentate gyrus, CA3 or CA1, or the recurrent collaterals within the CA3 network. The mossy fiber input from dentate gyrus to CA3 seems less likely, considering the NMDA receptor independence of plasticity in this pathway. The direct entorhinal cortical input to CA1 can maintain place firing (Brun et al., 2002), and in a highly differentiated novel environment where there are no pattern separation/completion demands, both CA3 and CA1 establish new ensembles to represent the new space, with CA1 representations stabilizing faster than CA3 (Leutgeb et al., 2004; Vazdarjanova & Guzowski, 2004). However the schaffer collateral input to CA1 is required for formation of CA1 place fields in a novel environment (Nakashiba et al., 2008), so this input pathway is still a likely mediator of the effects of LTD blockade seen here.

Our observation of altered acute formation dynamics in the presence of $GluA2_{3y}$ peptide is the first indication that synaptic plasticity may be required for normal rapid place field representation. We did not find evidence that place fields characteristics were abnormal, or instability in lap-by-lap dynamics. The alteration observed was in fact an enhancement of lap-by-lap stability, specifically in the early laps in a novel environment. In control rats, place field firing on the first lap was minimally correlated with the overall firing map for the 16-minute trial, and gradually gained stability over several more laps. In contrast, firing maps correlated with the overall map were observed on the first lap in $GluA2_{3y}$ treated rats, and by the second lap reached an asymptotic level. This suggests that LTD is involved in an early process of constraining place field recruitment. In absence of this constraint, fields appear more rapidly. The fact that no discernable deficits in firing specificity were observed implies that this specificity is either intrinsic or mediated by other plasticity mechanisms.

We have shown a proscribed role for LTD in both the development and maintenance of place fields in a novel environment. This effect is consistent with previous work implicating the NMDA receptor in place field consolidation, however the relative contributions of LTP and LTD could not be elucidated. We suggest that LTD, which is specifically recruited during novelty exposure, is at least in part responsible for constraining the recruitment of place cells into an ensemble representation of the novel place, a necessary step for the long-term maintenance of a contextual representation.

Chapter 4 General Discussion

The studies described in this thesis support a role for synaptic long-term depression mechanisms in the acquisition of novel spatial information in the hippocampus. Inhibition of activity-dependent AMPA receptor endocytosis with the peptide inhibitor tat-GluA2$_{3y}$, a validated and widely used specific inhibitor of long-term depression (Collingridge et al., 2010) blocked a novelty induced decrease in evoked fEPSPs in the hippocampus, blocked facilitation of inhibitory avoidance by contextual exposure, and impaired the maintenance of place field locations on re-exposure to a novel environment. In addition, acute LTD blockade altered the dynamics of place field formation in a novel environment. Place specific firing was unimpaired with LTD blockade, however stable fields developed more rapidly with LTD blockade, with spatial firing established after a single lap on a linear maze that remained highly correlated for the duration of the trial. In comparison, place fields from control animals developed over several laps, with an equivalent level of correlation reached only after four laps. These results demonstrate a role for LTD in the creation and maintenance of cellular representations of place in the hippocampus, suggesting a role for LTD in episodic memory formation generally.

There are two important conclusions that can be drawn from the observation that LTD was specifically observed in synapses to the stratum radiatum. The first is that the changes in evoked potential are not likely due to global alterations such as brain state changes or temperature change. The second is that the information transfer via inputs to basal and apical dendrites of CA1 pyramidal cells is differentially altered by novelty. Apical and Basal inputs are physiologically isolated (Sajikumar et al., 2007; Spruston, 2008), however both inputs contribute to the generation of the complex spiking in CA1 cells that code for place in a freely moving animal (Grienberger, Chen, & Konnerth, 2014). It is important to note that apical inputs are

collocated with direct inputs from entorhinal cortex, and functional interactions between these inputs influence spiking behavior (Bittner et al., 2015) and plasticity in both pathways (Dudman et al., 2007; Takahashi et al., 2009). LTD in the apical Schaffer collateral input is therefor uniquely positioned to govern the likelihood that coincident inputs from the entorhinal cortex will drive cell spiking (Jarsky et al., 2005).

4.1 Factors that Contribute to Facilitated LTD during Novelty

One of the fundamental observations concerning LTD is the difficulty in its induction, particularly in adult animals (Bashir & Collingridge, 1994). A narrow set of parameters is required to produce LTD in hippocampal slices (Dudek & Bear, 1992) or in anesthetized in vivo preparations (Heynan et al., 1996), including specific and prolonged induction stimulation patterns, a moderate afferent activation level and reduced feedforward inhibition. As a consequence, the comparative ease of inducing LTP has led to the expectation that potentiation is a more common feature of learning related synaptic plasticity. This generalization overlooks the fact that there are several unique circumstances that appear to facilitate the induction of LTD. In the hippocampus, exposure to stress or novelty facilitates the induction of LTD (Foy et al., 1987;Manahan-Vaughan & Braunwell, 1999), and as demonstrated by the data in the present thesis, no patterned input is required to see broad LTD in response to environmental novelty, where contextual learning is presumed to take place. The co-occurrence of LTD with behavioral learning suggests that it plays a central role in the plastic changes that mediate new learning.

Specific physiological changes mediate the pronounced metaplastic facilitation of LTD, although the mechanisms are not fully understood. Considerable work has established that multiple neuromodulators, including dopamine, serotonin, and noradrenaline (Kemp & Manahan-Vaughan, 2007b) are required for novelty induced LTD. Both dopamine and noradrenaline are elevated during novelty exposure (Ihalainen et al., 1999), and D1/5 antagonists as well as noradrenergic B2 antagonists block novelty facilitation of LTD, indicating that these receptors mediate the neuromodulator effects in the hippocampus. The mechanisms by which dopamine and noradrenaline facilitate LTD are unclear. Both D1/5 receptors and B2 receptors are canonical Gs coupled G-protein coupled receptors (GPCRs), that activate a protein kinase A

(PKA) signaling cascade, a pathway implicated in LTP facilitation but not LTD (Malenka & Bear, 2004). Indeed both D1/5 receptors and b2 receptors are traditionally implicated in the facilitation of LTP in the hippocampus (Otmakhova et al., 1996; Huang & Kandel, 1996). This suggests that neuromodulator signaling during novelty produces cellular effects distinct from those that have been observed in vitro. This could be due to additional novelty triggered changes such as glucocorticoid release, or novelty specific activity and excitability changes within the hippocampus, but these mechanisms remain to be determined.

4.2 Multiple Independent Roles for LTD in Learning

The primary findings of this research contribute to a growing body of evidence that implicates LTD in new hippocampal learning. There are several well-established roles for LTD in different learning and memory domains. One frequently observed role of LTD appears to be to suppress behavior guided by previously learned associations. Alternation deficits (Nicholls et al., 2008; Brigman et al., 2010) and reversal learning deficits (Duffy, Labrie, & Roder, 2008; Dalton et al., 2011) have frequently been observed with disruption of GluN2B function, effects attributed to a deficit in LTD (Dong et al., 2013). Extinction learning (Dalton et al., 2008) and time-dependent memory loss (Hardt et al., 2013; Dong et al., 2015; Migues et al., 2016) also require synaptic LTD. However there is accumulating evidence that certain forms of new learning also depend on LTD. While simple associative learning does not seem to be affected by LTD blockade (Dalton et al., 2008), recognition learning is strongly impaired (Griffiths et al., 2008; Cazakoff & Howland, 2011). In the hippocampus, administered the $GluA2_{3y}$ peptide immediately following 8 trials on the morris water maze showed impaired memory for the location of the escape platform when tested 24 hours later (Ge et al., 2010), suggesting that LTD blockade interferes with hippocampal guided navigation to this reference location. Rats administered $GluA2_{3y}$ prior to exposure to a novel object configuration show reduced behavioral habituation to the configuration on re-exposure as indicated by exploratory behaviors, indicating that memory of the configuration is impaired by LTD blockade (Dong et al., 2012). These effects suggest a role for LTD in the consolidation of a hippocampal representation of a location within a maze or a spatial configuration, as well as the representation of an object in the perirhinal cortex. Our current observation that LTD blockade interferes with contextual pre-exposure facilitation of inhibitory avoidance, as well as the maintenance of place field location firing

supports the idea that LTD is required for the maintenance of contextual representations. We also make an additional novel observation as to the role of LTD during the acquisition of contextual information. LTD is measurable during exposure to a novel environment, and blocking LTD while a rat explores a novel linear maze for the first time alters the dynamics of place fields that are forming within this environment. This suggests that in addition to a role for LTD in the consolidation of new spatial learning, LTD may participate in the initial formation of a contextual representation. Whether these two effects are in fact independent or simply represent the same process being affected at different times during the acquisition and consolidation process remains to be determined.

4.3 Alternative Accounts of Hippocampal LTD in Spatial Novelty

The pathway specific LTD that we observed in response to novel environment exploration is not necessarily responsible for the behavioral effects of systemic LTD blockade and the associated effects on single unit activity. Although the $GluA2_{3y}$ peptide is a specific inhibitor of AMPA receptor endocytosis, it is important to note that systemic administration would block LTD throughout the brain. Studies using intrahippocampal AP5 administration to block NMDA receptors have specifically implicated the hippocampus in the contextual learning component of inhibitory avoidance (Roesler et al., 2003; Matus-Amut et al., 2004; Malin & McGaugh, 2006), so the present effect of $GluA2_{3y}$ inhibition of AMPA receptor endocytosis is likely mediated by disruption of hippocampal function. Similarly, the effect of synaptic LTD blockade on CA1 place cells is most likely mediated by synapses directly on CA1 pyramidal cells or within the specific neural circuit that provides activity input to these cells. Every major hippocampal synaptic pathway appears to undergo metaplastic changes, and facilitation of both LTP and LTD have been observed following novelty exposure paradigms. This includes the perforant path input to the dentate gyrus (Wiescholleck & Manahan-Vaughan, 2014; Hansen & Manahan-Vaughan, 2015), the mossy fibre input from dentate gyrus to CA3 (Hagena & Manahan-Vaughan, 2011; Hagena & Manahan-Vaughan, 2012), and the associational commissural inputs between CA3 cells (Hagena & Manahan-Vaughan, 2011). It is important to note that different types of novelty exposure drove different metaplastic responses. Specifically, the presence and salience of objects changed the plasticity response, depending on the synapses. Generally, novel objects or a novel configuration of large objects facilitate LTD, while environmental changes without objects facilitates LTP. There are notable connectivity differences between entorhinal cortical inputs that are believed to code for spatial versus non-spatial features, with MEC inputs

and LEC inputs segregated along the dendritic arbor in DG and CA3 and segregated topographically in CA1 (Ito & Schuman, 2012). These input patterns could contribute to the differential metaplastic responses observed with and without objects, however a novelty magnitude effect is also possible. Thus although LTD induction driven by purely spatial novelty has not been demonstrated in these additional hippocampal pathways, they remain viable candidates for the effects observed here. The direct perforant path inputs to CA3 and CA1 are also candidates for novelty facilitated metaplasticity, and the CA1 input in particular has a well-studied dopamine mediated pre-synaptic inhibition effect (Otmakhova & Lisman, 1999), however it is unknown how these inputs are modified by behavioral novelty. These direct input synapses are necessary for place field firing (Brun et al., 2002), so plastic changes here could mediate place field formation in a novel environment. In summary, the network of hippocampal synapses seems to undergo a complex set of changes in response to novelty, and LTD blockade may affect the function of more than one of these pathways to affect learning and memory, depending on task parameters.

4.4 Place Field Formation as a Model of Episodic Memory Formation

Despite our support for the proposal that place field formation can serve as a model of episodic memory, it is important to consider the weaknesses and strengths of this model. Beyond the general deficiencies encountered in any animal model of a human cognitive process, place field formation in a novel environment diverges from episodic memory formation in several discrete ways. First, place field formation discounts the formation or elaboration of any non-spatial information into memory, a necessary feature of episodic memory. Second, episodic memory does not occur exclusively or even primarily within a novel context, and therefor the requirement for novelty as a key factor in encode typical episodic memory may be limited. Third, episodic memory has a strong temporal order component, with memory for event sequence being another cardinal feature. It is likely that this wider set of features is heavily dependent on hippocampal-cortical interactions (Dickerson & Eichenbaum, 2010; Preston & Eichenbaum, 2013), but within the hippocampus the mechanisms that support these features may differ in important ways from the mechanisms that support place field formation.

It is well known that hippocampal pyramidal cells encode multiple types of information, including non-spatial information, object features (Komorowski, Manns, & Eichenbaum, 2009), sounds (Moita et al., 2003; Itskov et al., 2012), behaviorally relevant events (Wood et al., 1999), and time (Eichenbaum, 2014). Many of these coding schemes are conjunctive with place, wherein individual cells fire only when the event or non-spatial feature co-occurs with a specific location. The conjunctive input cells often derive from existing cells firing within a context, where selectively develops both within an exposure (Komorowski et al., 2009) and across exposure days (McKenzie et al., 2013; McKenzie et al., 2014). These changes are reminiscent of partial remapping observed when specific environmental changes trigger change in place field

firing locally, but do not trigger a global change (Knierim, 2002). Progressive specification of firing across days is similarly reminiscent of place firing that diverges between two similar environments upon repeated exposure (Lever et al., 2002). It is likely that non-spatial coding develops in operant learning as the task drives attention to relevant features within a context (Kentros et al., 2004). However, when non-spatial selectively development has been observed within a trial, it seems to derive from a pre-existing spatial framework (Komorowski et al., 2009). These observations are consistent with the role of schema in episodic memory (Tse et al., 2007; van Kesteren et al., 2012). Pre-existing frameworks (schema) are known to facilitate memory, and this effect is attributed to the fact that new information can be elaborated into a schema (Tse et al., 2011). A small number of unique attributes distinguish a memory from a larger set of memories that share common elements. In turn, these memories share a broader similarity with other sets of memories, leading to an information-efficient hierarchical organization.

The requirement of LTD in place field formation was demonstrated in highly novel environments, where there was limited likelihood for partial remapping or interference from pre-existing contextual representations. It is unknown whether the incorporation of new information within an established representation requires LTD specifically, but synaptic plasticity in both in the hippocampus and elsewhere is necessary (Tse et al., 2007; Bethus, Tse, & Morris, 2010; Tse et al., 2011). This schema encoding is facilitated by activation of locus coeruleus afferents in the hippocampus, suggesting that while this learning does not trigger a broad novelty response, attentional modulation directed by the novel features might be a requirement (Takeuchi et al., 2016).

Time encoding has also been observed in hippocampal pyramidal cell firing patterns (Eichenbaum, 2014), and temporal order is a cardinal feature of episodic memory, requiring

hippocampal-cortical interactions (Hannesson et al., 2004). It has been suggested that temporal organization could be maintained in the hippocampus similarly to spatial trajectories: Serial activation of place fields encodes pathways through an environment, and temporal order might be maintained in a similar manner (Buzsaki & Moser, 2013). In fact a path sequence is intrinsically a temporal sequence, and the organization of events or actions temporally would be a natural consequence of organizing those same events or actions relative to a path sequence. Therefor the mechanisms that support sequence replay would likely be shared with the mechanisms that support temporal order (Carr, Jadhav, & Frank, 2011).

4.5 Accounting for LTD in Models of Place Field Formation

Although it seems unlikely that the broad decrease in synaptic strength observed in the current studies serves as a memory trace in any direct sense, models of place field formation nevertheless need to account for this synaptic change and explain its utility. Recent work has provided insight into the synaptic and cellular mechanisms that occur to support new place field firing. The active electrical properties of dendrites (Larkum et al., 1999; Schiller et al., 2000) are implicated in numerous cellular computations in pyramidal cells, including the induction of synaptic plasticity in area CA1 of the hippocampus (Jarsky et al., 2005; Spruston, 2008). The recording of these events in freely moving animals is a technical challenge, partially due to the depth of the laminar extension of apical dendritic tufts. However recent work (Harvey et al., 2009; Bittner et al., 2015) has demonstrated recordings of not only intracellular dynamics of the soma but of basal and apical dendrites as well. The results of these experiments strongly implicate dendritic plateau potentials as a necessary event in the rapid establishment of new place field firing (Bittner et al., 2015). The combined inputs of both schaffer collateral on proximal apical dendrites and entorhinal cortex on apical dendritic tufts is required to drive plateau potentials, and synaptic plasticity in both pathways is facilitated by coactivation (Dudman, Tsay, & Siegelbaum, 2007; Takahashi & Magee, 2009). This form of synaptic potentiation is proposed to support the newly established place field, in either one or both pathways.

How might our observed LTD in the schaffer collateral pathway be involved in this process? The experiments detailed above do not occur in a novel context, but instead are observations of randomly generated or experimentally induced place fields. In a novel context, there is presumable more incongruency in excitatory synaptic inputs, and crucially there is a

reduction in inhibitory tone, which critically shapes the response to excitatory inputs (Milstein et al., 2015; Basu et al., 2016; Grienberger et al., 2017). As we observed, most characteristics of new place field appeared normal under LTD blockade: Fields were not absent or spatially diffuse, but lack their characteristic formation dynamics. We suggest that LTD in schaffer collateral synapses serves to constrain place cell recruitment, thereby suppressing field formation in cells receiving a certain level of activation. Neuromodulator activity enables this LTD, and likely LTP as well, participating in the selection of CA1 cell activity into the representation. It is unknown whether active dendritic properties affect LTD induction as has been reported for LTP. Sub and supralinear calcium levels are thought to dictate the direction of plasticity in spike-timing dependent plasticity (Zucker, 1999), so its possible such a mechanism is active, however this model does not easily account for the observed dynamics of dendritic spikes, which are prototypically all or none events. We observe that a basal stimulation level is sufficient to induce LTD when in a novel environment. These extrinsic stimulations are not naturally convergent with entorhinal cortical inputs, so one possibility is that schaffer-collateral inputs that do not coincide with entorhinal cortical inputs in a novel environment might undergo LTD. These would represent most synapses in any particular environment, leading to the widespread LTD we observed.

These issues represent important future avenues of research. The acquisition of novel spatial information is one of the core functions of the hippocampus and a process to which many major brain systems contribute. A complex cognitive operation is triggered on exposure to a novel environment that begins with novelty recognition and ends with the elaboration of new spatial information. It requires behavioral exploration in concert with hippocampal activity patterns and neuromodulator release to produce synaptic plasticity that entrains this new

information. Only recently have the specific synaptic changes that occur begun to be described, though the general requirement of synaptic plasticity has long been hypothesized. The observations made in this thesis strongly support a specific role of synaptic depression in this process, contributing to our understanding of episodic memory formation.

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