NMDA RECEPTOR-MEDIATED ARC EXPRESSION IN DORSAL AND VENTRAL HIPPOCAMPUS CONTRIBUTES TO THE ACQUISITION OF TRACE AND CONTEXTUAL FEAR CONDITIONING

By

JENNIFER CZERNIAWSKI

A Dissertation submitted to the

Graduate School-New Brunswick

Rutgers, The State University of New Jersey

in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

Graduate Program in Psychology

Written under the direction of

Timothy Otto, PhD

and approved by

_____

_____

_____

_____

New Brunswick, New Jersey

May 2011

ABSTRACT OF DISSERTATION

NMDA receptor mediated expression of Arc in dorsal and ventral hippocampus contributes to the acquisition of contextual and trace fear conditioning

By JENNIFER CZERNIAWSKI

Dissertation Director:

Professor Timothy A. Otto

The dorsal and ventral subregions of the hippocampus are differentially involved in several of types of learning, including fear conditioning. For example, we have previously demonstrated that the integrity of ventral, but not dorsal, hippocampus is necessary for the acquisition and expression of trace fear conditioning while dorsal, but not ventral, hippocampus is critically involved in spatially-guided reinforced alternation (Czerniawski, Yoon & Otto, 2009). In contrast to the partially dissociable effects of either lesions or inactivation, however, several lines of research suggest that, in intact subjects, both subregions are normally involved in the acquisition of many hippocampal-dependent tasks.

The present studies investigated the molecular basis of these forms of learning by determining whether NMDA receptor-mediated immediate early

gene expression in dorsal vs. ventral hippocampus contributes to the acquisition and/or retention of trace and contextual fear conditioning. In the first set of experiments we examined the effect of NMDA receptor antagonism in dorsal or ventral hippocampus on the acquisition or expression of trace and contextual fear conditioning. Next we assessed if trace fear conditioning alters the transcription and/or translation of Arc, an immediate early gene thought to be critically involved in some forms of plasticity and learning. In addition we examined the effect of blocking Arc translation with antisense oligodeoxynucleotides on the acquisition of CS-US associations in this paradigm. Lastly, in the final experiment we determined if the learning induced increase in Arc translation is NMDA receptor-dependent.

The results of these studies suggest that both NMDA-receptor antagonism and the infusion of antisense oligodeoxynucleotides for the immediate early gene Arc (activity-regulated cytoskeletal protein) into dorsal or ventral hippocampus impair the acquisition of contextual and trace fear conditioning. In addition, trace fear conditioning enhances both Arc transcription and translation. Finally, pre-training infusions of either Arc antisense oligodeoxynucleotides or the NMDA receptor antagonist APV block the learning-induced enhancement of Arc. Together these studies support the notion that NMDA-receptor mediated expression of the immediate early gene Arc in both dorsal and ventral hippocampus may underlie the acquisition of a variety of forms of hippocampal dependent learning.

ACKNOWLEDGEMENTS

I would like to thank the members of my dissertation committee, Dr. Tracey Shors, Dr. John McGann, and Dr. Bonnie Firestein for all their time and advice. I would like to especially thank my advisor, Dr. Tim Otto for all of his patience, guidance, and dedication to making me a better scientist and for all that I have learned from him. Finally, I would like to thank my family, friends, fellow graduate students, and research assistants in the Otto Lab for making my graduate school experience enjoyable and gratifying.

TABLE OF CONTENTS

INTRODUCTION

It is now widely accepted that the hippocampus participates in a variety of tasks that require mnemonic function, including spatial navigation and learning (O'Keefe & Nadel, 1978; Jung et al., 1994; Moser & Moser, 1993; Moser & Moser, 1998), novel object recognition (Broadbent et al., 2004), working memory (Pothuizen et al., 2004; McHugh et al., 2008), and certain forms of fear conditioning (Kim & Fanselow, 1994; Richmond et al., 1999; Maren & Holt, 2004; Otto & Poon, 2006; Parsons & Otto, 2008; Czerniawski, Yoon & Otto, 2009). However, there are inconsistencies in the literature regarding the extent to which the hippocampus participates in these various types of learning and memory. Therefore, from a conceptual standpoint, it has been difficult to form a complete characterization of hippocampal function.

Some of the inconsistencies regarding hippocampal function may be reconciled by exploring the potentially differential functions of distinct subregions within the hippocampus rather than the whole hippocampus. Anatomically, the hippocampus can be divided along its septotemporal axis into dorsal and ventral hippocampus (Moser and Moser, 1998). While it has been proposed that these subregions work together to support a unitary function in memory (Squire and Zola-Morgan, 1991), recent evidence suggests that there is a functional, as well as anatomical, dissociation between these subregions (Moser & Moser, 1998; Bannerman et al., 1999;

Richmond et al., 1999; Pitkanen et al., 2000; Czerniawski et al., 2009). This dissociation between dorsal and ventral hippocampus will be discussed below, with respect to both structural and functional differences between these subregions and a focus on their contributions to fear conditioning.

Anatomical Dissociation of Dorsal and Ventral Hippocampus

Anatomically, the hippocampus can be divided along its septotemporal axis into dorsal and ventral subregions with the septal two-thirds comprising the dorsal subregion and the remaining one-third comprising the ventral subregion (Moser & Moser, 1998). While these subregions both consist of the CA1 and CA3 subfields and dentate gyrus (DG), they differ with respect to neuronal organization as well as both cortical and subcortical connections. For example, dorsal hippocampus receives primarily visual, auditory, and somatosensory via the entorhinal cortex while ventral hippocampus receives considerably less sensory input (Burwell & Amaral, 1998; Dolorfo & Amaral, 1998). However, ventral but not dorsal hippocampus has direct reciprocal connections with the amygdala (Pitkanen et al., 2000; Canteras & Swanson, 1992). While both ventral CA1 and CA3 receive direct projections from the amygdala, the different subfields of dorsal hippocampus are indirectly connected to the amygdala via ventral hippocampus (Pitkanen et al., 2000). Furthermore, unlike dorsal hippocampus, ventral CA1/subiculum projects to amygdala, prefrontal cortex, and nucleus accumbens shell (Ishikawa & Nakamura, 2006; Verwer et al., 1997; Groenwegen et al., 1987).

The difference in anatomical connections between dorsal and ventral hippocampus suggests that these subregions may also serve different functions in memory. For instance, dorsal hippocampus may be preferentially recruited for spatial learning compared to ventral hippocampus because of the extensive sensory information it receives. Conversely, the direct reciprocal connections between ventral hippocampus and the amygdala (and other subcortical structures) suggest that ventral hippocampus may be preferentially involved in emotional learning and anxiety relative to dorsal hippocampus. Finally, because dorsal hippocampus establishes anatomical connections with the amygdala only via ventral hippocampus, it is likely that any behavior which requires interactions between dorsal hippocampus and amygdala should also require an intact ventral hippocampus. Thus, while these subregions may vary with respect to their anatomical connections, they may still work in tandem to support some forms of learning.

Functional Dissociation of Dorsal and Ventral Hippocampus

In recent years there has been accumulating evidence supporting a possible functional dissociation between dorsal and ventral hippocampus (Moser & Moser, 1998, Bannerman et al., 1999; Yoon & Otto, 2007; Czerniawski et al., 2009). Specifically, dorsal, but not ventral, hippocampus appears to be critical for spatial learning (Czerniawski et al., 2009; O'Keefe & Nadel, 1978; Jung et al., 1994; Moser & Moser, 1993; Mao & Robinson, 1998; Ferbinteanu & McDonald, 2001). This follows from the aforementioned anatomical connections relaying sensory information to dorsal hippocampus

(Dolorfo & Amaral, 1998). Although there are "place cells" in both dorsal and ventral hippocampus, there is a greater proportion of place cells in dorsal hippocampus and with better spatial selectivity per cell than in ventral hippocampus (Jung et al., 1994). These data suggest that while both subregions may participate in spatial learning, the dorsal hippocampus is more importantly involved.

On the other hand, ventral, and not dorsal, hippocampus appears to be preferentially involved in anxiety-related behaviors (Bannerman et al., 2004; Trivedi & Cooper, 2004; Kjelstrup et al., 2002; McHugh et al., 2004). This is not surprising considering the aforementioned dense and reciprocal connections between ventral hippocampus and the amygdala. We, and others, have recently observed that this functional dissociation between dorsal and ventral hippocampus also extends to fear conditioning (Yoon & Otto, 2007; Czerniawski et al., 2009; Rogers et al., 2005; Maren & Holt, 2004).

The Hippocampus and Fear Conditioning

Pavlovian fear conditioning is a widely used paradigm to investigate the neural substrates of associative learning. In Pavlovian fear conditioning a neutral conditioned stimulus (CS), such as a tone, light, or odor, is paired with an aversive unconditioned stimulus (US), such as a mild footshock, which evokes an unconditioned response (UR). After one or more pairings of the CS and US, subsequent presentation of the CS alone or to the training chamber in which they were paired elicits a conditioned response (CR) that is

topographically similar to the UR. In rats, these behavioral responses include fear potentiated startle, ultrasonic vocalizations, and increased autonomic nervous system activity (see Kim & Jung, 2006 for review). However, the most frequently measured behavior is "freezing", which is thought to reflect an internal state of fear (Fanselow, 1980).

While there is a general consensus that the amygdala is critical for the acquisition and maintenance of fear conditioning (Phillips & LeDoux, 1992; Maren et al., 1996; Kim & Jung, 2006), there is conflicting data concerning the role of the hippocampus in these forms of associative learning. Although there is now a general agreement that interactions between the hippocampus and amygdala underlie certain types of Pavlovian fear conditioning, hippocampal involvement appears to depend on a variety of factors. These include the extent and location of damage, the type of manipulation used (i.e. lesion, inactivation, NMDA-receptor antagonism, etc.), the timing of these manipulations, and whether or not a "context", "delay" or "trace" fear conditioning paradigm is used. Dorsal and ventral hippocampal contributions to these forms of associative learning are described below.

Delay Fear Conditioning

Dorsal Hippocampus is Not Critically Involved in the Acquisition or Retrieval of Delay Fear Conditioning

In delay conditioning, the CS typically overlaps and coterminates with the US. This is generally considered an implicit memory task and not

dependent on the hippocampus. Converging evidence from a number of studies employing lesions, temporary inactivation, or NMDA-receptor antagonism all indicate that dorsal hippocampus is not necessary for the *acquisition* (Phillips & LeDoux, 1992; 1994; Richmond et al., 1999; Lee & Kesner, 2004; Burman et al., 2006; Daumas et al., 2006; Wanisch et al., 2005; Misane et al., 2005; Hunsaker & Kesner, 2008; Parsons et al., 2008; Seo et al., 2008; Schenberg et al., 2008; but see Maren et al., 1997) or the *retrieval/expression* (Kim & Fanselow, 1992; Maren et al., 1997; Anagnostaras et al., 1999; Quinn et al., 2002; Chowdhury et al., 2005; Burman et al., 2006; Maren & Holt, 2004; Daumas et al., 2006; but see Quinn et al., 2008) of delay fear conditioning.

Ventral Hippocampus May Participate in Delay Fear Conditioning

The extent to which ventral hippocampus participates in delay fear conditioning is less clear. While some studies report that ventral hippocampus is important for the acquisition of delay fear conditioning (Richmond et al., 1999; Bast et al., 2001; Zhang et al., 2001; Maren & Holt, 2004; Esclassan et al., 2009), others report that it is not necessary (Bast et al., 2001; Zhang et al., 2001). Although the data are mixed, these studies collectively suggest that ventral hippocampus may play a limited role in associating a discrete CS with a US in a delay fear conditioning paradigm. Specifically, while pre-testing inactivation of ventral hippocampus has no effect on the retrieval or expression of delay fear conditioning, post-training lesions produce a mild impairment in freezing to the CS in this paradigm

(Maren & Holt, 2004). However, it should be noted that these electrolytic lesions included extensive damage to the ventral subiculum and medial entorhinal cortex. Therefore, it is possible that ventral hippocampus may contribute to the acquisition or expression of delay fear conditioning, while dorsal hippocampus is not necessary for any aspect of delay fear conditioning.

Trace Fear Conditioning

Dorsal Hippocampus is Not Necessary for the Acquisition of Trace Fear Conditioning

In trace conditioning there is a trace interval between the offset of the CS and the onset of the US during which no stimulus is presented. Although it is generally accepted that the hippocampus is necessary for trace fear conditioning (McEchron et al., 1998), until recently few studies have critically examined the effect of selective manipulations of dorsal vs. ventral hippocampus on the acquisition of trace fear conditioning. recent work from our laboratory has shown that neither pre-training lesions nor inactivation of dorsal hippocampus affect the acquisition of trace fear conditioning (Yoon & Otto, 2007; Czerniawski, Yoon, Otto, 2009; Rogers et al., 2006). Thus, integrity of the dorsal hippocampus is not necessary to acquire CS-US associations in a trace fear conditioning paradigm; these data are consistent with a number of studies examining dorsal hippocampal contributions to contextual fear conditioning (see below).

NMDA Receptor Activation within Dorsal Hippocampus is Important for Acquisition of Trace Fear Conditioning

It is important to note that while neither lesions nor inactivation of dorsal hippocampus affect the acquisition of trace fear conditioning, recent evidence suggests that pre-training administration of the NMDA-receptor antagonist *dl*-2-phosphonovaleric acid (APV) into dorsal hippocampus reliably impairs the acquisition of this form of learning (Misane et al., 2005; Quinn et al., 2005; Wanisch et al., 2005). These data, which are consistent with the effect of similar manipulations on the acquisition of contextual conditioning (see below) raise the intriguing possibility that in the presence of APV, the intact dorsal hippocampus may participate in CS-US processing via uncompromised AMPA receptors. However, without NMDA receptor-mediated plasticity, it cannot support the plastic mechanisms required for the acquisition of associations between those stimuli. It is tempting to speculate that dorsal hippocampus may be the main site of processing for contextual and trace fear conditioning but that ventral hippocampus may compensate in the absence of an intact dorsal hippocampus. Additional evidence suggesting that dorsal hippocampus may participate in the acquisition of trace fear conditioning comes from studies using electrophysiological recordings to examine the response properties of dorsal hippocampal neurons during learning. Specifically, learning related changes in the firing rats of dorsal hippocampal neurons to the CS and US (but not during trace interval) were observed in dorsal DG and CA1 (Gilmartin & McEchron, 2005). Collectively,

these data indicate that while the integrity of dorsal hippocampus is not necessary for the acquisition of trace fear conditioning, plasticity within an intact dorsal hippocampus may normally occur.

Dorsal Hippocampus Contributes to the Retrieval/Expression of Trace Fear Conditioning

While several studies have reported an impairment in the retention or expression of trace fear conditioning following post-training lesions of the dorsal hippocampus (Quinn et al., 2002; Chowdhury et al., 2005; Yoon & Otto, 2007), others have reported no effect of post-training lesions or temporary inactivation (Burman et al., 2006; Czerniawski et al., 2009). Therefore it is currently difficult to form a complete characterization of dorsal hippocampal involvement in the maintenance or retrieval in trace fear conditioning. However, if dorsal hippocampus is normally engaged during acquisition of trace fear conditioning, it seems likely that this structure may also be necessary for retrieving recently acquired trace fear memories.

Ventral Hippocampus is Critical for the Both the Acquisition and Retrieval of Trace Fear Conditioning

Despite the direct reciprocal connections between ventral hippocampus and the amygdala, few studies have focused selectively on ventral hippocampal contributions to trace fear conditioning. Recent data from our laboratory have demonstrated that pre-training OR pre-testing lesions or inactivation of ventral hippocampus result in attenuated freezing during

testing (Rogers et al., 2006; Yoon & Otto, 2007; Czerniawski et al., 2009; Esclassan et al., 2009). Together these data suggest that ventral hippocampus is critical for both the acquisition and recall or expression of trace fear memories. However, these studies have used lesions or temporary inactivation to assess ventral hippocampal involvement in this form of associative learning. Therefore, while the integrity of ventral hippocampus is important, it is currently unknown whether or not it is a primary site of plasticity for learning these associations.

Contextual Fear Conditioning

Dorsal Hippocampus is Not Critically Involved in the Acquisition of Contextual Fear Conditioning

Contextual fear conditioning is a commonly used paradigm to explore hippocampal function. In most contextual fear conditioning paradigms, subjects are trained in one context (typically defined as the behavioral chamber) where they are presented with signaled or unsignaled footshocks. Subjects are subsequently returned to that training chamber in order to asses whether they have developed a conditioned fear response to that context. Although there is general agreement that hippocampus is importantly involved in contextual fear conditioning, a precise characterization of its role in this form of emotional learning remains to be determined.

The extensive evidence demonstrating a prominent role for dorsal hippocampus in spatial learning suggests that the dorsal hippocampus may also play an important role in associating a shock with the location in which it was delivered. However, there are clear discrepancies in the literature regarding the effect of dorsal hippocampal manipulations on contextual fear conditioning. Specifically, several studies have that dorsal hippocampus is important for the *acquisition* of contextual fear conditioning (Phillips & LeDoux, 1992, 1994; Young et al., 1994; Maren et al., 1997; McEchron et al., 1998; Frankland et al., 1998). Contrary to that finding, however, are many other reports indicating that dorsal hippocampus is *not* necessary for the acquisition of context-US associations (Maren et al., 1997; Richmond et al., 1999; Matus-Amat et al., 2004; Quinn et al., 2008; Esclassan et al., 2009). It is important to note that in these studies, pre-training *electrolytic* lesions of dorsal hippocampus tend to produce deficits in the acquisition of contextual fear conditioning (Phillips & LeDoux, 1992, 1994; Maren et al., 1997; Frankland et al., 1998), while pre-training *excitotoxic* lesions or temporary inactivation tend to have no effect (Matus-Amat et al., 2004; Maren et al., 1997; Richmond et al., 1999; Quinn et al., 2008; but see Young et al., 1994). This distinction between electrolytic and excitotoxic lesions may explain this inconsistency in the literature because unlike excitotoxic lesions or temporary inactivation, electrolytic lesions may damage fibers of passage in addition to cell bodies. Therefore, the deficits observed following pre-training electrolytic lesions of dorsal hippocampus may not be due to the disruption dorsal hippocampal processing per se, but instead due to

damaging fibers of passage between dorsal hippocampus and other structures. Thus, dorsal hippocampus does not appear to be necessary for the acquisition of contextual fear conditioning

Conversely, post-training manipulations of dorsal hippocampus have consistently been observed to attenuate freezing during context testing, suggesting an important role for this subregion in the retention or expression of fear to a context (Kim & Fanselow, 1992; Matus-Amat et al., 2004; Maren et al., 1997; Frankland et al., 1998; Anagnostaras et al., 1999; Quinn et al., 2002; Quinn et al., 2008; but see Maren & Holt, 2004; Chowdhury et al., 2005). In these studies, deficits are observed regardless of whether dorsal hippocampus was lesioned (electrolytic or excitotoxic) or pharmacologically inactivated. Together, the data suggest that dorsal hippocampus likely plays a crucial role in the retrieval, maintenance, or expression of contextual fear conditioning.

NMDA Receptor Activation within Dorsal Hippocampus is Important for Acquisition of Contextual Fear Conditioning

Interestingly, while the data are mixed regarding the effects of lesions or temporary inactivation of dorsal hippocampus on the acquisition of contextual fear conditioning, disrupting NMDA receptor activity reliably produce deficits in this paradigm. Specifically, pre-training administration of the NMDA receptor antagonists APV or MK-801 results in attenuated freezing to the context during retention testing (Young et al., 1994; Bast et al., 2003; Wiltgen & Fanselow, 2003; Schenberg et al., 2008). As NMDA receptors are

thought to be critical for some forms of neural plasticity, these data indicate that plasticity within dorsal hippocampus may normally play a potentially important role in the acquisition of contextual fear. Collectively, these findings suggest that while dorsal hippocampus may not be integral for the acquisition of contextual fear conditioning, it may normally participate in forming these associations.

Ventral Hippocampal Contributions to Contextual Fear Conditioning

To date, much of the research on contextual fear learning has focused exclusively on dorsal hippocampus. This is somewhat surprising, given the anatomical connections between ventral hippocampus and amygdala (Pitkanen et al., 2000), which has repeatedly been shown to be critical for the acquisition and expression of conditioned fear (Phillips & LeDoux, 1992; LeDoux, 1995; Maren & Holt, 2004; Kim & Jung, 2006). As noted previously, ventral hippocampus has direct and reciprocal connections with amygdala, whereas dorsal hippocampus gains access to amygdalar processing only through its connections with ventral hippocampus (Pitkanen et al., 2000). Therefore, it is likely that the integrity of ventral hippocampus is necessary for any behavior that may depend on communication between dorsal hippocampus and amygdala, even if ventral hippocampus itself is not integral for the task. Thus, it is possible that the main site of information processing regarding the context may be within dorsal hippocampus, but manipulations of ventral hippocampus may have an effect because they disrupt the flow of information from dorsal hippocampus to amygdala.

There is emerging evidence suggesting that ventral hippocampus does participate in contextual fear conditioning but, similar to dorsal hippocampus, these findings have been mixed. For example, there have been several reports that manipulations of ventral hippocampus made before training (i.e. lesions or infusions of muscimol, TTX, or MK-801) impair the acquisition of contextual fear conditioning (Richmond et al., 1999; Bast et al., 2001; Zhang et al., 2001; Rudy & Matus-Amat, 2005). However, it has also been reported that pre-training inactivation of ventral hippocampus do not affect the acquisition of contextual fear conditioning (Maren & Holt, 2004). Few studies have examined ventral hippocampal contributions to the retrieval of contextual fear conditioning. In one study, pre-testing electrolytic lesions but not inactivation attenuated the retrieval or expression of contextual fear conditioning (Maren & Holt, 2004). It is important to note that these electrolytic lesions included damage to the ventral subiculum and medial entorhinal cortex. Therefore, while there are several reports supporting the notion of ventral hippocampal involvement in contextual fear conditioning, its precise role in this form of associative learning remains to be fully characterized.

In summary, dorsal and ventral hippocampus both appear to be involved in contextual fear conditioning, albeit to different extents. Specifically, dorsal hippocampus seems to be important for the retrieval while ventral hippocampus seems to be important for the acquisition of contextual fear conditioning. However, there are numerous inconsistencies

in the literature, with some studies suggesting that dorsal hippocampus may participate in acquisition. In addition, the literature is scant with respect to ventral hippocampal contributions to the retrieval of contextual fear conditioning as well as whether or not plasticity within ventral hippocampus is required to form associations between the US and contextual stimuli.

Methodological Differences in Fear Conditioning Experiments

Experiments examining dorsal and ventral hippocampal contributions to fear conditioning are inconsistent not only in their findings, but also in their methods. For instance, parametric differences in the duration of CS presentation or trace interval, the number of trials, and the intensity and duration of footshock could all contribute to discrepancies in the literature. In addition, in comparison to the literature on dorsal hippocampus alone, few studies have directly compared dorsal and ventral hippocampus involvement in these paradigms (Richmond et al., 1999; Maren & Holt, 2004; Burman et al., 2006; Rogers et al., 2006; Yoon & Otto, 2007; Hunsaker & Kesner, 2008; Czerniawski et al., 2009). Thus, it is often difficult to compare data across studies because any inconsistencies in findings may be due to methodological differences, not to differences in dorsal and ventral hippocampal functioning.

In an effort to reduce this variability in methodological differences, one goal of our laboratory is to use a common set of parameters to systematically compare the function dorsal and ventral hippocampus. In one systematic study in our laboratory, pre-training or pre-testing inactivation of dorsal hippocampus had no effect on trace fear conditioning but, in the same

subjects, dramatically impaired performance on spatial reinforced alternation (Czerniawski et al., 2009). In the same study, pre-training or pre-testing inactivation of ventral hippocampus dramatically impaired trace fear conditioning but, in the same subjects, had no effect on spatial reinforced alternation performance. This double dissociation of dorsal and ventral hippocampal contributions to trace fear conditioning and spatial delayed reinforced alternation provide compelling evidence that dorsal and ventral hippocampus are functionally dissociable. More studies systematically comparing dorsal and ventral hippocampus using a variety of methods, such as lesions, inactivation, NMDA receptor antagonism, and gene and protein expression analyses will help to better characterize both the extent to which these subregions contribute to different aspects of fear conditioning as well as provide insight into the fundamental neurobiological mechanisms supporting learning.

The Arc of Fear Conditioning

Immediate early genes (IEGs) are genes which are rapidly and transiently activated following synaptic stimulation. A number of these IEGs, including c-fos, zif268, and Arc, have been implicated in hippocampal synaptic plasticity and memory consolidation (see Guzowski, 2002 for review). Among these, Arc (activity-regulated cytoskeleton-associated protein) has been of particular interest because, unlike other IEGs, it is rapidly and robustly induced after neural activity and then quickly transported to activated synaptic zones where it undergoes local protein

synthesis (Lyford et al., 1995; Link et al., 1995; Steward et al., 1998). *In vitro,* the expression of Arc is tightly coupled to the induction of LTP and is NMDA-receptor dependent (Lyford et al., 1995; Link et al., 1995). This is important with respect to learning and memory because associative LTP is synapse-specific, mediated in part by NMDA receptors, and is widely regarded as a putative mechanism underlying long-term memory formation and maintenance. Importantly, Arc mRNA rapidly migrates and selectively accumulates in the recently-activated synaptic zone (Steward et al., 1998). Thus, Arc could potentially contribute to functional and structural modifications that lead to selective, long-lasting, synapse specific alterations of synaptic efficacy.

An increase in Arc expression in the hippocampus has been observed following spatial learning and exploration of a novel environment (Guzowski et al., 2001; Fletcher et al., 2006). However, this increase in gene expression is correlative and does not provide direct evidence linking Arc to synaptic plasticity or memory consolidation. Providing support for an importance of Arc in learning, Arc knockout mice fail to form long-lasting memories in a variety of paradigms including spatial learning, fear conditioning, and novel object recognition (Plath et al., 2006). Recently, antisense oligodeoxynucleotides (ODNs) have been used to block translation of Arc; these data suggest that infusion of Arc antisense ODNs into the hippocampus block the maintenance of LTP, and also block the consolidation of spatial and inhibitory avoidance learning (Guzowski et al., 2000; McIntyre

et al., 2005). Furthermore, blocking Arc translation in the amygdala also blocks the consolidation of fear conditioning (Ploski et al., 2008). Collectively, these studies provide compelling support that Arc expression may play a critical role in the plastic processes underlying some forms of learning, and that this role may extend to trace and contextual fear conditioning.

PROJECT OBJECTIVE

We have previously observed that ventral but not dorsal hippocampus is crucial for both the acquisition and expression of trace fear conditioning (Yoon & Otto, 2007; Czerniawski et al., 2009). One goal of the present study was to examine the extent to which NMDA-receptor mediated plasticity within these subregions is also a necessary precursor to the acquisition of trace and contextual fear conditioning. Therefore, we aimed to replicate the finding that administration of the NMDA receptor antagonist APV into dorsal hippocampus impairs the acquisition of contextual and trace fear conditioning and to additionally examine whether NMDA receptor-mediated plasticity in ventral hippocampus is also necessary for forming or maintaining associations in these paradigms (Experiment 1).

A second major goal was to investigate the role of Arc in the acquisition of trace fear conditioning. In order to do so we assessed whether training in trace fear conditioning alters Arc mRNA and protein levels (Experiment 2). In addition, we examined the effect of administering antisense oligodeoxynucleotides for Arc into dorsal or ventral hippocampus on the acquisition of trace (Experiment 2) or delay (Experiment 3) fear conditioning.

In order to further investigate the extent to which learning-related alterations in Arc and NMDA receptor activation are interdependent, we infused APV into dorsal or ventral hippocampus before trace fear conditioning and subsequently assessed levels of Arc protein to establish if the learning

induced enhancement in Arc translation is NMDA receptor-dependent (Experiment 4).

Based on the results of previous studies, we anticipated that blocking NMDA receptors in dorsal hippocampus before conditioning (but not before testing) would attenuate the acquisition of trace and contextual fear conditioning, but that blocking NMDA receptors in ventral hippocampus would only attenuate the acquisition of trace fear conditioning. We also predicted that trace fear conditioning would enhance Arc transcription and translation in both dorsal and ventral hippocampus and that blocking Arc translation with antisense oligodeoxynucleotides would also block the acquisition of trace and contextual, but not delay, fear conditioning. Finally, because the induction of Arc is tightly coupled to the induction of some forms of plasticity and is NMDA-receptor dependent *in vitro*, we expected that the learning-induced increase in Arc expression would be blocked by NMDA receptor antagonism in dorsal and ventral hippocampus.

Together with previous research from our laboratory these studies will help reconcile some of the inconsistencies in the literature regarding dorsal and ventral hippocampal contributions to fear conditioning. Furthermore, the Arc experiments will help to ascertain its putative role in learning and as a downstream mechanism for memory consolidation in different subregions of the hippocampus. Collectively, these proposed studies will contribute to a greater characterization of hippocampal processing in learning and memory.

METHODS

All procedures have been approved by Rutgers University's Institutional Animal Care and Use Committee.

General Methods

Methods that are common to all experiments are described below. Detailed methods specific to each experiment are described in the methods sections for that particular experiment.

Subjects

Naive male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 250-300g at the time of surgery served as subjects. All subjects were housed individually in plastic cages in a colony room with a 12 h light/dark cycle with lights on at 7 a.m. All behavioral testing occurred during the light cycle. Subjects had access to food and water *ad libitum* and were handled for two minutes daily for five days prior to surgical procedures and behavioral training.

Apparatus

Fear conditioning chambers. Auditory trace fear conditioning and context testing were conducted in a behavioral chamber (30 X 24 X 27 cm) enclosed in a sound-attenuating enclosure (56 X 41 X 42 cm). The floor of the chamber was composed of 16 stainless steel rods equally spaced by 1.9 cm which were connected to a shock generator (model H13-15, Coulbourn

Instruments, Allentown, PA) designed to administer footshock US (0.6 mA). Two of the opposing walls were composed of transparent Plexiglas and the other two were aluminum. When appropriate, a computer-generated tone (3.9 kHz, 80 dB) was presented through a speaker mounted 14 cm above the floor on the outside one of the aluminum chamber walls. A single light bulb (29 V, 0.04 A) was located 24.5 cm above the floor. A one-way glass window on the front door of the sound attenuating enclosure allowed an experimenter to observe and score the behavioral measure of freezing using a hand switch that was connected to the computer controlling all paradigmatic events. The training chamber was cleaned with a commercially available cage cleaner (Research Laboratories Inc.) between sessions.

The testing session for trace fear conditioning took place in a novel chamber located in a different experimental room. The testing chamber had the same measurements and configuration as the training chamber but was differentiated from the training chamber in that the entire floor was covered with black Plexiglas and a black and white striped panel was attached to one of the opposing walls. A one-way glass window on the front door of the sound attenuating enclosure allowed for an observer to manually score freezing behavior with a hand switch. The testing chamber was cleaned with alcohol between sessions.

Open field chamber. Locomotor activity was assessed one week after trace fear conditioning in an open-field chamber (85 X 85 X 30 cm) made of black Plexiglas. The floor of the chamber was divided into 36 squares (14 cm).

The chamber was located in a room lit with a single fixture (65 W), and a video camera placed approximately 1.5 m above the center of the chamber was used to record each session. An experimenter unaware of the experimental condition of each subject watched the video on a TV screen in a different room and manually recorded locomotor activity.

Procedure

Surgery. All subjects who underwent cannula implantation surgery were first anesthetizes with an i.p. administration of a ketamine (80 mg/kg)-xylazine (12 mg/kg) mixture. The subject's head was shaved, mounted in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA), and cleaned with alcohol and Betadine. Subcutaneous injections of Marcaine (0.1 ml, 25%) in several locations below the scalp served as a local anesthetic and vasoconstrictor. The scalp was then incised and retracted. Six small burr holes were drilled into the skull. For subjects receiving infusions into DH, double guide cannulae (22-gauge, 11 mm, Plastics1, Roanake, VA) were implanted bilaterally into the DH (AP: -3.8 mm, ML: ± 2.5 mm from bregma; DV: -2.2 mm from dura). For subjects receiving infusions into VH, single guide cannulae (22-gauge, 11 mm, Plastics1, Roankae, VA) were implanted bilaterally into VH (AP: -5.2 mm, ML: ±5 mm from bregma; DV: -5.5 mm from dura). The cannulae were affixed with acrylic cement and anchored to the skull via four stainless steel screws. The incision was then closed with stainless steel surgical staples and obdurators were placed into the guide cannula. All animals were closely monitored during the 7 – 10 d post surgical

recovery period. Before behavioral testing subjects were randomly assigned to the different experimental groups for each of the experiments.

Auditory trace fear conditioning and testing. Trace fear conditioning was conducted 7 – 10 days after surgery. Auditory trace fear conditioning took place in a single session consisting of 7 pairings of a tone (16 s, 3.9 KHz, 80 dB) and footshock (2 s, 0.6 mA), with a trace interval of 28 s between the offset of the tone and onset of the shock. The first tone was presented after a 2 min acclimation period and subsequent trials were separated by a 2 min intertrial interval (ITI). The behavioral response of freezing, defined as a rigid posture and lack of movement except that required for respiration, was recorded throughout the entire conditioning session by an observer blind to the subjects' condition. These raw data were subsequently transformed into the percentage of time spent freezing for each minute of the training session.

Conditioned fear to the training context was assessed 24 h after conditioning by placing each subject into the chamber in which conditioning occurred for 6 min. Freezing was recorded during the entire session by an observer blind to the subjects' condition. No stimuli (i.e. tone, shock) were presented during this session.

The testing session for trace fear conditioning was conducted in a novel chamber 48 h after conditioning (24 h after context testing) in one session consisting of three trials. The procedure was the same as during conditioning except that footshock was not presented. As during

conditioning, the behavioral measure of freezing was recorded throughout the entire testing session.

Locomotor Activity. In order to examine whether our manipulations affected basal levels of activity, locomotion was assessed in an open field in three sessions over three consecutive days one week after trace conditioning. At the start of each session subjects were placed in a corner of the open chamber, and they were then allowed to explore freely (10 min in Experiment 1, 6 min in Experiment 2). Each session was recorded by a video camera placed approximately 1.5 m above the open field and scored by an observer who watched the video on a TV screen in another room. The experimenter, who was unaware of the experimental condition of the subjects, recorded both ambulation, defined as the crossing of all four legs from one square to another, and rearing, defined as lifting the two front legs off the floor. On the second day of locomotor activity testing, subjects received microinfusions of the same solution that was administered before behavioral testing; no infusions occurred on the first or third day of locomotor activity testing.

Histology. Following completion of all behavioral testing, animals were administered a sub-lethal dose of sodium pentobarbital (100 mg/kg, i.p.) and perfused transcardially with 0.9% saline followed by buffered 10% formalin. The brain was removed and placed in a 10% formalin-30% sucrose solution for at least three days. The brain was then frozen and sliced into coronal sections with a thickness of 50 μm using a cryostat. Every other slice

throughout the DH or VH was mounted on gelled glass microscope slides and subsequently stained with cresyl violet and coverslipped. An observer blind to the subject's condition verified cannula placement throughout the DH or VH. Subjects with inaccurate cannula placement or extensive damage were excluded from data analysis.

Experiment 1. Effect of NMDA receptor antagonism in dorsal or ventral hippocampus on the acquisition and/or retrieval of trace and contextual fear conditioning.

The present experiment examined the effect of infusion of the NMDA receptor antagonist APV into dorsal or ventral hippocampus on the acquisition and/or expression of trace and contextual fear conditioning. Subjects received surgery prior to all behavioral experiments and were randomly assigned to one of four experimental groups (see Figure 1). They are as follows: aCSF before training and testing (aCSF-aCSF), APV before training and aCSF before testing (APV-aCSF), aCSF before training and APV before testing (aCSF-APV), and APV before training and testing (APV-APV).

Methods

Subjects. One hundred male Sprague Dawley rats served as subjects. Initial sample sizes for subjects with cannula in dorsal hippocampus were: aCSF-aCSF (n=11), aCSF-APV (n= 11), APV-aCSF (n=12), APV-APV (n=11). Initial sample sizes for subjects with cannula in dorsal hippocampus were: aCSF-aCSF (n=14), aCSF-APV (n= 14), APV-aCSF (n=14), APV-APV (n=13).

Final sample sizes for each group were determined following histological assessment of cannula placement (see Cannula Placement, below).

Infusions of APV or aCSF. Subjects received microinfusions of either aCSF (Harvard Apparatus) or APV (10 µg/µl; Sigma, St Louis, MO, pH 7.4). The infusions were administered via insertion of an infusion cannula into the guide cannula targeted at the dorsal or ventral hippocampus. The infusion cannula protruded 1 mm beyond the tip of the guide cannula, and was connected via polyethylene tubing to a 10 µl Hamilton syringe mounted in an infusion pump (Harvard Apparatus). A volume of 0.5 µl (0.25 µl/min) was infused bilaterally for a total volume of 1 µl for all subjects. The infusion cannula was left in position for four minutes following completion of infusion to allow for diffusion of the APV or aCSF. Subjects then had the infusion cannula replaced with a dummy cannula and were subsequently transferred to an experimental room to undergo behavioral training or testing as described in Figure 1. One week later, locomotor activity was assessed for all subjects in an open field for three consecutive days. On the first and third days of locomotor testing, subjects were placed directly into the open field without receiving any infusions. On the second day of locomotor testing, subjects received an infusion (APV or aCSF) of whichever they received before the testing sessions in the same manner described above.

Statistical Analysis. Data were analyzed using separate one- or two-way analyses of variance (ANOVAs) as appropriate. An alpha level of .05 was

used for all statistical analyses. Post hoc comparisons, where necessary, were conducted using Student-Newman-Keul's (SNK) post hoc test.

Results

Cannula placement. Following histological examination, nine subjects with cannula in dorsal hippocampus and ten subjects with cannula in ventral hippocampus were excluded due to incorrect cannula placement. Thus, the final group numbers for subjects with cannula in dorsal were: aCSF-aCSF (n=9), aCSF-APV (n=10), APV-aCSF (n=9) and APV-APV (n=8). The final group numbers for subjects with cannula in ventral hippocampu were as: aCSF-aCSF (n=14), aCSF-APV (n=9), APV-aCSF (n=12) and APV-APV (n=10). Figure 3a illustrates the cannula placement for subjects with cannula in dorsal (Figure 2a) or ventral (Figure 2b) hippocampus.

The effect of NMDA receptor antagonism in dorsal or ventral hippocampus on the acquisition and expression during trace and contextual fear conditioning

The results of pre-training or pre-testing infusions of APV or aCSF are described below. Although the context testing session occurred prior to the testing session for trace fear conditioning, the data from the context test will be presented after the data for trace fear conditioning and testing for the sake of clarity.

Trace fear conditioning: effect of APV or aCSF infusions into dorsal hippocampus on freezing during training.

The mean (±SEM) percentage of freezing exhibited by different infusion groups during conditioning is illustrated in Figure 3. For analysis of data during the conditioning session, subjects were collapsed into two groups for training: those that received pre-training infusions of APV into dorsal hippocampus (APV-APV and APV-aCSF, n=17) and those that received pre-training infusions of aCSF (aCSF-aCSF and aCSF-APV, n=19). Because freezing for each subject was stable across trials during conditioning, the data for each subject after the first US presentation was combined into one value (trials 2-7). For the ITI, a two way ANOVA with infusion condition as the between subjects factor and time point (baseline vs. trials 2-7) as the within subjects factor revealed there was a main effect for infusion condition, $F(1,34) = 6.91$, $p = 0.0128$, a main effect for time point, $F(1,71) = 262.92$, $p < 0.0001$, and a significant interaction between infusion condition and time point, $F(1,71) = 8.69$, $p = 0.005$ during conditioning (Figure 3a). Subsequent SNK post hoc analyses revealed that group APV exhibited significantly less freezing than group aCSF during trials 2-7 but not during baseline ($p < 0.05$). In addition, both groups exhibited significantly higher levels of freezing during trials 2-7 than they did during baseline ($p < 0.05$).

For the CS, a two way ANOVA with infusion condition as the between subjects factor and time point (baseline vs. trials 2-7) as the within subjects factor ANOVA revealed there was a main effect for time point, $F(1,71) = 104.02$, $p < 0.001$, but no main effect for infusion condition, $F(1,34) = 0.516$, $p = 0.477$,and no significant interaction between infusion condition

and time point, $F(1,71) = 0.683$, $p = 0.414$ (Figure 3b). Subsequent SNK post hoc analyses revealed that both groups exhibited significantly higher levels of freezing during trials 2-7 of the CS than they did during the first trial (baseline), before the first shock was presented ($p < 0.05$).

For the trace interval, a two way ANOVA with infusion condition as the between subjects factor and time point (baseline vs. trials 2-7) as the within subjects factor ANOVA revealed there was a main effect for infusion condition, $F(1,34) = 10.46$, $p = 0.002$ and a main effect for time point, $F(1,71) = 0.228.59$, $p < 0.0001$, but no significant interaction between infusion condition and time point, $F(1,71) = 2.58$, $p = 0.117$ (Figure 3c). However, subsequent SNK post hoc analyses revealed that both groups exhibited significantly higher levels of freezing during trials 2-7 of the trace interval than they did during the first trial (baseline), before the first shock was presented ($p < 0.05$). In addition subjects that received aCSF infusions exhibited significantly higher levels of freezing during the trace interval than those that received APV infusions into dorsal hippocampus before conditioning.

Trace fear conditioning: effect of APV or aCSF infusions into ventral hippocampus on freezing during training.

The mean (±SEM) percentage of freezing exhibited by different infusion groups during conditioning is illustrated in Figure 4. For analysis of data during the conditioning session, subjects were collapsed into two groups for training: those that received pre-training infusions of APV into ventral

hippocampus (APV-APV and APV-aCSF, n=22) and those that received infusions of aCSF (aCSF-aCSF and aCSF-APV, n=23). For the ITI, a two way ANOVA with infusion condition as the between subjects factor and time point (baseline vs. trials 2-7) as the within subjects factor ANOVA revealed there was a main effect for infusion condition, $F(1,43) = 34.0$, $p < 0.0001$, a main effect for time point, $F(1,89) = 419.8$, $p < 0.0001$, and a significant interaction between infusion condition and time point, $F(1,89) = 64.7$, $p < 0.0001$ for the ITI during conditioning (Figure 4a). Subsequent SNK post hoc analyses revealed that group APV exhibited significantly less freezing than group aCSF during trials 2-7 but not during baseline ($p < 0.05$). In addition, both groups exhibited significantly higher levels of freezing during trials 2-7 than they did during baseline ($p < 0.05$).

For the CS, a two way ANOVA with infusion condition as the between subjects factor and time point (baseline vs. trials 2-7) as the within subjects factor ANOVA revealed there was a main effect for infusion condition, $F(1,43) = 33.3$, $p < 0.0001$, a main effect for time point, $F(1,89) = 151.3$, $p < 0.0001$, and a significant interaction between infusion condition and time point, $F(1,89) = 37.7$, $p < 0.0001$ for the ITI during conditioning (Figure 4b). Subsequent SNK post hoc analyses revealed group APV exhibited significantly less freezing than group aCSF during trials 2-7 but not during baseline ($p < 0.05$). In addition, both groups exhibited significantly higher levels of freezing during the CS presentations during trials 2-7 than they did during baseline ($p < 0.05$).

For the trace interval, a two way ANOVA with infusion condition as the between subjects factor and time point (baseline vs. trials 2-7) as the within subjects factor ANOVA revealed there was a main effect for infusion condition, $F(1,43) = 4.82$, $p = 0.033$, a main effect for time point, $F(1,89) = 439.15$, $p < 0.0001$, and a significant interaction between infusion condition and time point, $F(1,89) = 48.56$, $p < 0.0001$ for the ITI during conditioning (Figure 4c). Subsequent SNK post hoc analyses revealed that group APV exhibited significantly less freezing than group aCSF during trials 2-7 but not during baseline ($p < 0.05$). In addition, both groups exhibited significantly higher levels of freezing during the trace interval of trials 2-7 than they did during baseline ($p < 0.05$).

Trace fear conditioning: effect of APV or aCSF infusions into dorsal hippocampus on freezing during testing.

The expression of conditioned fear exhibited during testing for subjects with cannula implanted in dorsal hippocampus is depicted in Figure 5. The mean (±SEM) percentage of freezing exhibited during the 2 min ITI is shown in Figure 5a. The first trial of the ITI consisted of the first two minutes in the novel chamber before any CS presentations and thus served as a period during which baseline levels of freezing were measured. A one-way ANOVA revealed there was no statistically significant difference between groups during the first ITI (baseline), $F(3,35) = 2.34$, $p = 0.0923$, but there was a significant difference in the percentage of time spent freezing during trials 2-3 of the ITI, $F(3,35) = 19.8$, $p < 0.0001$. Subsequent post hoc analyses

(SNK) revealed that there was no significant difference between groups aCSF-aCSF and aCSF-APV, nor was there a difference between groups APV-aCSF and APV-APV. However groups aCSF-aCSF & aCSF-APV were significantly different from groups APV-aCSF & APV-APV ($p < 0.05$), thus indicating that only subjects who received infusions of APV into dorsal hippocampus before conditioning froze significantly less during the ITI presentations during testing 48 h later.

The mean (±SEM) percentage of freezing exhibited during the 16 s CS presentation is shown in Figure 5b. A one-way ANOVA revealed there was a significant difference between groups in the percentage of time spent freezing during the CS, $F(3,35) = 8.55$, $p = 0.003$. Subsequent post hoc analyses (SNK) revealed that there was no significant difference between groups aCSF-aCSF and aCSF-APV, nor was there a difference between groups APV-aCSF and APV-APV. However groups aCSF-aCSF & aCSF-APV were significantly different from groups APV-aCSF & APV-APV ($p < 0.05$), thus indicating that only subjects who received infusions of APV into dorsal hippocampus before conditioning froze significantly less to the CS presentations during the testing session 48 h later.

The mean (±SEM) percentage of freezing exhibited during the 28 s trace interval is shown in Figure 5c. A one-way ANOVA revealed there was a significant difference between groups in the percentage of time spent freezing during the trace interval, $F(3,35) = 24.4$, $p < 0.0001$. Subsequent post hoc analyses (SNK) revealed that there was no significant difference

between groups aCSF-aCSF and aCSF-APV, nor was there a difference between groups APV-aCSF and APV-APV. However groups aCSF-aCSF & aCSF-APV were significantly different from groups APV-aCSF & APV-APV ($p < 0.05$), thus indicating that only subjects who received infusions of APV into dorsal hippocampus before conditioning froze significantly less during the trace interval in the testing session 48 h later.

Trace fear conditioning: effect of APV or aCSF infusions into ventral hippocampus on freezing during testing.

The expression of conditioned fear exhibited during the testing session for subjects with cannula implanted in ventral hippocampus is depicted in Figure 6. The mean (±SEM) percentage of freezing exhibited during the 2 min ITI is shown in Figure 6a. A one-way ANOVA revealed there was a statistically significant difference between groups during the first ITI (baseline), $F(3,44) = 3.34$, $p = 0.0284$. Subsequent post hoc analyses revealed that group APV-aCSF froze significantly less than group aCSF-aCSF ($p < 0.05$) but that there were no other group differences. A one-way ANOVA revealed there was a significant difference in the percentage of time spent freezing during trials 2-3 of the ITI, $F(3,44) = 7.83$, $p = 0.003$. Subsequent post hoc analyses (SNK) revealed that there was a significant difference between group aCSF-aCSF and all other groups ($p < 0.05$), thus indicating that subjects who received infusions of APV into ventral hippocampus before conditioning OR before testing froze significantly less than control subjects during the ITI of the testing session.

The mean (±SEM) percentage of freezing exhibited during the 16 s CS presentation is shown in Figure 6b. A one-way ANOVA revealed there was a significant difference between groups in the percentage of time spent freezing during the CS, $F(3,44) = 5.41$, $p = 0.003$. Subsequent post hoc analyses (SNK) revealed that there was a significant difference between group aCSF-aCSF and all other groups ($p < 0.05$), thus indicating that subjects who received infusions of APV into ventral hippocampus before conditioning OR before testing froze significantly less than control subjects during the CS presentations during the testing session 48 h later.

The mean (±SEM) percentage of freezing exhibited during the 28 s trace interval is shown in Figure 6c. A one-way ANOVA revealed there was a significant difference between groups in the percentage of time spent freezing during the trace interval, $F(3,44) = 12.6$, $p < 0.0001$. Subsequent post hoc analyses (SNK) revealed that there was a significant difference between group aCSF-aCSF and all other groups ($p < 0.05$), thus indicating that subjects who received infusions of APV into ventral hippocampus before conditioning OR before testing froze significantly less than control subjects during the trace interval in the testing session 48 h later.

Context testing

For statistical analysis during testing, data from only the first three minutes of context testing were used because the behavior at the beginning of the session was least likely affected by extinction. The mean (±SEM) percentage freezing for each of the experimental groups is shown in Figure 7.

For subjects receiving infusions in dorsal hippocampus, a one-way ANOVA revealed a significant difference in freezing between groups, $F(3,35) = 7.81$, $p = 0.0005$ (Figure 7a). Subsequent post hoc analyses (SNK) revealed that there was no significant difference between groups aCSF-aCSF and aCSF-APV, nor was there a difference between groups APV-aCSF and APV-APV. However groups aCSF-aCSF & aCSF-APV were significantly different than groups APV-aCSF & APV-APV ($p < 0.05$), thus indicating that only subjects who received infusions of APV into dorsal hippocampus before conditioning froze significantly less during context testing 24 h later.

For subjects receiving infusions into ventral hippocampus, a one-way ANOVA revealed a significant difference in freezing between groups, $F(3,44) = 5.44$, $p = 0.003$ (Figure 7b). Subsequent post hoc analyses (SNK) revealed that there was a significant difference between group aCSF-aCSF and all other groups ($p < 0.05$), thus indicating that subjects who received infusions of APV into ventral hippocampus before conditioning OR before testing froze significantly less than control subjects during the context testing session.

Locomotor activity

Subjects were placed into the open field on Days 1 and 3 of locomotor testing without receiving any infusions beforehand. Subjects were infused with whatever substance they received before testing on Day 2 of the locomotor activity test. Therefore, prior to locomotor testing on Day 2, subjects from group aCSF-aCSF or APV-aCSF received infusions of aCSF and

were combined into one group (aCSF), and subjects from group aCSF-APV or APV-APV received infusions of APV and were combined into a separate group (APV). The final group numbers for subjects who received infusions of aCSF or APV into dorsal hippocampus were 15 and 14, respectively. The final group numbers for subjects who received infusions of aCSF or APV into ventral hippocampus were 24 and 17, respectively.

The mean (±SEM) ambulation counts for subjects with infusions of APV or aCSF into dorsal hippocampus are depicted in Figure 8a. A two-way ANOVA with day as the within-subjects factor and infusion condition as the between-subjects factor failed to reveal a significant main effect for condition, $F(1,27) = 0.083$, $p = 0.775$, or for day, $F(2, 86) = 2.91$, $p = 0.063$. There was no interaction between condition and day, $F(2,86) = 0.278$, $p = 0.757$. All subjects with cannula in dorsal hippocampus exhibited similar levels of ambulation throughout all sessions of locomotor activity assessment, regardless of infusion condition.

The mean (±SEM) rearing counts for subjects with infusions of APV or aCSF into dorsal hippocampus are depicted in Figure 8b. A two-way ANOVA with day as the within-subjects factor and infusion condition as the between-subjects factor failed to reveal a significant main effect for condition, $F(1,27) = 0.619$, $p = 0.438$, but did reveal a significant main effect for day, $F(2, 86) = 4.622$, $p = 0.014$. There was no interaction between condition and day, $F(2,84) = 0.131$, $p = 0.877$, indicating that the difference in rearing across days did not depend on the infusion condition.

The mean (±SEM) ambulation counts for subjects with infusions of APV or aCSF into ventral hippocampus are depicted in Figure 9a. A two-way ANOVA with day as the within-subjects factor and infusion condition as the between-subjects factor failed to reveal a significant main effect for condition, $F(1,39) = 0.158$, $p = 0.693$, or for day, $F(2, 122) = 0.127$, $p = 0.880$. There was no interaction between condition and day, $F(2,122) = 0.093$, $p = 0.911$. All subjects with cannula in ventral hippocampus exhibited similar levels of ambulation throughout all sessions of locomotor activity assessment, regardless of infusion condition.

The mean (±SEM) rearing counts for subjects with infusions of APV or aCSF into dorsal hippocampus are depicted in Figure 9b. A two-way ANOVA with day as the within-subjects factor and infusion condition as the between-subjects factor failed to reveal a significant main effect for condition, $F(1,39) = 0.061$, $p = 0.805$, or for day, $F(2, 122) = 0.405$, $p = 0.667$. There was no interaction between condition and day, $F(2,122) = 0.055$, $p = 0.946$. All subjects with cannula in ventral hippocampus exhibited similar levels of rearing throughout all sessions of locomotor activity assessment, regardless of infusion condition.

Summary and Discussion

Effect of NMDA receptor antagonism in dorsal or ventral hippocampus on freezing during trace fear conditioning

During the trace fear conditioning session, subjects that received pre-training infusions of the NMDA receptor antagonist APV exhibited lower levels of freezing during the ITI, CS, and trace interval compared to subjects who received infusions of aCSF. This reduction in freezing was observed regardless of whether APV was administered into dorsal or ventral hippocampus. This observation is similar to that reported by others (Quinn et al., 2005). However, it is important to point out that all subjects, regardless of infusion condition or brain region, froze significantly more during trials 2-7 than they did during trial 1 (before the first US presentation). This suggests that blocking NMDA receptors did not affect sensory processing of the auditory CS or footshock. Instead, it is plausible that NMDA receptor antagonism in dorsal or ventral hippocampus disrupted short term memory processing during acquisition.

NMDA receptor antagonism of dorsal hippocampus selectively impairs the acquisition but not expression of trace fear conditioning

In the present study, pre-training but not pre-testing infusions of APV into dorsal hippocampus produced a robust decrease in the conditioned fear response (freezing) compared to subjects who received aCSF infusions. This was observed during the ITI, CS, and trace interval of the testing session.

This finding is in stark contrast to previous research from our laboratory in which neither lesions nor temporary inactivation of dorsal hippocampus affected the acquisition of trace fear conditioning (Yoon & Otto, 2007; Czerniawski et al., 2009). However, it is consistent with numerous reports that blocking NMDA receptors in dorsal hippocampus before trace fear conditioning attenuates acquisition of this paradigm (Misane et al., 2005; Quinn et al., 2005; Wanisch et al., 2005). Therefore, our data are consistent with the notion that although the integrity of dorsal hippocampus is not necessary, plasticity within this region may contribute to the formation of CS-US associations in a trace paradigm.

Although there have been many inconsistencies with data regarding dorsal hippocampal contributions to trace fear conditioning, we have now shown within our own laboratory that pre-training lesions or inactivation have no effect on acquisition while pre-training NMDA receptor antagonism of dorsal hippocampus dramatically attenuates learning CS-US associations in trace fear conditioning. This further supports the notion that dorsal hippocampus may participate in these memory processes if it is intact during learning while simultaneous inhibiting other neural structures. Thus, in the event of lesions or inactivation there should be a disinhibition thereby allowing other regions to potentially compensate for a lack of dorsal hippocampal integrity. In the presence of APV these structures may still be inhibited and dorsal hippocampus may participate in forming associations via uncompromised AMPA receptors. However, without NMDA-receptor

activation, dorsal hippocampus may not be able to support the plastic mechanisms necessary to acquire associations between these stimuli (cf. Wiltgen & Fanselow, 2003). If so, this may help explain why lesions or inactivation of dorsal hippocampus typically have no effect on the acquisition of contextual or trace fear conditioning but blocking NMDA receptors does. Collectively, these findings suggest that while dorsal hippocampus may not be integral for acquisition, it normally participates in forming these associations.

NMDA receptor antagonism in ventral hippocampus impairs the acquisition and expression of trace fear conditioning

To date, this is the first study that has examined the potential role that NMDA receptors in ventral hippocampus may play in trace fear conditioning. We have previously demonstrated that pre-training or pre-testing lesions or inactivation of ventral hippocampus dramatically impair the acquisition and expression of trace fear conditioning (Yoon & Otto, 2007; Czerniawski et al., 2009). We now report that pre-training or pre-testing NMDA receptor antagonism of ventral hippocampus also robustly attenuates the acquisition of this type of associative learning. Together, these data provide compelling support that the integrity of, and possibly NMDA receptor-mediated plasticity within, ventral hippocampus is critical for various aspects of trace fear conditioning.

The observation that NMDA receptor antagonism in ventral hippocampus attenuated the expression of trace fear memories is somewhat

surprising, given that NMDA receptors are thought to be particularly important for the induction of some forms of neuronal plasticity and not normal synaptic transmission (Morris et al., 1990). Thus it would be expected that NMDA receptor antagonism should only affect acquisition and we would only observe a behavioral deficit during testing after pre-training but not pre-testing infusions of APV. However, there have been several reports that NMDA receptor antagonism in the amygdala attenuates both the acquisition and expression of fear conditioning (Maren et al., 1996; Lee & Kim, 1998; Lee et al., 2001). This suggests that NMDA receptors may play a more prominent role in normal synaptic transmission than previously thought. In addition, there is the likely possibility that NMDA receptor activation is an important feature underlying the new learning that occurs during extinction. Thus, the same system that is activated during the initial experience may also be engaged during subsequent related experiences.

It is interesting that in the current study pre-testing NMDA receptor antagonism in ventral, but not dorsal, hippocampus affected the expression and/or retrieval of contextual and trace fear conditioning. As previously mentioned, ventral hippocampus has direct reciprocal connections with the amygdala whereas dorsal hippocampus gains access to amygdalar processing through its connections with ventral hippocampus (Pitkanen et al., 2000). It is intriguing to speculate that blocking NMDA receptors in ventral hippocampus before testing may disrupt connections with amygdala, thereby hindering the retrieval, extinction or reconsolidation processes of contextual

and trace fear conditioning. However, the testing sessions in the current study were only 6 min (context) or 3 trials (tone), making it difficult to ascertain a clear role for NMDA receptor activation in the various processes involved in the extinction of associative learning. Nevertheless, it is clear that NMDA receptor antagonism in ventral hippocampus impairs the acquisition and expression of contextual and trace fear conditioning.

NMDA receptor antagonism in dorsal hippocampus selectively impairs the acquisition but not expression of contextual fear conditioning

Pre-training, but not pre-testing, infusions of APV into dorsal hippocampus resulted in an attenuation of the conditioned fear response during testing to the context. With respect to dorsal hippocampus, this finding is consistent with other reports that NMDA receptor antagonism in this hippocampal subregion disrupts the acquisition but not expression of contextual fear conditioning (Bast et al., 2003; Misane et al., 2005; Wanisch et al., 2005; Quinn et al al., 2005; Matus-Amat et al., 2007; Schenberg et al., 2008). Although the data are mixed regarding the effects of lesions or temporary inactivation (Maren et al., 1997; Richmond et al., 1999; Young et al., 1994; Phillips & LeDoux, 1992, 1994), blocking NMDA receptors in dorsal hippocampus before training consistently impairs the acquisition of conditioned fear to a context. Our findings further support the notion that while the integrity of dorsal hippocampus may not be necessary to acquire context-US associations in this paradigm, if it is intact during learning NMDA-

receptor activation normally occurs in this hippocampal subregion and is important for the acquisition of contextual fear conditioning.

NMDA receptor antagonism of ventral hippocampus impairs the acquisition and expression of contextual fear conditioning

While there have been numerous studies exploring the putative role of NMDA receptor-mediated plasticity in dorsal hippocampus, few have examined a potentially important role of NMDA receptor-mediated plasticity in ventral hippocampus for contextual fear conditioning. It has been previously been reported that administration of the noncompetitive NMDA receptor antagonist MK-801 into ventral hippocampus disrupts the acquisition of contextual fear conditioning (Zhang et al., 2001). In the present study, we have demonstrated that the competitive NMDA receptor antagonist APV also blocks learning in this paradigm. We have also extended that finding to include an important role for NMDA receptors in the retrieval or expression of contextual fear conditioning. This was somewhat surprising because although there have been some reports that pre-training manipulations (lesions, temporary inactivation) of ventral hippocampus attenuate the acquisition of contextual fear conditioning, pre-testing inactivation of ventral hippocampus has no effect (Maren & Holt, 2004). In that study, there was only a deficit in freezing to the context after large pre-testing electrolytic lesions that included damage to the ventral subiculum and medial entorhinal cortex.

It can be postulated that the integrity of ventral hippocampus is necessary for any behavior that may depend on communication between dorsal hippocampus and the amygdala. This is due to the fact that there are direct reciprocal connections between ventral, but not dorsal, hippocampus and the amygdala (Pitkanen et al., 2000). However, few studies have examined whether plasticity within ventral hippocampus is required to form associations between contextual stimuli and an aversive US. Data in the present study suggest that NMDA receptor-mediated plasticity in ventral hippocampus may mediate contextual fear conditioning, indicating it is likely more than just a "relay" between dorsal hippocampus and the amygdala. Together, the present study and others strongly suggest that NMDA receptor activation is critical in both dorsal and ventral hippocampus for the acquisition of contextual fear conditioning. However, only in ventral hippocampus is NMDA receptor activation important for the expression of fear to a context.

Infusions of APV into dorsal or ventral hippocampus do not affect locomotor activity

Manipulations of the hippocampus have been shown to induce hyperactivity (Good & Honey, 1997). This is a key concern because we interpret a reduction in freezing as learning deficit when it could potentially be due to hyperactivity induced by our manipulations. In the present study, animals receiving infusions of APV into either dorsal or ventral hippocampus were not hyperactive, as measured by ambulation and rearing counts in the

open field. In addition, subjects receiving infusions of APV before both training and testing exhibited significantly less freezing during testing compared to control subjects. Because all animals with pre-training APV infusions exhibited attenuated freezing responses during testing regardless of their pre-testing infusion condition, our results cannot be interpreted as a state-dependent effect of drug infusions. Thus, any behavioral observations of reduced freezing in the experimental groups likely reflect a learning and not performance deficit.

Summary

We have observed that pre-training NMDA receptor antagonism of both dorsal and ventral hippocampus impair the acquisition of contextual and trace fear conditioning. In addition, pre-testing NMDA receptor antagonism of ventral, but not dorsal, hippocampus attenuated the expression of these memories. In the following experiments, we wanted to further explore the neural processes that may be normally involved in an intact dorsal or ventral hippocampus in the acquisition of contextual and trace fear conditioning. To this end, we have focused on the putative role of Arc, an immediate early gene which has been implicated in synaptic plasticity and memory consolidation (see Guzowski, 2002 for review), underlying memory formation in these hippocampal subregions.

Experiment 2. The role of Arc in trace fear conditioning.

In the present experiment we sought to address the following questions 1) does trace fear conditioning enhance Arc transcription and/or translation in dorsal or ventral hippocampus? 2) does blocking Arc protein translation block this potential learning-induced increase in Arc protein? and 3) does blocking Arc protein translation in the hippocampus block the acquisition of trace fear conditioning? First, we performed quantitative real-time PCR to assess levels of Arc mRNA in dorsal or ventral hippocampus in subjects that had been trained in our trace fear conditioning paradigm. Then, in separate subjects, we infused Arc antisense or scrambled oligodeoxynucleotides into dorsal or ventral hippocampus three hours before trace fear conditioning. While most of the subjects were also subjected to context and testing sessions 24 and 48 hrs later, respectively, a subset were sacrificed one hour after conditioning in order to quantify Arc protein levels via western blots (see Figure 10). The procedures are described below.

Methods

Quantitative real-time PCR

Subjects. Fourteen male Sprague Dawley rats served as subjects. Arc gene expression was assessed in both dorsal and ventral hippocampus for each subject. Therefore there were 7 subjects in the trained condition and 7 in the untrained condition. Two ventral hippocampal samples from each the trained and untrained group were excluded for technical reasons. Therefore

the final group numbers were: dorsal hippocampus trained (n=7), dorsal hippocampus untrained (n=7), ventral hippocampus trained (n=5) and ventral hippocampus untrained (n=5).

Behavioral Training and Brain extractions. Subjects were handled 2 min per day for five days. They were then trained in the trace fear conditioning paradigm described above and then sacrificed 15 min after the end of training. This time point was chosen based on the results of pilot studies in which there was a robust increase in Arc mRNA in the hippocampus 15 min after training compared to other time points. Separate subjects serving as untrained control subjects were removed from their home cage and sacrificed without undergoing any training. For all subjects, the brain was removed after rapid decapitation. The hippocampus was removed and dissected into dorsal and ventral subregions which were then placed into nuclease-free cryogenic tubes and flash-frozen in liquid nitrogen and stored at -80°C until processed.

RNA Isolation, Purification, and cDNA Synthesis. Total RNA was isolated using Promega SV Total RNA Isolating System (Promega Corporation) according to the manufacturer's instructions. RNA purification using the RNeasy Mini Kit (Qiagen) was performed according to the manufacturer's instructions. Absorbance values for the samples were determined using a Nanodrop ND-1000 (NanoDrop Technologies, Wilmington, DE) spectrophotometer. RNA concentration was determined using UV absorption at 260nm. RNA purity was assessed using the ratio of absorbance at 260/280 nm and 260/230 nm.

Samples not within 260/280 nm and ratio between 1.8 and 2.15 were removed from further analysis. Total RNA (1uL) was reverse transcribed using iScript cDNA Synthesis Kit (Bio-Rad) according to the manufacturer's instructions to a total volume of 67.5uL. Samples were then run in a thermal cycler (Applied BioSystems Thermal Cycler Modeler 2720, Foster City, CA) at the following conditions: 25°C for 10 min, 49°C for 30 min, and 95°C for 5 min. The samples were stored at -20°C after the reaction was complete.

qRT-PCR. Quantitative real-time PCR was performed in a 7900HT Real-Time PCR System (Applied Biosystems) using the Syber Green Mastermix (Bio-Rad). The primers used were for Arc (forward: CCCTGCAGCCCAAGTTCAAG; reverse: GAAGGCTCAGCTGCCTGCTC) and GAPDH (forward: GCATCCTGCACC ACCAACTG, reverse: ACGCCACAGCTTTCCAGAGG). Each sample was run in triplicate per gene of interest. The samples were run under the following conditions: 50°C for 4 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec and 60°C for one minute.

Quantification of Gene Expression. Relative quantification of Arc mRNA was determined using the Δ ΔC_T method (Livak & Schmittgen, 2001). The triplicate values of threshold cycle (C_T, cycle at which sample reached the threshold fluorescence level) for each sample were averaged. Mean C_T values for GAPDH, endogenous control, were subtracted from mean C_T values of Arc for each subject. This served as the ΔC_T. The subject with the highest ΔC_T from the home cage untrained control group served as the calibrator and was subtracted from the ΔC_T of the remaining subjects ($\Delta\Delta C_T$). The fold

change in the expression of Arc, normalized to the endogenous control (GAPDH), was then determined using the formula $2^{-\Delta\Delta Ct}$. The fold change values were then averaged for each of the respective groups (i.e. dorsal hippocampus trained, dorsal hippocampus untrained, ventral hippocampus trained, and ventral hippocampus untrained).

Infusions and Behavioral Testing

Subjects. Sixty-five male Sprague Dawley rats served as subjects in the following experiment. For subjects with cannula implanted in dorsal hippocampus, initial sample sizes were: Scrambled (n=15) and Arc Antisense (n=17). For subjects with cannula implanted in ventral hippocampus, initial sample sizes were: Scrambled (n=15) and Arc Antisense (n=18). A subset of these subjects (Scrambled (n=4), Arc Antisense (n=5) for both dorsal and ventral hippocampus) were sacrificed 1 hour after training for western blot analysis of Arc protein expression.

Antisense oligodeoxynucleotide Preparation and Infusions

Subjects received infusions of antisense oligodeoxynucleotides for *Arc* or scrambled oligodeoxynucleotides. These antisense oligodeoxynucleotides were synthesized by Integrated DNA Technologies and obtained through DNA Synthesis and Core Facility at UMDNJ (Piscataway, NJ). These oligodeoxynucleotides were prepared to encode antisense or scrambled sequences for Arc mRNA starting at the sequence near the translation start site. For the experimental group, base sets of 20 mer antisense

oligodeoxynucleotides with a sequence from bases 209 to 228 of the published Arc sequence were used. For the control group, the sequence was the same base composition in a randomized (scrambled) order. All oligodeoxynucleotides were prepared using HPLC purification and then resuspended in aCSF.

The oligodeoxynucleotide infusions were administered via insertion of an infusion cannula into the guide cannula targeted at the dorsal or ventral hippocampus according to the same protocol described above for the APV infusions. A volume of 0.5µl (0.1 µl/min) was infused bilaterally into either dorsal or ventral hippocampus for a total volume of 1µl. The infusion cannula was replaced by the dummy cannula 2 minutes after the infusion was complete. Subjects were returned to their cages for 3 hours at which point they were transported into the training room to undergo trace fear conditioning (described above). This time point was chosen because it has previously been shown to be an effective time for the oligodeoxynucleotides to effectively innervate the cells and block protein translation (Guzowski et al., 2000; McIntyre et al., 2005). Subjects were infused with aCSF before both the context and tone testing sessions and 48 h later, respectively.

Western Blots

Brain extractions and tissue preparation. Subjects were infused with Arc antisense or scrambled oligodeoxynucleotides and then underwent trace fear conditioning as described above. A subset of these subjects was sacrificed 1hour after conditioning. Separate subjects serving as untrained control

subjects were removed from their home cage and sacrificed without undergoing any training. For all subjects, the brain was removed after rapid decapitation. The hippocampus was removed and dissected into dorsal and ventral subregions which were then homogenized in ice cold 1% lysis buffer with EDTA and protease inhibitor cocktail. Samples were then placed on agitator plate for 2 hours at 4°C, followed by 20 min in the centrifuge at 12000rpm at 4°C. The supernatant was then placed into separate microcentrifuge tubes and placed in liquid nitrogen prior to being stored at -80°C until further processed.

Gel electrophoresis & transfer to membrane. Protein concentration for each sample was assessed and normalized using a standard Bradford Assay. 5% Beta-Mercaptoethanol-Laemmli Sample Buffer (Bio-Rad) was added to each sample, and then the samples were placed in the water bath for 10 min at 70°C. The samples were then loaded into 12% Mini-PROTEAN TGX Precast Gels for gel electrophoresis at a constant current of 30mAmps for 2 hours or until the lanes reached the bottom of the gel. The gel was then transferred to a nitrocellulose membrane (Ready-Blot sandwiches, Bio-Rad) at 500mAmps for 1 hour.

Blocking and Antibody Incubatons. The membrane was then blocked in TBST buffer with 5% BSA for 1.5 hours at room temperature. Following blocking, the membrane was incubated with mouse anti-Arc monoclonal antibody (1:5000; Santa Cruz Biotechnology) and rabbit anti-actin polyclonal antibody (1:20000; Sigma-Aldrich) overnight at 4°C. After incubating the primary

antibody, the membrane was then incubated in both anti-rabbit and anti-mouse conjugated to horseradish peroxidase (1:10000; Santa Cruz) for 1 hour at room temperature.

Visualization and quantification of the blots. The blots were developed using chemiluminescent substrate ECL reagent (Pierce laboratories) according to manufacturer's instructions. The membrane was then placed in a film cassette and imaged with the Fluor-S Scanner (Bio-rad). Optical densities were determined by Quantity One software. To control for any inconsistencies in loading, the optical densities were normalized to actin for each sample. Data for subjects that received oligodeoxynucleotide infusions and were trained in trace fear conditioning are expressed as a percentage increase in optical density compared to home cage untrained control subjects.

Results

Quantitative real-time PCR

The fold increase in Arc mRNA relative to home cage untrained subjects is shown in Figure 11. A two-way ANOVA with brain region (dorsal or ventral hippocampus) and training condition (trained or untrained) as factors revealed that there was no main effect for brain region, $F(1,23) = 0.385$, $p = 0.542$, nor was there an interaction between region and condition, $F(1,23) = 0.171$, $p = 0.684$. There was a main effect for condition, $F(1,23) = 12.789$, $p = 0.0014$, indicating that there was a significant fold increase in

Arc mRNA in trained compared to untrained subjects in both dorsal and ventral hippocampus.

Western Blots

The final subject numbers were the same for infusions into dorsal or ventral hippocampus: Arc antisense oligodeoxynucleotides (n=5) and scrambled oligodeoxynucleotides (n=4). We measured Arc protein levels in both dorsal and ventral hippocampus, regardless of the brain region in which the cannula was implanted. Figure 12 depicts a representative blot showing Arc and actin bands for subjects with cannula in dorsal (Figure 12a) or ventral (Figure 12b) hippocampus. The mean (±SEM) percent increase in Arc protein expression compared to actin and relative to home cage controls is shown in Figure 13. For subjects receiving infusions of scrambled or Arc antisense oligodeoxynucleotides into dorsal hippocampus before trace fear conditioning, a two-way ANOVA with brain region and infusion condition as the main factors revealed there was a significant main effect for brain region, $F(1,17) = 19.0$, $p = 0.0007$ and for infusion condition, $F(1,17) = 26.9$, $p = 0.0006$. There was also a significant interaction between brain region and infusion condition, $F(1,17) = 19.8$, $p = 0.0006$. Subsequent post hoc analyses revealed that subjects with ARC antisense oligodeoxynucleotide infusions in dorsal hippocampus expressed significantly less Arc protein levels than all other groups ($p < 0.05$) (Figure 13a).

For subjects receiving infusions of scrambled or Arc antisense oligodeoxynucleotides into ventral hippocampus before trace fear

conditioning, a two-way ANOVA with brain region and infusion condition as the main factors revealed there was a significant main effect for brain region, $F(1,17) = 9.41$, $p = 0.0083$ and for infusion condition, $F(1,17) = 10.40$, $p = 0.0061$. There was also a significant interaction between brain region and infusion condition, $F(1,17) = 13.40$, $p = 0.0026$. Subsequent post hoc analyses revealed that subjects with ARC antisense oligodeoxynucleotide infusions in ventral hippocampus expressed significantly less Arc protein levels than all other groups ($p < 0.05$) (Figure 13b).

Cannula placement. Following histological examination, five subjects with cannula in dorsal hippocampus and six subjects with cannula in ventral hippocampus were excluded due to incorrect cannula placement. Thus, the final group numbers for subjects with cannula in dorsal hippocampus were as follows: Arc Antisense (n=9) and Scrambled (n=9). The final group numbers for subjects with cannula in ventral hippocampus were: Arc Antisense (n=11) and Scrambled (n=7). A schematic representation of cannula placement throughout dorsal and ventral hippocampus is shown in Figure 14.

The effect of Arc antisense or scrambled oligodeoxynucleotides in dorsal or ventral hippocampus on the acquisition of trace and contextual fear conditioning

The results of pre-training infusions of Arc antisense or scrambled oligodeoxynucleotides are described below. Although the context testing session occurred prior to the testing session for trace fear conditioning, the

data from the context test will be presented after the data for trace fear conditioning and testing for the sake of clarity.

Trace fear conditioning: effect of pre-training Arc antisense or scrambled oligodeoxynucleotide infusions into dorsal hippocampus on freezing during training.

The mean (±SEM) percentage of freezing exhibited by different infusion groups during conditioning is illustrated in Figure 15. The first US occurred after the first trial, thus trial 1 of the ITI, CS, and trace interval serves as a baseline measure of freezing. For the ITI, a two-way ANOVA with infusion condition (scrambled or Arc antisense oligodeoxynucleotides) as the between subjects factor and time point (baseline vs. trials 2-7) as the within subjects factor revealed a main effect for time point, $F(1,35) = 401.055$, $p < 0.0001$, but not for infusion condition, $F(1,16) = 0.702$, $p = 0.414$ (Figure 15a). There was not a significant interaction between condition and trial, $F(1,35) = 0.193$, $p = 0.666$, indicating that all subjects exhibited significantly more freezing during trials 2-7 of the ITI than during baseline (trial 1 – before the first shock presentation) regardless of whether they received pre-training infusions of scrambled or Arc antisense oligodeoxynucleotides into dorsal hippocampus.

For the CS, a two-way ANOVA with infusion condition (scrambled or Arc antisense oligodeoxynucleotides) as the between subjects factor and time point (baseline vs. trials 2-7) as the within subjects factor revealed a main effect for time point, $F(1,35) = 68.236$, $p < 0.0001$, but not for infusion

condition, $F(1,16) = 0.707$, $p = 0.412$ (Figure 15b). There was not a significant interaction between condition and trial, $F(1,35) = 1.521$, $p = 0.235$, indicating that all subjects exhibited significantly more freezing during CS presentations of trials 2-7 than during baseline (trial 1 – before the first shock presentation) regardless of whether they received pre-training infusions of scrambled or Arc antisense oligodeoxynucleotides into dorsal hippocampus.

For the trace interval, a two-way ANOVA with infusion condition (scrambled or Arc antisense oligodeoxynucleotides) as the between subjects factor and time point (baseline vs. trials 2-7) as the within subjects factor revealed a main effect for time point, $F(1,35) = 142.561$, $p < 0.0001$, but not for infusion condition, $F(1,16) = 0.158$, $p = 0.696$ (Figure 15c). There was not a significant interaction between condition and trial, $F(1,35) = 0.592$, $p = 0.452$, indicating that all subjects exhibited significantly more freezing during the trace interval of trials 2-7 than during baseline (trial 1 – before the first shock presentation) regardless of whether they received pre-training infusions of scrambled or Arc antisense oligodeoxynucleotides into dorsal hippocampus.

Trace fear conditioning: effect of pre-training Arc antisense or scrambled oligodeoxynucleotide infusions into ventral hippocampus on freezing during training.

The mean (±SEM) percentage of freezing exhibited by different infusion groups during conditioning is illustrated in Figure 16. For the ITI, a

two-way ANOVA with infusion condition (scrambled or Arc antisense oligodeoxynucleotides) as the between subjects factor and time point (baseline vs. trials 2-7) as the within subjects factor revealed a main effect for time point, $F(1,35) = 393.179$, $p < 0.0001$, but not for infusion condition, $F(1,16) = 0.026$, $p = 0.873$ (Figure 16a). There was not a significant interaction between condition and trial, $F(1,35) = 1.396$, $p = 0.254$, indicating that all subjects exhibited significantly more freezing during trials 2-7 of the ITI than during baseline (trial 1 – before the first shock presentation) regardless of whether they received pre-training infusions of scrambled or Arc antisense oligodeoxynucleotides into ventral hippocampus.

For the CS, a two-way ANOVA with infusion condition (scrambled or Arc antisense oligodeoxynucleotides) as the between subjects factor and time point (baseline vs. trials 2-7) as the within subjects factor revealed a main effect for time point, $F(1,35) = 54.68$, $p < 0.0001$, but not for infusion condition, $F(1,16) = 0.744$, $p = 0.401$ (Figure 16b). There was not a significant interaction between condition and trial, $F(1,35) = 0.009$, $p = 0.924$, indicating that all subjects exhibited significantly more freezing during CS presentations of trials 2-7 than during baseline (trial 1 – before the first shock presentation) regardless of whether they received pre-training infusions of scrambled or Arc antisense oligodeoxynucleotides into ventral hippocampus.

For the trace interval, a two-way ANOVA with infusion condition (scrambled or Arc antisense oligodeoxynucleotides) as the between subjects

factor and time point (baseline vs. trials 2-7) as the within subjects factor revealed a main effect for time point, $F(1,35) = 75.09$, $p < 0.0001$, but not for infusion condition, $F(1,16) = 0.013$, $p = 0.910$ (Figure 16c). There was not a significant interaction between condition and trial, $F(1,35) = 0.557$, $p = 0.466$, indicating that all subjects exhibited significantly more freezing during the trace interval of trials 2-7 than during baseline (trial 1 – before the first shock presentation) regardless of whether they received pre-training infusions of scrambled or Arc antisense oligodeoxynucleotides into ventral hippocampus.

Trace fear conditioning: effect of pre-training Arc antisense or scrambled oligodeoxynucleotide infusions into dorsal hippocampus on freezing during testing.

The expression of conditioned fear exhibited during the testing session for subjects with cannula implanted in dorsal hippocampus is depicted in Figure 17. The mean (±SEM) percentage of freezing exhibited during the 2 min ITI is shown in Figure 17a. A *t*-test revealed there was so significant difference between the two groups during the first trial (baseline) of the ITI, $t(1,16) = 0.313$, $p = 0.758$, but that infusions of Arc antisense oligodeoxynucleotides into dorsal hippocampus resulted in significantly less freezing during trials 2-3 of the ITI during testing, $t(1,16) = 3.71$, $p = 0.0019$.

The mean (±SEM) percentage of freezing exhibited during the 16 s CS presentation is shown in Figure 17b. A *t*-test revealed that subjects who

received Arc antisense oligodeoxynucleotide infusions into dorsal hippocampus before conditioning froze significantly less to the CS presentations during testing compared to those that received scrambled oligodeoxynucleotide infusions, $t(1,16) = 2.55$, $p = 0.0212$.

The mean (±SEM) percentage of freezing exhibited during the 28 s trace interval is shown in Figure 17c. A *t*-test revealed that subjects who received Arc antisense oligodeoxynucleotide infusions into dorsal hippocampus before conditioning froze significantly less to the trace interval during testing compared to those that received scrambled oligodeoxynucleotide infusions, $t(1,16) = 4.94$, $p = 0.0001$.

Trace fear conditioning: effect of pre-training Arc antisense or scrambled oligodeoxynucleotide infusions into ventral hippocampus on freezing during testing.

The expression of conditioned fear exhibited during the testing session for subjects with cannula implanted in ventral hippocampus is depicted in Figure 18. The mean (±SEM) percentage of freezing exhibited during the 2 min ITI is shown in Figure 18a. A *t*-test revealed there was so significant difference between the two groups during the first trial (baseline) of the ITI, $t(1,16) = 1.54$, $p = 0.144$, but that infusions of Arc antisense oligodeoxynucleotides into ventral hippocampus resulted in significantly less freezing during trials 2-3 of the ITI during testing, $t(1,16) = 4.11$, $p = 0.0008$.

The mean (±SEM) percentage of freezing exhibited during the 16 s CS presentation is shown in Figure 18b. A *t*-test revealed that subjects who received Arc antisense oligodeoxynucleotide infusions into ventral hippocampus before conditioning froze significantly less to the CS presentations during testing compared to those that received scrambled oligodeoxynucleotide infusions, $t(1,16) = 3.95$, $p = 0.0011$.

The mean (±SEM) percentage of freezing exhibited during the 28 s trace interval is shown in Figure 18c. A *t*-test revealed that subjects who received Arc antisense oligodeoxynucleotide infusions into ventral hippocampus before conditioning froze significantly less to the trace interval during testing compared to those that received scrambled oligodeoxynucleotide infusions, $t(1,16) = 3.76$, $p = 0.0017$.

Context testing

For statistical analysis during testing, data from only the first three minutes of context testing were used because the behavior at the beginning of the session was least likely affected by extinction. The mean (±SEM) percentage freezing during the context test is shown in Figure 19. For subjects receiving infusions in dorsal hippocampus, a *t*-test revealed that subjects with Arc antisense infusions into dorsal hippocampus before conditioning froze significantly less during context testing compared to those that received the scrambled oligodeoxynucleotides, $t(1,16) = 6.88$, $p < 0.0001$ (Figure 19a). Similarly, subjects who received Arc antisense infusions into ventral hippocampus before conditioning also froze significantly

less during context testing compared to those that received the scrambled oligodeoxynucleotides, $t(1,16) = 3.35$, $p = 0.004$ (Figure 19b).

Locomotor activity

Subjects were infused with whatever substance they received before conditioning on Day 2 of the locomotor activity test. The final group numbers for subjects who received infusions of scrambled or Arc antisense oligodeoxynucleotide infusions into dorsal hippocampus were 9 and 9, respectively. The final group numbers for subjects who received infusions of scrambled or Arc antisense oligodeoxynucleotide infusions into ventral hippocampus were 7 and 10, respectively.

The mean (±SEM) ambulation counts for subjects with infusions of scrambled or Arc antisense oligodeoxynucleotides into dorsal hippocampus are depicted in Figure 20a. A two-way ANOVA with day as the within-subjects factor and infusion condition as the between-subjects factor failed to reveal a significant main effect for condition, $F(1,16) = 0.17$, $p = 0.686$, or for day, $F(2,53) = 1.099$, $p = 0.345$. There was no interaction between condition and day, $F(2,53) = 0.310$, $p = 0.735$. All subjects with cannula in dorsal hippocampus exhibited similar levels of ambulation throughout all sessions of locomotor activity assessment, regardless of infusion condition.

The mean (±SEM) rearing counts for subjects with infusions of scrambled or Arc antisense oligodeoxynucleotides into dorsal hippocampus are depicted in Figure 20b. A two-way ANOVA with day as the within-

subjects factor and infusion condition as the between-subjects factor failed to reveal a significant main effect for condition, $F(1,16) = 0.046$, $p = 0.833$, but did reveal a significant main effect for day, $F(2,53) = 4.455$, $p = 0.019$. There was no interaction between condition and day, $F(2,53) = 1.959$, $p = 0.157$, indicating that the difference in rearing across days did not depend on the infusion condition.

The mean (±SEM) ambulation counts for subjects with infusions of scrambled or Arc antisense oligodeoxynucleotides into ventral hippocampus are depicted in Figure 21a. A two-way ANOVA with day as the within-subjects factor and infusion condition as the between-subjects factor failed to reveal a significant main effect for condition, $F(1,15) = 0.094$, $p = 0.762$, or for day, $F(2, 50) = 0.860$, $p = 0.433$. There was no interaction between condition and day, $F(2,50) = 0.075$, $p = 0.927$. All subjects with cannula in ventral hippocampus exhibited similar levels of ambulation throughout all sessions of locomotor activity assessment, regardless of infusion condition.

The mean (±SEM) rearing counts for subjects with infusions of scrambled or Arc antisense oligodeoxynucleotides into ventral hippocampus are depicted in Figure 21b. A two-way ANOVA with day as the within-subjects factor and infusion condition as the between-subjects factor failed to reveal a significant main effect for condition, $F(1,15) = 0.071$, $p = 0.793$, or for day, $F(2, 50) = 1.136$, $p = 0.334$. There was no interaction between condition and day, $F(2,50) = 0.634$, $p = 0.537$. All subjects with cannula in

ventral hippocampus exhibited similar levels of rearing throughout all sessions of locomotor activity assessment, regardless of infusion condition.

Summary and Discussion

Learning induces Arc transcription in dorsal and ventral hippocampus

By using quantitative real-time PCR, we have observed an increase in Arc mRNA in both dorsal and ventral hippocampus compared to untrained home cage control subjects. This finding is consistent with other reports of an induction of Arc mRNA in the hippocampus following various forms of learning including spatial learning, exploration of a novel environment, contextual fear conditiooning and avoidance (Guzowski et al.; 1999, Kelly & Deadwyler, 2002; Montag-Sallaz & Montag, 2003; Huff et al., 2006; Fletcher et al., 2006). In addition, there is an upregulation of Arc mRNA in the amygdala after delay fear conditioning (Ploski et al., 2008). To our knowledge, we are the first to observe an increase in Arc gene expression in the hippocampus after trace fear conditioning.

There were no subregional differences in Arc mRNA expression; trace fear conditioning resulted in an enhancement in Arc mRNA in both dorsal and ventral hippocampus. This adds further support to the notion that both subregions normally participate in the acquisition of trace and contextual fear conditioning. Although it may be argued that an increase in Arc mRNA is not due to learning processes *per se*, this is not likely for the following reasons. In addition to trace and contextual fear conditioning, we have also observed

an increase in Arc mRNA expression after novel context exposure, but not after delay fear conditioning (Ramamoorthi & Otto, in preparation). In addition, Huff et al. (2006) observed a similar upregulation of Arc mRNA in the hippocampus after contextual fear conditioning or novel context exposure but not after immediate shock. Together these strongly imply that the observed enhancement in Arc gene expression is specifically due to learning.

Leaning induces Arc translation in dorsal and ventral hippocampus

Consistent with the observation of an upregulation of Arc gene expression, there is also an upregulation of Arc protein expression induced by trace fear conditioning. This enhancement in Arc protein expression was observed in trained subjects 1 hour after conditioning relative to untrained home cage control subjects, with each sample standardized to actin. Similar to the learning-induced increase in Arc gene expression, there was an increase in Arc protein in both dorsal and ventral hippocampus after trace fear conditioning.

Importantly, infusions of Arc antisense oligodeoxynucleotides significantly attenuated this learning-induced increase in Arc protein expression in both dorsal and ventral hippocampus. The sequence, design, and administration of the oligodeoxynucleotides used in the current study were the same as that previously employed by other researchers (Guzowski et al., 2000; McIntyre et al., 2005; Ploski et a;., 2008). We selected this sequence because in the aforementioned studies it has been shown to

selectively target and block Arc protein translation without causing any changes in normal synaptic transmission.

There is the possibility that the spread of oligodeoxynucleotides diffused greater than we had anticipated. However, this is highly unlikely because there was a reduction in Arc protein only in the subregion in which the antisense oligodeoxynucleotides were infused. For example, there was a significant increase in Arc protein expression relative to untrained controls in the ventral hippocampus in subjects who received infusions of Arc antisense or scrambled oligodeoxynucleotides into dorsal hippocampus. Likewise, there was a significant increase in Arc protein expression in the dorsal hippocampus of subjects that received infusions into ventral hippocampus, regardless of infusion condition. Moreover, there was a remarkably consistent ~33% increase in Arc protein expression after infusions of scrambled oligodeoxynucleotides and in the non-cannulated hippocampal subregion. This indicates that the spread of infusion was confined to the targeted subregion and that there were no adverse effects of cannula implantation or infusion of scrambled oligodeoxynucleotides on the learning-induced enhancement of Arc protein. Together with the gene expression data, we observed a consistent and reliable increase in Arc transcription and translation in dorsal and ventral hippocampus after trace fear conditioning.

Blocking Arc protein translation with antisense oligodeoxynucleotides blocks the acquisition of trace and contextual fear conditioning

The learning-induced enhancement of both Arc gene and protein expression in dorsal and ventral hippocampus strongly suggests that it is crucial for memory formation. However, these data are correlative and do not provide a direct causal link between Arc expression and learning. Therefore, we examined the effect of blocking Arc translation on the acquisition and subsequent expression of fear conditioning. Pre-training infusions of Arc antisense oligodeoxynucleotides into dorsal or ventral hippocampus resulted in dramatically attenuated freezing during the context and tone testing sessions. It is important to note that administration of these antisense oligodeoxynucleotides did not completely block Arc translation after conditioning. Although it is significantly less than the ~33% increase observed in the hippocampus after scrambled oligodeoynuxleotide infusions, our western blots revealed there was still a ~13% increase in Arc after trace fear conditioning compared to untrained home cage controls after Arc antisense oligodeoxynucleotide infusions. However, we observed a robust behavioral deficit after this attenuated enhancement of Arc protein, strongly suggesting an important role for Arc in forming associations in trace and contextual conditioning.

The effects of blocking Arc translation before learning greatly paralleled the effects of NMDA receptor antagonism during both the context and tone tests. However, unlike APV infusions, there was no effect of blocking Arc translation on freezing during the conditioning session. However, freezing during conditioning ought to be intact because although

Arc expression is induced by trace fear conditioning, any role of Arc in memory acquisition should be due to the function of Arc shortly after, but not during, the conditioning session. In addition, it has previously been shown that the antisense oligodeoxynucleotide sequence and design used in the current study impair the maintenance but not induction of LTP (Guzowski et al., 2000). This suggests that while NMDA receptor activation may be important during the conditioning session itself, Arc translation is likely important for processes that occur after the conditioning session enabling the maintenance of these associations. This is consistent with the aforementioned data regarding an increase in both Arc mRNA and protein 15 minutes or 1 hour after conditioning, respectively. Moreover, administration of Arc antisense oligodeoxynucleotides into the hippocampus disrupts spatial learning and inhibitory avoidance (Guzowski et al., 2001; McIntyre et al., 2005). Furthermore, blocking Arc protein translation in the amygdala prior to delay fear conditioning impairs the conditioned fear response during testing but not training (Ploski et al., 2008). We have now extended this finding to include a critical role for Arc protein translation in the hippocampus for forming-associations in contextual and trace fear conditioning.

There were no observed behavioral differences between groups receiving infusions into dorsal or ventral hippocampus. However the observed learning deficits were likely due to blocking Arc translation in the targeted subregion because the western blots revealed there was no difference in Arc protein levels in dorsal hippocampus for those subjects

infused with antisense or scrambled oligodeoxynucleotides into ventral hippocampus. The same holds true for the ventral hippocampus of subjects that received infusions into dorsal hippocampus. Additionally, when a behavioral measure, such as freezing, is used as an index for learning it is important that any manipulations due not alter locomotor activity. In the current study, Arc antisense oligodeoxynucleotides were administered 24 or 48 hours before testing and were therefore not present in the hippocampus at the time during which freezing was reduced. Furthermore, there were no differences in locomotor activity as determined by ambulation and rearing counts the day of or after Arc or scrambled oligodeoxynucleotide infusions during the locomotor activity testing.

The present study supports the finding in Experiment 1 that dorsal and ventral hippocampus are both important for learning contextual and trace fear conditioning However, there are still questions regarding the role of Arc in memory formation in the hippocampus. For instance, we administered the oligodeoxynucleotides before conditioning only but it is possible, although unlikely, that Arc translation in the hippocampus is also important for the expression or extinction of trace and/or contextual fear conditioning. It is intriguing to speculate whether the parallel effects of pre-training APV and Arc antisense oligodeoxynucleotides also exist with respect to pre-testing infusions. That is, whether pre-testing Arc antisense oligodeoxynucleotide infusions into ventral but not dorsal hippocampus attenuate the expression of trace and contextual fear conditioning in a manner similar to pre-testing APV

infusions. There is also the question of whether the effects of blocking Arc translation were specific to the hippocampus. Do Arc antisense oligodeoxynucleotides disrupt only forms of learning that are generally thought to be hippocampal-dependent? We address this question in the following experiment in which we blocked Arc protein translation in ventral hippocampus before delay fear conditioning. Nevertheless, it is clear that Arc protein translation is important for forming associations in contextual and trace fear conditioning in dorsal and ventral hippocampus.

Experiment 3. The effect of pre-training antisense oligodeoxynucleotide infusions into ventral hippocampus before DELAY fear conditioning.

We have shown in the preceding experiment that pre-training infusions of Arc antisense oligodeoxynucleotides into dorsal or ventral hippocampus block the acquisition of trace and contextual fear conditioning. The present study examines the effect of pre-training antisense oligodeoxynucleotides into ventral hippocampus on the acquisition of delay fear conditioning. As the hippocampus has been shown to be necessary for trace, but not delay conditioning (Czerniawski et al., 2009; Phillips & LeDoux, 1992; Bast et al., 2001; Zhang et al., 2001), we anticipated that blocking Arc protein translation with antisense oligodeoxynucleotides in ventral hippocampus would not alter the acquisition of delay fear conditioning.

Methods

Subjects. 20 male Sprague Dawley rats served as subjects in this experiment (Scrambled, n=10; Arc Antisense, n=10).

Antisense oligodeoxynucleotide infusions

The antisense oligodeoxynucleotide design, preparation, and infusion procedure was identical to that described in Experiment 2 above. In addition, the surgical procedure and coordinates were the same as subjects which received Arc antisense or scrambled oligodeoxynucleotide infusions into ventral hippocampus before trace fear conditioning.

Delay Fear Conditioning

The delay fear conditioning and testing parameters were identical to those described above, expect that instead of 28s trace interval between the offset of the CS and onset of the US, the US coterminated with the CS. Therefore, conditioning consisted of 7 trials of a 2 min ITI, 16s auditory CS, and 2s footshock US. Context testing was conducted exactly as described above: 6 min in the conditioning chamber with no stimuli presentations, 24 hours after training. Testing to the CS was also conducted as described above: 3 trials identical procedurally to delay fear conditioning except no footshock was presented. Because there was no trace interval, only data during the ITI and CS were collected and analyzed.

Results

Cannula placement. Following histological examination, four subjects with cannula in ventral hippocampus were excluded due to incorrect cannula placement. Thus, the final group numbers for subjects with cannula in ventral hippocampus were: Arc Antisense (n=9) and Scrambled (n=7). A schematic representation of cannula placement throughout dorsal and ventral hippocampus is shown in Figure 22.

Delay fear conditioning: effect of pre-training Arc antisense or scrambled oligodeoxynucleotide infusions into ventral hippocampus on freezing during training.

The mean (±SEM) percentage of freezing exhibited by different infusion groups during conditioning is illustrated in Figure 23. For the ITI, a two-way ANOVA with infusion condition (scrambled or Arc antisense oligodeoxynucleotides) as the between subjects factor and time point (baseline vs. trials 2-7) as the within subjects factor revealed a main effect for time point, $F(1,31) = 954.53$, $p < 0.0001$, but not for infusion condition, $F(1,14) = 1.09$, $p = 0.314$ (Figure 23a). There was not a significant interaction between condition and trial, $F(1,31) = 1.39$, $p = 0.258$, indicating that all subjects exhibited significantly more freezing during trials 2-7 of the ITI than during baseline (trial 1 – before the first shock presentation) regardless of whether they received pre-training infusions of scrambled or Arc antisense oligodeoxynucleotides into ventral hippocampus.

For the CS, a two-way ANOVA with infusion condition (scrambled or Arc antisense oligodeoxynucleotides) as the between subjects factor and time point (baseline vs. trials 2-7) as the within subjects factor revealed a main effect for time point, $F(1,31) = 231.866$, $p < 0.0001$, but not for infusion condition, $F(1,14) = 0.066$, $p = 0.800$ (Figure 23b). There was not a significant interaction between condition and trial, $F(1,31) = 0.216$, $p = 0.648$, indicating that all subjects exhibited significantly more freezing during CS presentations of trials 2-7 than during baseline (trial 1 – before the first shock presentation) regardless of whether they received pre-training infusions of scrambled or Arc antisense oligodeoxynucleotides into ventral hippocampus.

Delay fear conditioning: effect of pre-training Arc antisense or scrambled oligodeoxynucleotide infusions into ventral hippocampus on freezing during testing.

The expression of conditioned fear exhibited during the testing session for subjects who received Arc antisense or scrambled oligodeoxynucleotide infusions into ventral hippocampus before delay fear conditioning is depicted in Figure 24. The mean (±SEM) percentage of freezing exhibited during the 2 min ITI is shown in Figure 24a. A *t*-test revealed there was no significant difference in freezing between the two groups during the first trial (baseline) of the ITI, $t(1,14) = 0.403$, $p = 0.693$, nor was there a significant difference during trials 2-3 of the ITI during testing, $t(1,14) = 0.498$, $p = 0.625$.

The mean (±SEM) percentage of freezing exhibited during the 16 s CS presentation is shown in Figure 24b. A *t*-test revealed there was no significant difference between the two groups, $t(1,14) = 0.257$, $p = 0.801$. Thus, subjects who received Arc antisense oligodeoxynucleotide infusions into ventral hippocampus before delay fear conditioning exhibited similar levels of freezing during CS presentations during testing compared to those that received scrambled oligodeoxynucleotide infusions.

Context testing

For statistical analysis during testing, data from only the first three minutes of context testing were used because the behavior at the beginning of the session was least likely affected by extinction. The mean (±SEM) percentage freezing during the context test is shown in Figure 25. A *t*-test revealed that subjects with Arc antisense infusions into ventral hippocampus before conditioning froze significantly less during context testing compared to those that received the scrambled oligodeoxynucleotides, $t(1,14) = 3.10$, $p = 0.007$.

Summary and Discussion

In the present study, subjects receiving pre-training infusions of Arc antisense oligodeoxynucleotides exhibited similar levels of freezing during the test to those that received scrambled oligodeoxynucleotide infusions. Therefore, blocking Arc translation in ventral hippocampus did not block the formation of CS-US associations in delay fear conditioning. This is consistent

with the general view that the hippocampus is not necessary to learn CS-US associations in a delay fear conditioning paradigm (Phillips & LeDoux, 1992; McEchron et al., 1998; Lee & Kesner, 2004; Wanisch et al., 2005). Furthermore, these data indicate that the antisense oligodeoxynucleotides are not diffusing into the amygdala because if they were we should see a similar deficit observed by Ploski et al. (2008) after blocking Arc protein translation the amygdala before delay fear conditioning. Although there have been a few reports suggesting that ventral hippocampus may participate in some aspects of delay fear conditioning (Richmond et al., 1999; Bast et al., 2001; Maren & Holt, 2004), the data here suggest that Arc protein translation is not a necessary component of ventral hippocampal involvement in delay fear conditioning.

Although we did not asses protein levels in ventral hippocampus after administration of oligodeoxynucleotides before delay fear conditioning, we can be reasonably confident of their efficacy for several reasons. First, they were the same sequence, design, and (in most cases) the same batch as those used in Experiment 2 in which we demonstrated that blocking Arc protein translation blocked the learning-induced enhancement of Arc protein. In addition, while administration of Arc antisense oligodeoxynucleotides into ventral hippocampus did not alter freezing levels during testing, it robustly attenuated freezing during the context test. Additionally, because animals froze discriminatively during the different testing sessions, it is highly unlikely that any observed behavioral deficits are due to any possible effect

of the oligodeoxynucleotides on locomotor activity and/or sensory processing during acquisition. This dissociation provides compelling evidence that Arc translation is essential for memory formation of trace and contextual, but not delay, fear conditioning.

Experiment 4. The effect of NMDA receptor antagonism on the learning-induced increase in Arc protein

We have previously shown that 1) blocking NMDA receptors in dorsal or ventral hippocampus before conditioning attenuates the acquisition of trace fear conditioning and 2) there is a learning-induced increase in Arc protein, and 3) this learning-induced enhancement of Arc is blocked by administration of Arc antisense oligodeoxynucleotides. *In vitro,* the induction of Arc is tightly coupled to the induction of LTP and is NMDA-receptor dependent (Lyford et al., 1995; Link et al., 1995). However, to our knowledge, there has been no study investigating if the induction of Arc is NMDA receptor-dependent *in vivo.* Therefore, in the current study we infused APV into dorsal or ventral hippocampus before trace fear conditioning and subsequently performed western blots to measure Arc protein levels.

Methods

Subjects. 16 male Sprague Dawley rats served as subjects in the present experiment. For subjects receiving APV or aCSF infusions into dorsal hippocampus, sample sizes were: APV (n=8) and aCSF (n=8). For subjects

receiving APV or aCSF infusions into ventral hippocampus, sample sizes were: APV (n=8) and aCSF (n=8).

Procedure. Surgical procedures and APV infusions were identical to those described in Experiment 1. Subjects received infusions of APV (10ug/ul) into dorsal or ventral hippocampus 6 min prior to trace fear conditioning (the same paradigm used in Experiments 1 and 2). Subjects were sacrificed one hour after conditioning. The brain was removed and dissected into dorsal and ventral hippocampus. Then, western blots were performed on the tissue, identical to the procedure described in Experiment 2.

Results

A representative blot showing Arc and actin bands is depicted in Figure 26 for subjects with cannula in dorsal (Figure 26a) or ventral (Figure 26b) hippocampus. The final subject numbers were the same for infusions into dorsal or ventral hippocampus: APV (n=8) and aCSF (n=8). We measured Arc protein levels in both dorsal and ventral hippocampus, regardless of which brain region the cannula was implanted. The mean (±SEM) percent increase in Arc protein expression compared to actin and relative to home cage controls is shown in Figure 27. For subjects who received infusions of APV or aCSF into dorsal hippocampus before trace fear conditioning, a two-way ANOVA with brain region and infusion condition as the main factors revealed there was a significant main effect for brain region, $F(1,31) = 38.9.0$, $p < 0.0001$, and for infusion condition, $F(1,31) = 39.7$, $p < 0.0001$. There was also a significant interaction between brain region and infusion

condition, $F(1,31) = 18.9$, $p = 0.0002$. Subsequent post hoc analyses revealed that subjects who received pre-training APV infusions into dorsal hippocampus expressed significantly less Arc protein levels than all other groups ($p < 0.05$) (Figure 27a).

For subjects who received infusions of APV or aCSF into ventral hippocampus before trace fear conditioning, a two-way ANOVA with brain region and infusion condition as the main factors revealed there was a significant main effect for brain region, $F(1,31) = 20.9$, $p < 0.0001$ and for infusion condition, $F(1,31) = 36.2$, $p = < 0.0001$. There was also a significant interaction between brain region and infusion condition, $F(1,31) = 33.2$, $p < 0.0001$. Subsequent post hoc analyses revealed that subjects who received pre-training APV infusions into ventral hippocampus expressed significantly less Arc protein levels than all other groups ($p < 0.05$) (Figure 27b).

Summary and discussion

Western blots revealed significantly less Arc protein in subjects that received APV infusions into dorsal or ventral hippocampus compared to subjects that received aCSF infusions. Thus, NMDA receptor antagonism blocks the learning-induced increase of Arc protein in the hippocampus. Although the induction of Arc has been shown to be NMDA-receptor dependent *in vitro* (Lyford et al., 1995; Link et al., 1005), this is the first study to our knowledge to demonstrate that this is also the case *in vivo*.

We observed parallel effects of NMDA receptor antagonism and Arc antisense oligodeoxynucleotide infusions on Arc protein levels after trace fear conditioning. However it is difficult to determine if the knockdown of Arc protein expression was because of blocking NMDA-receptor activation *per se* or because learning was blocked in these subjects. In an attempt to dissociate the induction of Arc from neural activity vs. plasticity, Fletcher et al. (2006) observed that lesions of the fornix prevented the behavioral induction of Arc mRNA while leaving Arc transcription intact after potentiation of the medial perforant pathway. This suggests that Arc transcription is specific to learning and not just cellular activity. In Experiment 1 we demonstrated that NMDA receptor antagonism blocks learning. In experiment 2 we demonstrated that this same type of learning enhances Arc protein expression, and that blocking Arc translation impairs learning. Therefore, the reduction in Arc protein after NMDA receptor antagonism is likely due to a blockade of NMDA receptor-dependent learning. Collectively, these data support the notion that these processes are tightly coupled and likely interdependent.

GENERAL DISCUSSION

Parallel effects of NMDA receptor antagonism and blockade of Arc translation on trace and contextual fear conditioning

In the current studies we observed parallel effects of NMDA receptor antagonism and blocking Arc translation on learning. Specifically, pre-training infusions of APV or Arc antisense oligodeoxynucleotides into dorsal or ventral hippocampus impaired the conditioned fear response during testing of both the CS-US and context-US associations. This suggests that NMDA receptor activation and Arc expression may be interdependent processes working in tandem to support experience-dependent alterations in synaptic strengths that underlie long term memory formation. Consistent with this notion, activity-dependent Arc expression is blocked by NMDA receptor antagonism *in vitro* (Link et al., 1995; Lyford et al., 1995; Steward et al., 1998). In the present study we observed that a learning-induced increase in Arc expression is also blocked by NMDA receptor antagonism *in vivo.* Together these studies strongly suggest that the induction of Arc is NMDA-receptor dependent.

One characteristic of Arc is that it is quickly transported (300uM/hour) to dendrites where it undergoes local translation (Link et al., 1995; Lyford et al., 1995). A remarkable feature is that Arc accumulates at recently activated synapses (Steward et al., 1998; Steward & Worley, 2001). This is important because if changes in gene expression play a prominent role in mediating changes in synaptic strength that may underlie the formation of

specific memories, activation of these genes should selectively affect specific synapses. Steward & Worley (2001) observed *in vitro* that in the presence of an NMDA receptor antagonist (APV or MK-801), newly synthesized Arc mRNA was transported to the dendrites but was diffusely distributed. Thus, the docking of Arc mRNA to recently activated synapses appears to be dependent on NMDA receptor activation, while the transport of Arc mRNA from the cell body to the dendrites does not. Therefore NMDA receptor activation appears to play a prominent role in both the induction of Arc mRNA and its selective accumulation near recently activated synaptic sites. Thus it is likely that the learning-induced enhancement in Arc observed in the present study was mediated in part by NMDA receptor activation.

Learning: What's Arc got to do with it?

The main finding from the current set of studies indicates that NMDA receptor-mediated expression of Arc in dorsal and ventral hippocampus is critical for trace and contextual fear conditioning. Converging data support that notion that Arc is important for forming memories in a variety of types of learning (Guzowski et al., 2001; McIntyre et al., 2005; Ploski et al., 2008). What is not clear, however, is the precise role that Arc plays in learning. It is widely thought that memory acquisition and retention are due to long-lasting changes in synaptic strength and that these alterations are in part mediated by changes in gene expression. However, there is a question of how the activation of a set of synapses leads to protein synthesis-dependent changes of those synapses when the effector genes for those proteins are expressed

in the nucleus. While there are numerous immediate early genes implicated in memory processes, Arc is of particular interest because it is activity-dependent and is rapidly transported to recently activated synapses (Steward et al., 1998; Steward & Worley, 2001). But does Arc play a functional role in processes underlying memory formation and retention or is it merely a marker of synaptic plasticity or neuronal activity?

Arc has been implicated in LTP, LTD, and homeostatic plasticity (see Bramham et al., 2010 for review). Specifically, the maintenance and consolidation of LTP requires sustained Arc expression (Guzowski et al., 2001; Messaoudi et al., 2007), while the induction of LTD is attenuated in Arc knockout mice (Plath et al., 2006). There is also evidence suggesting that increases in Arc expression modulates the expansion of the F-actin network in dendritic spines which is important for the enlargement of the synapse (Messaoudi et al., 2007). This indicates that one way in which Arc may contribute to modifying synaptic strengths is through effects on post-synaptic density.

Arc is also involved in AMPA receptor endocytosis (Rial Verde et al., 2006; Chowdhury et al., 2006; Waung et al., 2008). This indicates that Arc may play a role in homeostatic scaling, in which a reduction of the expression AMPA receptors leads to a weakening of excitatory signaling without changing the relative strengths of the inputs (Turrigiano, 2008). AMPA receptors also downregulate Arc expression, suggesting a negative feedback loop at the level of Arc induction. This suggests that Arc could potentially act

to preserve the stability of activity- dependent changes in synaptic strengths and prevent hyperexcitability.

A speculative hypothesis about how Arc function may lead to bidirectional effects on synaptic efficacy is depicted in Figure 28. The induction of Arc is mediated by NMDA receptor activation and varying downstream signaling pathways including ERK, PKA, and PKC. Arc is then transported and subsequently docked at recently activated zones in dendritic synapses where it undergoes local protein synthesis. At this point Arc can then result in the enhancement of synaptic efficacy (e.g., LTP) by its effects on translational regulation of other IEGs, expansion of the post-synaptic density, or enlargement of dendritic spines. Conversely, Arc may also result in a reduction of synaptic efficacy (e.g., LTD) through AMPA receptor endocytosis. This dynamic ability of Arc, along with the fact that its expression is tightly regulated, suggests that Arc could potentially be involved in altering and refining synaptic strengths within a network to maintain and update existing information while simultaneously allowing for the capacity to form new memories.

The precise function of Arc may depend on the timing, location, and amount of Arc protein synthesis induced by neuronal activity. There is a rapid degradation of Arc mRNA and protein suggesting that these factors are critical for Arc functioning (Giorgi et al., 2007). It is possible that in some instances Arc contributes to synapse specific alterations of synaptic efficacy and other processes that enhance the excitability of specific synapses while

at other times acting as a feedback mechanism to prevent saturation of synaptic enhancement or reducing overall excitability via AMPA receptor endocytosis. What is not known, however, is how Arc alternates between regulating these excitatory and inhibitory processes. The amount and duration of NMDA receptor activation may be an important factor in determining the specific function of Arc at the cellular level. Perhaps rapid and transient increases in Arc result in excitatory signaling while prolonged increases lead to more inhibitory effects. It is also possible that sustained increases in Arc may instead lead to continued excitatory activation of synapses. At the behavioral level, perhaps there is a robust sustained increase in Arc during conditioning that supports memory formation. Conversely, repeated exposure to stimuli, such as the training context or CS used in the current paradigm, may result in a different pattern and amount of Arc expression that leads to AMPA receptor endocytosis and, in turn, a reduction of synaptic efficacy. Although our data do not address this question, differential activation and amount of Arc expression may underlie various components of learning, such as acquisition, maintenance, retrieval and extinction. In addition to the timing and amount of Arc, the location of Arc expression may be meaningful. For instance, repeated behavioral exploration of an environment enhances Arc transcription in the same discrete set of hippocampal neurons, while exposure to two completely different environments induces a partly nonoverlapping neuronal set of synapses (Vazdarjanova & Guzowski, 2004). This suggests that experience-dependent Arc expression may play a prominent role in processes accounting

for retrieval, and possibly habituation or extinction. Although it is difficult to dissociate and test experimentally, it is possible that Arc activation in the same, different, or partially overlapping set/population of synapses in the hippocampus mediates both trace and contextual fear conditioning. An increase in Arc expression in partially overlapping population of neurons may help explain how various aspects of an event, such as the place and timing, are formed during learning to create a representation of a unified yet complex memory.

One way in which the differential effects of Arc on synaptic signaling may be reconciled is by the effect of Arc on dendritic spine density. Arc significantly changes spine density and regulates spine morphology (Peebles et al., 2010). Specifically, Arc increases spine density and the proportion of thin spines, which are often referred to as "learning spines" because they are likely to change shape in response to activity. Interestingly, this increase in spine density is tightly coupled to Arc's role in AMPA receptor endocytosis (Peebles et al., 2010). Therefore Arc might lead to a reduction synaptic strength by decreasing AMPA receptors while at the same time increasing structural plasticity by increasing the proportion of thin spines. It is intriguing to speculate that this may be a mechanism by which Arc contributes to both homeostatic and Hebbian plasticity. However, much of the research on the function of Arc has been conducted *in vitro,* so it is unclear if Arc behaves the same way *in vivo.* Converging evidence suggests that the regulation and dynamic function of Arc is important for synaptic

plasticity and learning but the precise mechanisms through which this may occur remain to be elucidated.

NMDA receptor antagonism and blockade of Arc protein translation in both dorsal and ventral hippocampus impair contextual and trace fear conditioning: an argument against a functional dissociation?

Pre-training NMDA receptor antagonism and blockade of Arc protein translation produced similar learning deficits regardless of what hippocampal subregion was manipulated, suggesting that dorsal and ventral hippocampus play a similar role in these forms of learning. This is in contrast to numerous reports of a functional dissociation between dorsal and ventral hippocampus (Moser & Moser, 1998; Bannerman et al., 1999; Richmond et al., 1999; Pitkanen et al., 2000; Yoon & Otto, 2007; Czerniawski et al., 2009). While we have previously demonstrated that there is a clear dissociation in that the integrity of ventral, but not dorsal hippocampus, is necessary for trace fear conditioning (Yoon & Otto, 2007; Czerniawski et al., 2009), data from the present study indicate that both subregions are normally involved in forming associations in this paradigm. Specifically, NMDA receptor activation and Arc protein translation play an integral role in both dorsal and ventral hippocampus in the acquisition of both trace and contextual fear conditioning.

Both dorsal and ventral hippocampus appear to be important for learning the "where" or "when" of a stimulus or event. Thus, while dorsal and ventral hippocampus may be differentially involved in various paradigms,

this does not necessarily mean that they are completely functionally dissociable from a conceptual standpoint. Different types of learning that are deemed to be dependent on the hippocampus typically involve forming relationships between temporally or spatially diffuse stimuli that can be used to influence behavior. For example, in spatial learning paradigms that typically depend on dorsal hippocampus, it is essential to form a unified representation of the various stimuli that comprise the place. In addition, anxiety-related behaviors that tend to depend on the integrity of ventral hippocampus also include forming relationships between stimuli, including motivational state. There is a common thread of reducing ambiguity in the variety of paradigms that appear to be differentially mediated by dorsal or ventral hippocampus. Thus, it is likely that dorsal and ventral hippocampus exhibit similar processing of information but are sometimes preferentially recruited for different types of learning based on their differing anatomical connections. It is possible that, while not necessary, both subregions are typically engaged in the same types of learning as a potential 'backup' mechanism. For instance, we theorize that dorsal hippocampus normally participates in the acquisition of trace and contextual fear conditioning and that there is a compensatory mechanism if it is not intact during learning, as is the case with lesions and inactivation. Perhaps that mechanism entails a compensatory upregulation of Arc or other genes in ventral hippocampus. We did not observe a greater amount of Arc expression in ventral hippocampus after APV or Arc antisense infusions into dorsal hippocampus compared to vehicle controls. However, it is not inconceivable to speculate

that there may be an even greater enhancement in Arc gene and protein expression in ventral hippocampus after lesions or inactivation of dorsal hippocampus. Future studies will be aimed at investigating the potentially dynamic interaction between these subregions involved in memory formation.

Do contextual and trace fear conditioning tap a common underlying hippocampal function?

The fact that both NMDA-receptor antagonism and blockade of Arc protein translation impair both contextual and trace fear conditioning indicates that learning and memory in these paradigms may engage the hippocampus in a similar fashion and depend upon a common underlying chemistry. Why is the hippocampus important for contextual and trace conditioning but not delay conditioning? One possibility is that learning associations in trace and contextual conditioning is more difficult than in delay conditioning. However this is unlikely because all three of these forms of associative learning can be learned in a single trial (Misane et al., 2005). Another possible explanation involves an important distinction between trace and delay conditioning. Unlike in delay conditioning, the CS and US are discontiguous in trace conditioning (Bangasser et al., 2006). Thus, during the trace interval, the hippocampus may maintain a memory 'trace' of the CS that can later be associated with the US. It is important to point out that the training context may be used during the trace interval to bridge the CS with the US because it is temporally diffuse and contiguous with both stimuli. In

fact, during the trace interval the only cues present are the static multimodal cues that comprise the context. Therefore, in both contextual and trace conditioning, the hippocampus may serve to integrate temporally and/or spatially diffuse multi-modal information (Wallenstein et al., 1998). Although traditional context learning may depend more on spatial processing and trace conditioning more on temporal processing, the hippocampus may play a general role in putting together the "what", "where", and "when" of an event (Ergorul & Eichenbaum, 2004). Manipulations of dorsal hippocampus reliably disrupt the acquisition of discriminative contextual conditioning (Frankland et al., 1998; Anagnostaras et al., 1999, 2001; Antoniadis & McDonald, 2000). Trace conditioning requires the ability to discriminate between the trace interval and intertrial interval. Thus, the hippocampus may be important for forming configural multi-modal representations that enable animals to better learn about, and discriminate between, places and events.

Summary and Conclusion

We have previously shown that the integrity of ventral, but not dorsal, hippocampus is necessary for the acquisition of trace fear conditioning. Now, however, we demonstrate that NMDA receptor activation in both dorsal and ventral hippocampus is critical for the acquisition of trace and contextual fear conditioning. In addition, trace fear conditioning induces both Arc gene and protein expression in both of these hippocampal subregions. Moreover, blocking Arc translation blocks both the learning-induced enhancement of Arc

protein in both dorsal and ventral hippocampus and memory formation in trace and contextual fear conditioning. Meanwhile, blocking Arc translation in ventral hippocampus does not affect the acquisition or consolidation of delay fear conditioning. The parallel effects of NMDA receptor antagonism and blockade of Arc translation suggest that NMDA receptor-dependent plasticity and Arc protein translation participate critically in the acquisition and maintenance of trace and contextual fear conditioning in the hippocampus. Given that NMDA receptor antagonism attenuates the learning-dependent increase in Arc translation, these processes appear to be tightly coupled and likely interdependent.

REFERENCES

Anagnostaras SF, Maren S, Fanselow MS. 1999. Temporally graded retrograde amnesia of contextual fear after hippocampal damage in rats: within subjects examination. Journal of Neuroscience 19: 1106-1114.

Antoniadis EA, McDonald RJ. 2000. Amygdala, hippocampus and discriminative fear conditioning to context. Behavioral Brain Research 108: 1-19.

Bangasser DA, Waxler DE, Santollo J, Shors TJ. 2006. Trace conditioning and the hippocampus: the importance of contiguity. Journal of Neuroscience 26(34): 8702-8706.

Bannerman DM, Rawlins, JNP, McHugh SB, Deacon RMJ, Yee BK, Bast T, Zhang WN, Pothuizen HHJ, Feldon J. 2004. Regional dissociations within the hippocampus – memory and anxiety. Neuroscience and Behavioral Reviews 28:273-283.

Bannerman DM, Grubb M, Deacon RMJ, Yee BK, Feldon J, Rawlins JNP. 2003. Ventral hippocampal lesions affect anxiety but not spatial learning. Behavioral Brain Research 139: 197-213.

Bannerman DM, Deacon RMJ, Offen S, Friswell J, Grubb M, Rawlins JNP. 2002. Double dissociation of function within the hippocampus: spatial memory and hyponeophagia. Behavioral Neuroscience 116(5): 884-901.

Bannerman DM, Yee BK, Good MA, Heupe, MJ, Iversan SD, & Rawlins, JNP. 1999. Double dissociation of function within the hippocampus: a comparison of dorsal, ventral, and complete hippocampal cytotoxic lesions. Behavioral Neuroscience 113(6):1170-1188.

Bast T, Zhang WN, Feldon J. 2003. Dorsal hippocampus and classical fear conditioning to tone and context in rats: effects of local NMDA-receptor blockade and stimulation. Hippocampus 13: 657-675.

Bast T, Zhang WN, Feldon J. 2001b. The ventral hippocampus and fear conditioning in rats: Different anterograde amnesias of fear after tetrodotoxin inactivation and infusion of the GABAa agonist muscimol. Experimental Brain Research 139:39-52.

Bertoglio LJ, Lourenco Joca SR, Guimaraes FS. 2006. Further evidence that anxiety and memory are regionally dissociated within in the hippocampus. Behavioral Brain Research 175: 183-188.

Broadbent NJ, Squire LR, Clarke RE. 2004. Spatial memory, recognition memory, and the hippocampus. Proc Natl Acad Arts and Sciences 101(40): 14515-14520.

Burman MA, Starr MJ, Gewirtz JC. 2006. Dissociable effects of hippocampus lesions on expression of fear and trace fear conditioning memories in rats. Hippocampus 16:103-113.

Burwell RD, Amaral DG. 1998. Cortical afferents of the perirhinal, postrhinal, and entorhinal cortices of the rat. Journal of Comparative Neurology 398: 179-205.

Canteras NS, Swanson LW. 1992. Projections of ventral subiculum to the amygdala, septum and hypothalamus: a PHAL anterograde tract-tracing study in the rat. Journal of Comparative Neurology 324: 180-194.

Chowdhury S, Shepherd JD, Okuno H, Lyford G, Petralia RS, Plath N, Kuhl D, Huganir RL, Worley PF. 2006. Arc/Arg3.1 interacts with the endocytic machinery to regulate AMPA receptor trafficking. Neuron 52(3): 445-459.

Chowdhury N., Quinn JJ, Fanselow MS. 2005. Dorsal hippocampus involvement in trace fear conditioning with long, but not short, trace intervals in mice. Behavioral Neuroscience 119(5):1396-1402.

Czerniawski J, Yoon T, Otto T. 2009. Dissociating space and trace in dorsal and ventral hippocampus. Hippocampus 19: 20-32.

Daumas S, Halley H, Frances B, Lassalle JM. 2006. Encoding, consolidation, and retrieval of contextual memory: differential involvement of dorsal CA3 and CA1 hippocampal subregions. Learning and Memory 12: 375-382.

Dolorfo CL, Amaral DG. 1998. Entorhinal cortex of the rat: topographic organization of the cells of origin of the perforant path projection to the dentate gyrus. Journal of Comparative Neurology 398: 25-48.

Ergorul C, Eichenbaum H. The hippocampus and memory for "what", "where", and "when". Learning and Memory 11: 397-405.

Esclassan F, Coutureau E, Di Scala G, Marchand AR. 2009. Differential contribution of dorsal and ventral hippocampus to trace and delay fear conditioning. Hippocampus 19: 33-44.

Fanselow MS. 1980. Conditional and unconditional components of postshock freezing. Pavlovian Journal of Biological Sciences 15: 177-182.

Ferbinteanu J, McDonald RJ. 2001. Dorsal/ventral hippocampus, fornix, and conditioned place preference. Hippocampus 11:187-200.

Fletcher BR, Calhoun ME, Rapp PR, Shapiro ML. 2006. Fornix lesions decouple the induction of hippocampal Arc transcription from behavior but not plasticity. Journal of Neuroscience 26(5): 1507-1515.

Frankland PW, Cestari V, Filipkowski RK, McDonald RJ, Silva AJ. The dorsal hippocampus is essential for context discrimination but not for contextual conditioning. Behavioral Neuroscience 112(4): 863-874.

Gilmartin MR, McEchron MD. 2005. Single neurons in the dentate gyrus and CA1 of the hippocampus exhibit inverse patterns of encoding during trace fear conditioning. Behavioral Neuroscience 119: 164-179.

Giorgi C, Yeo GW, Stone ME, Katz DB, Burge C, Turrigiano G, Moore MJ. 2007. The EJC factor eIF4AIII modulates synaptic strength and neuronal protein expression. Cell 130(1): 179-191.

Good M, Honey RC. 1997. Dissociable effects of selective lesions to hippocampal subsystems on exploratory behavior, context learning, and spatial learning. Behavioral Neuroscience 111(3):487-493.

Groenewegen HJ, Vermeulen-Van der Zee E, te Kortschot A, Witter MP. 1987. Organization of the projections from the subiculum to the ventral striatum in the rat. A study using anterograde transport of *Phaseolus vulgaris* leucoagglutinin. Neuroscience 23: 103-120.

Guzowski JF. 2002. Insights into immediate-early gene function in hippocampal memory consolidation using antisense oligonucleotide and fluorescent imaging approaches. Hippocampus 12: 86-104.

Guzowski JF, Setlow B, Wagner EK, McGaugh JL. 2001. Experience-dependent gene expression in the rat hippocampus following spatial learning: a comparison of the immediate early genes *Arc, c-fos,* and *zif268.* Journal of Neuroscience 21:5089-5098.

Guzowski JF, Lyford GL, Stevenson GD, Houston FP, McGaugh JL, Worley PF, Barnes CA. 2000. Inhibition of activity-dependent Arc protein expression in the rat hippocampus impairs the maintenance of long-term potentiation and consolidation of long-term memory. Journal of Neuroscience 20: 3993-4001.

Guzowski JF, McNaughton BL, Barnes CA, Worley PF. 1999. Environment-specific expression of the immediate-early gene Arc in hippocampal neuronal ensembles. Nature Neuroscience 2: 1120-1124.

Huff NC, Frank M, Wright-Hardesty K, Sprunger D, Matus-Amat, Higgins E, Rudy JW. 2006. Amygdala regulation of immediate-early-gene expression in the hippocampus induced by contextual fear conditioning. Journal of Neuroscience 26(5): 1616-1623.

Hunsaker MR, Kesner RP. 2008. Dissociations across the dorsal-ventral axis of CA3 and CA1 for encoding and retrieval of contextual and auditory-cued fear. Neurobiology of Learning and Memory 89: 61-69.

Ishikawa A, Nakamura S. 2006. Ventral hippocampal neurons project axons simultaneously to the medial prefrontal cortex and amygdala in the rat. Journal of Neurphysiology 96: 2134-2138.

Jung MW, Wiener SI, McNaughton BL. 1994. Comparison of spatial firing characteristics of units in dorsal and ventral hippocampus of the rat. Journal of Neuroscience 14(12): 7347-7356.

Kelly MP, Deadwyler SA. 2002. Acquisition of a novel behavior induces higher levels of Arc mRNA than does overtrained performance. Neuroscience 110:617-626.

Kim JJ, Jung MW. 2006. Neural circuits and mechanisms involved in Pavlovian fear conditioning: a critical review. Neuroscience and Biobehavioral Reviews 30(2): 188-202.

Kim JJ, Fanselow MS. 1992. Modality-specific retrograde amnesia of fear. Science 256: 675-677.

Kjelsrup KG, Tuvnes FA, Steffenach H, Murison R, Moser E, Moser M. 2002. Reduced fear expression after lesions of the ventral hippocampus. Proc Natl Acad Arts and Sciences 99: 10825-10830.

Lee I, Kesner RP. 2004. Differential contributions of dorsal hippocampal subregions to memory acquisition and retrieval in contextual fear-conditioning. Hippocampus 14: 301-310.

Link W, Konietzko U, Kauselmann G, Krug M, Schwanke B, Frey U, Kuhl D. 1995. Somatodendritic expression of an immediate early gene is regulated by synaptic activity. Proc Natl Acad Arts and Sciences 92: 5734-5738.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C}_{T}$ method. Methods 25: 402-408.

Lyford GL, Yamagata K, Kaufman WE, Barnes CA, Sanders LK, Copeland NG, Gilbert DJ, Jenkins NA, Lanahan AA, Worley PF. 1995. Arc, a growth factor and activity-regulated gene, encodes a novel cytoskeleton-associated protein that is enriched in neuronal dendrites. Neuron 14: 433-445.

Mao J, Robinson JK. 1998. Microinjection of GABA-A agonist muscimol into the dorsal but not the ventral hippocampus impairs non-mnemonic measures of delayed non-matching-to-position performance in rats. Brain Research 784:139-147.

Maren S, Holt WG. 2004. Hippocampus and Pavlovian fear conditioning in rats: muscimol infusions into the ventral but not dorsal, hippocampus impair the acquisition of conditioned freezing to an auditory conditioned stimulus. Behavioral Neuroscience 118: 97-110.

Maren S, Aharonov G, Fanselow MS. 1997. Neurotoxic lesions of the dorsal hippocampus and Pavlovian fear conditioning in rats. Behavioral Brain Research 88: 261-274.

Maren S, Aharonov G, Fanselow MS. 1996. Retrograde abolition of conditioned fear after excitotoxic lesions in the basolateral amygdala of rats: absence of a temporal gradient. Behavioral Neuroscience 15: 7548-7564.

Matus-Amat P, Higgins EA, Barrientos RM, Rudy JW. 2004. The role of the dorsal hippocampus in the acquisition and retrieval of context memory representations. Journal of Neuroscience 24(10): 2431-2439.

McEchron MD, Bouwmeester H, Tseng W, Weiss C, Disterhoft JF. 1998. Hippocampectomy disrupts auditory trace fear conditioning in the rat. Hippocampus 8:638-646.

McHugh SB, Campbell TG, Taylor AM, Rawlins JNP, Bannerman DM. 2008. A role for dorsal and ventral hippocampus in inter-temporal choice cost-benefit decision making. Behavioral Neuroscience 122: 1-8.

McHugh SB, Deacon RMJ, Rawlins JNP, Bannerman DM. 2004. Amygdala and ventral hippocampus contribute differentially to mechanisms of fear and anxiety. Behavioral Neuroscience 118(1): 63-78.

McIntyre CK, Miyashita T, Setlow B, Marjon KD, Steward O, Guzowski JF, McGaugh JL. 2005. Memory-influencing intra-basolateral amygdala drug infusions modulate expression of Arc protein in the hippocampus. Proc Natl Acad Arts and Sciences 102(30): 10718-10723.

Messaoudi E. Kanhema T, Soule J, Tiron A, Dagyte G, da Silva B, Bramham CR. 2007. Sustained Arc/Arg3.1 synthesis controls long-term potentiation consolidation through regulation of local actin polymerization in the dentate gyrus in vivo. Journal of Neuroscience 27(39): 10445-10455.

Miettinen M, Savandar V, Pitkanen A. 1996. Projections for lateral nucleus of the amygdala to entorhinal cortex in the rat. Society for Neuroscience Abstract 22: 2050.

Misane I, Tovote P, Meyer M, Spiess J, Ogren SO, Steidl O. 2005. Time-dependent involvement of the dorsal hippocampus in trace fear conditioning in mice. Hippocampus 15:418-426.

Montag-Sallaz M, Montag D. 2003. Learning-induces Arg3.1/Arc mRNA expression in the mouse brain. Learning and Memory 10(2): 99-107.

Moser E, Moser M, Anderson P. 1993. Spatial learning impairment parallels the magnitude of dorsal hippocampal lesions, but is hardly present following ventral lesions. Journal of Neuroscience 13:3916-3925.

Moser M, Moser,EI. 1998. Functional differentiation in the hippocampus. Hippocampus 8:608-619.

Moyer JR, Deyo RA, Disterhoft JF. 1990. Hippocampectomy disrupts trace eye-blink conditioning in rabbits. Behavioral Neuroscience 104(2): 243-252.

O'Keefe J, Nadel L. 1978. The hippocampus as a cognitive map. New York: Oxford University Press.

Otto T, Poon P. 2006. Dorsal hippocampal contributions to unimodal contextual conditioning. Journal of Neuroscience 26:6603-6609.

Panja D, Dagyte G, Bidinosti MT, Kristiansen AM, Sonenberg N, Bramham CR. 2008. Translation control pathways underlying LTP maintenance in the dentate gyrus in vivo. Society for Neuroscience Abstracts 433.14.

Parsons TC, Otto T. 2008. Temporary inactivation of dorsal hippocampus attenuates explicitly nonspatial, unimodal, contextual fear conditioning. Neurobiology of Learning and Memory 90: 261-268.

Peebles CL, Yoo J, Thwin MT, Palop JJ, Noebels JL, Finkbeiner S. 2010. Arc regulates spine morphology and maintains network stability in vivo. Proc Nat Acad Arts and Sciences 107(42): 18173-18178.

Phillips RG, LeDoux JE. 1992. Differential contributions of amygdala and hippocampus to cued and contextual fear conditioning. Behavioral Neuroscience 106: 274-285.

Phillips RG, LeDoux JE. 1994. Lesions of dorsal hippocampal formation interfere with background but not foreground contextual fear conditioning. Learning and Memory 1: 34-44.

Pitkanen A, Pikkarainen M, Nurminen N, Ylinen A. 2000. Reciprocal connections between the amygdala and hippocampal formation, perirhinal cortex, and postrhinal cortex in rat. Annals of the New York Academy of Sciences 911:369-391.

Plath N, Ohana O, Dammermann B, Errington ML, Schmitz D, Gross C, Mao X, Engelsberg A, Mahika C, Welzl H, Bick-Sander A, Therstappen E, Cooke SF, Blanquet V, Wurst W, Salmen B, Bosl MR, Lipp HP, Grant SG, Bliss TV, Wolfer DP, Kuhl D. 2006. Arc/Arg3.1 is essential for the consolidation of synaptic plasticity and memories. Neuron 52(3): 437-444.

Ploski JE, Pierre VJ, Smucny J, Park K, Monsey MS, Overeem KA, Schafe GE. 2008. The activity-regulated cytoskeletal-associated protein (Arc/Arg3.1) is required for memory consolidation of Pavlovian fear conditioning in the lateral amygdala. Journal of Neuroscience 28(47): 12383-12395.

Pothuizen HH, Zhang WN, Jongen-Relo AC, Feldon J, Yee BK. 2004. Dissociation of the function between the dorsal and ventral hippocampus in

spatial leaning abilities of the rat: a within-subjects, within-task comparison of reference and working spatial memory. European Journal of Neuroscience 19:705-712.

Quinn JJ, Wied HM, Ma QD, Tinsley MR, Fanselow MS. 2008. Dorsal hippocampus involvement in delay fear conditioning depends on the strength of the tone-footshock association. Hippocampus 18: 640-654.

Quinn JJ, Oommen SS, Morrison GE, Fanselow MS. 2002. Post-training excitotoxic lesions of the dorsal hippocampus attenuate forward trace, backward trace, and delay fear conditioning in a temporally specific manner. Hippocampus 12:495-504.

Quinn JJ, Loya F, Ma QD, Fanselow MS. Dorsal hippocampus NMDA receptors differentially mediate trace and contextual fear conditioning. Hippocampus 2005;5(5):665-74.

Rial Verde EM, Lee Osbourne J, Worley PF, Malinow R, Cline HT. 2006. Increased expression of the immediate-early gene arc/arg3.1 reduces AMPA receptor-mediated synaptic transmission. Neuron 52(3): 461-474.

Richmond MA, Yee BK, Pouzet B, Veenman L, Rawlins JN, Feldon J et al. 1999. Dissociating context and space within the hippocampus. Effects of complete, dorsal, and ventral excitotoxic lesions on conditioned freezing and spatial learning. Behavioral Neuroscience 113:1189-1203.

Rogers JL, Hunsaker MR, Kesner RP. 2006. Effects of dorsal and ventral CA1 subregional lesions on trace fear conditioning. Neurobiology of Learning and Memory 8:72-81.

Rudy JW, Matus-Amat P. 2005. The ventral hippocampus supports a memory representation of context and contextual fear conditioning: implications for a unitary function of the hippocampus. Behavioral Neuroscience 119(1): 154-163.

Sakamoto T, Takatsuki K, Kawahara S, Kirino Y, Niki H, Mishina M. 2005. Role of hippocampal NMDA receptors in trace eyeblink conditioning. Bran Research 1039: 130-136.

Schenberg EE, Oliveira MGM. 2008. Effects of pre or posttraining dorsal hippocampal D-AP5 injection on fear conditioning to tone, background, foreground context. Hippocampus 18(11): 1089-1093.

Seo DO, Pang MH, Shin MS, Kim HT, Choi JS. 1998. Hippocampal NMDA receptors are necessary for auditory trace fear conditioning measured with conditioned hypoalgesia in rats. Behavorial Brain Research 192(2):264-268.

Squire LR, Zola-Morgan S. 1991. The medial temporal lobe memory system. Science 253:1380-1386.

Steward O, Worley PF. 2001. Selective targeting of newly synthesized Arc mRNA to active synapses requires NMDA receptor activation. Neuron 30:227-240.

Steward O, Wallace CS, Lyford GL, Worley PF. 1998. Synaptic activation causes the mRNA for the IEG Arc to localize selectively near activated postsynaptic sites on dendrites. Neuron 21(4): 741-751.

Trivedi MA, Coover GD. 2004. Lesions of the ventral hippocampus, but not dorsal hippocampus, impair conditioned fear expression and inhibitory avoidance on the elevated T-maze. Neurobiology of Learning and Memory 81:172-184.

Turrigiano GG. 2008. The self-tuning neuron: synaptic scaling of excitatory synapses. Cell 135(3): 422-435.

Verwer RWH, Meijer RJ, Van Uum HFM, Witter MP. 1997. Collateral projections from the rat hippocampal formation to the lateral and medial prefrontal cortex. Hippocampus 7(4): 397-402.

Wanisch K, Tang J, Mederer A, Wotjak C. 2005. Trace fear conditioning depends on NMDA receptor activation and protein synthesis within the dorsal hippocampus of mice. Behavioral Brain Research 157: 63-69.

Waung MW, Pfeiffer BE, Nosyreva ED, Ronesi JA, Huber KM. 2008. Rapid translation of Arc/Arg3.1 selectively mediates mGluR-dependent LTD through persistent increases in AMPAR endocytosis rate. Neuron 59(1): 84-97.

Wiltgen BJ, Fanselow MS (2003) A model of hippocampal-cortical-amygdala interactions based on contextual fear conditioning. In: The neurobiology of spatial behaviour (Jeffery KJ, ed), pp 83–103. New York: Oxford UP.

Yoon T, Otto T. 2007. Differential contributions of the dorsal and ventral hippocampus in rats to trace fear conditioning. Neurobiology of Learning and Memory 87:464-475.

Young SL, Bohenek DL, Fanselow MS. 1994. NMDA processes mediate anterograde amnesia of contextual fear conditioning induced by hippocampal damage: immunization against amnesia by context preexposure. Behavioral Neuroscience 108(1): 19-29.

Zhang WN, Bast T, Feldon J. 2001. The ventral hippocampus and fear conditioning in rats: different anterograde amnesias of fear after infusion of N-methyl-D-aspartate or its noncompetitive antagonist MK-801 into the ventral hippocampus. Behavioral Brain Research 126: 159-174.

Figure 1

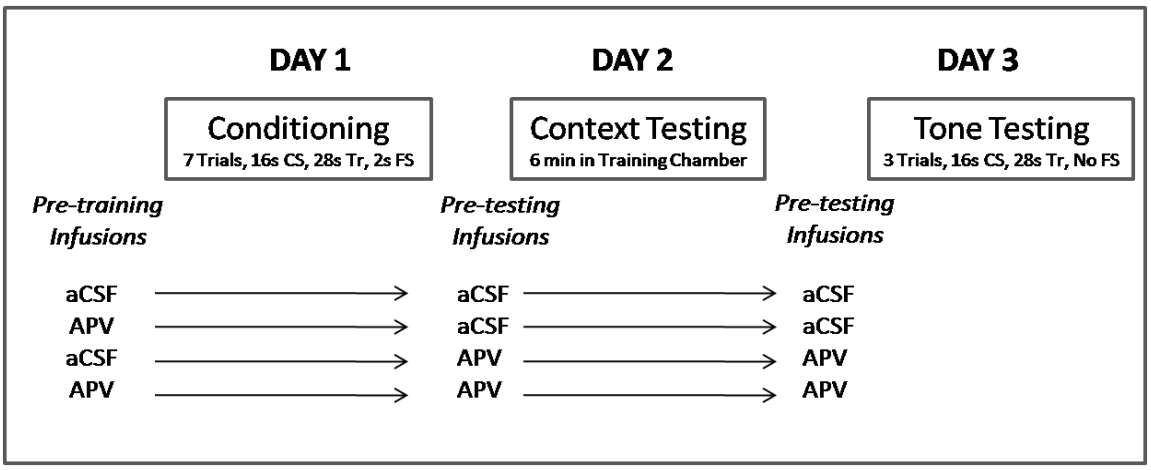
DAY 1
DAY 2
DAY 3
Conditioning
7 Trials, 16s CS, 28s Tr, 2s FS
Context Testing
6 min in Training Chamber
Tone Testing
3 Trials, 16s CS, 28s Tr, No FS
Pre-training Infusions
Pre-testing Infusions
Pre-testing Infusions
aCSF
APV
aCSF
APV
aCSF
aCSF
APV
APV
aCSF
aCSF
APV
APV

Figure 2

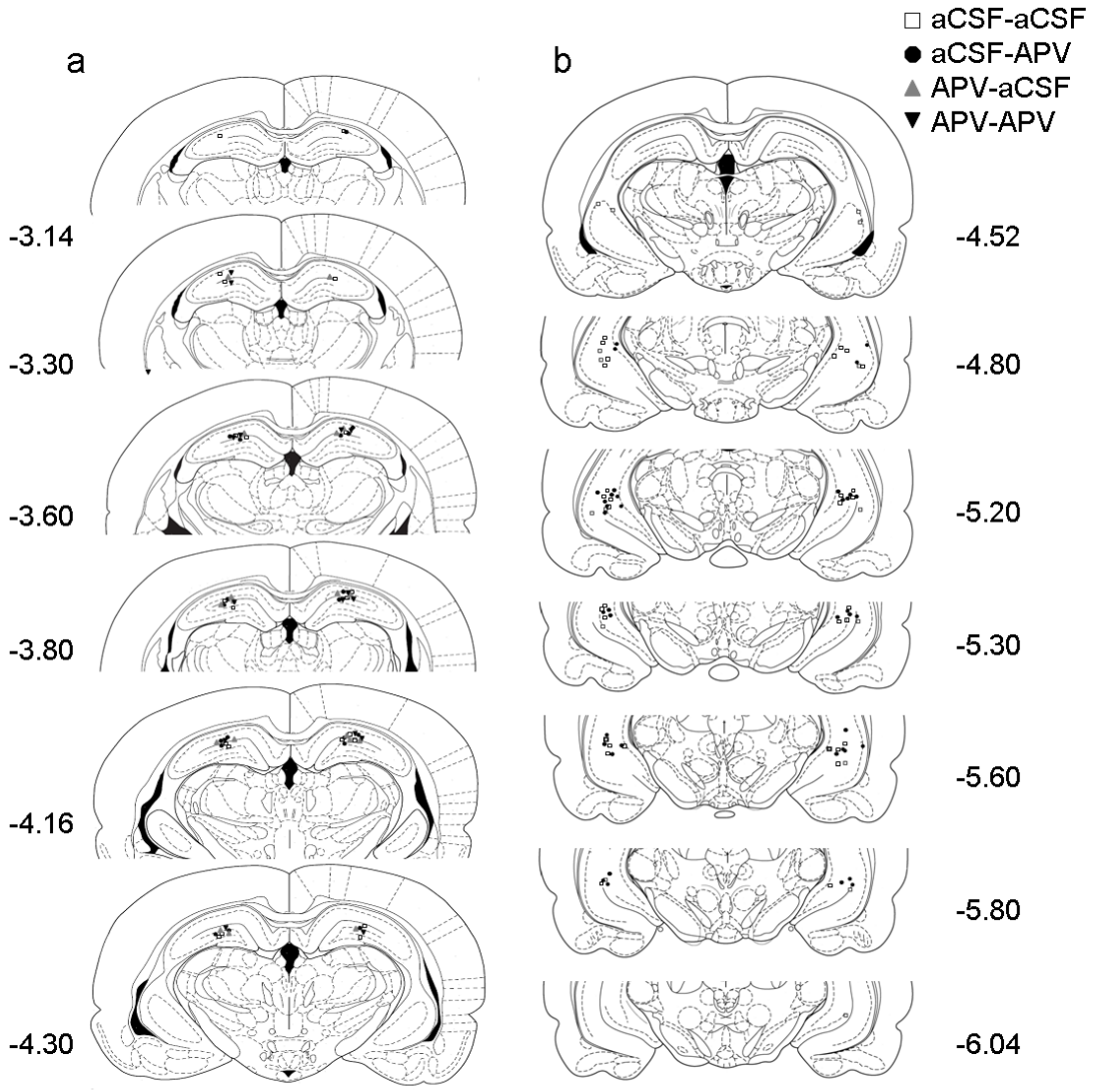

Figure 2. Schematic representation of cannula placement in coronal sections of a) dorsal or b) ventral hippocampus for Experiment 1.

Figure 3

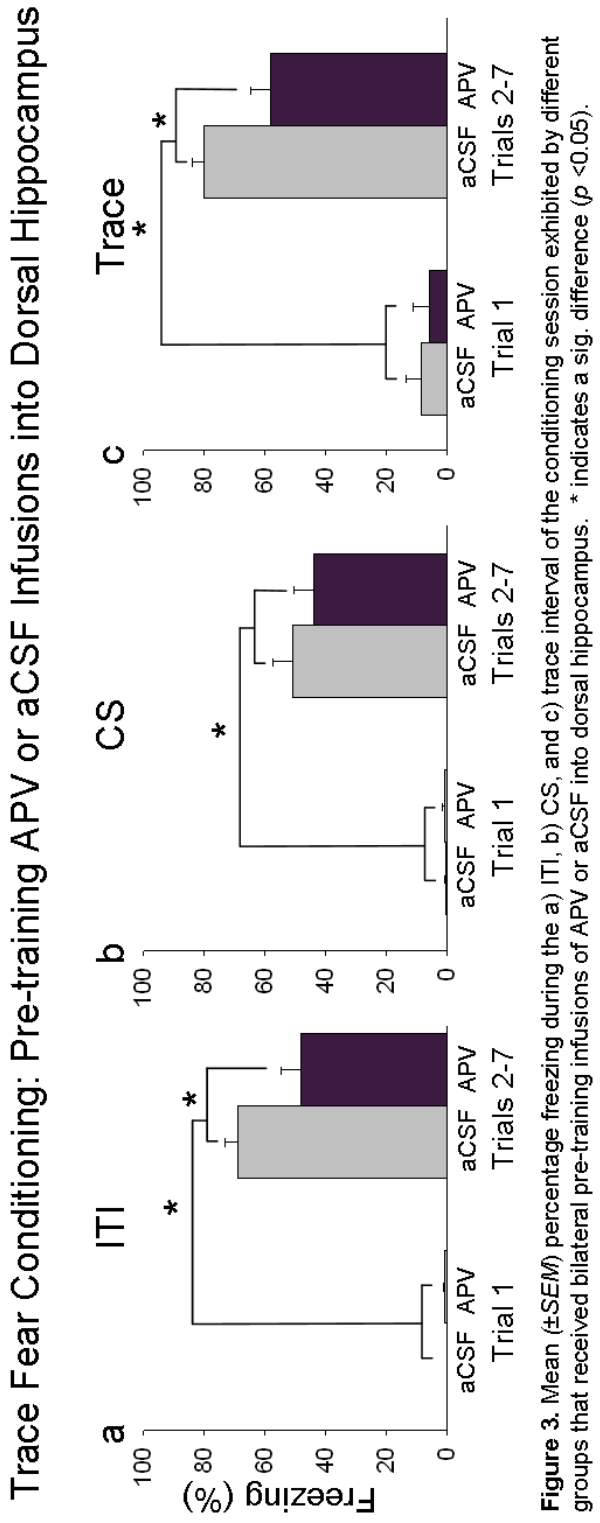

Figure 3. Mean (±*SEM*) percentage freezing during the a) ITI, b) CS, and c) trace interval of the conditioning session exhibited by different groups that received bilateral pre-training infusions of APV or aCSF into dorsal hippocampus. * indicates a sig. difference (p <0.05).

Figure 4

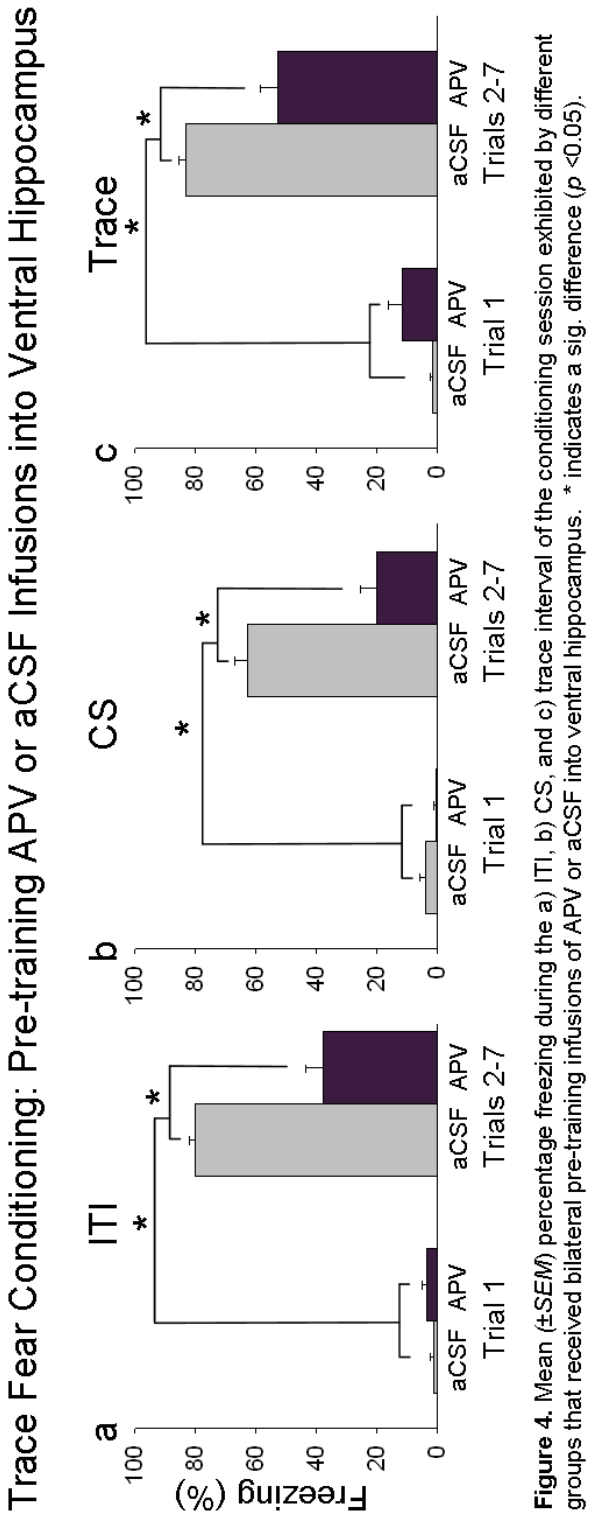

Figure 4. Mean (±*SEM*) percentage freezing during the a) ITI, b) CS, and c) trace interval of the conditioning session exhibited by different groups that received bilateral pre-training infusions of APV or aCSF into ventral hippocampus. * indicates a sig. difference (p <0.05).

Figure 5

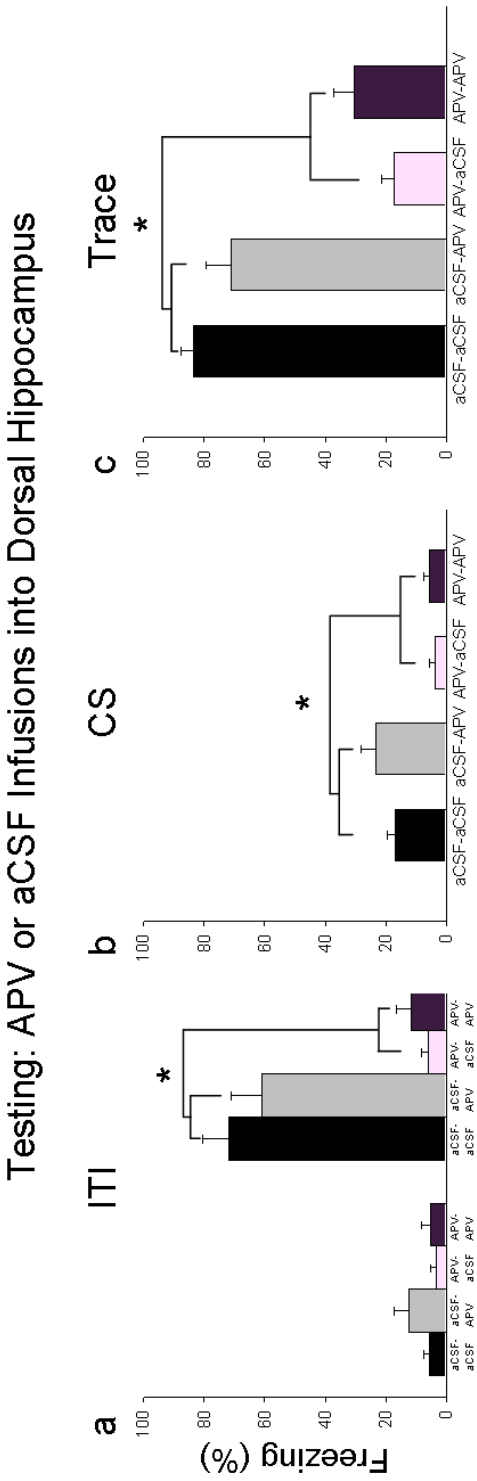

Figure 5. Mean (±*SEM*) percentage freezing during the a) ITI, b) CS, and c) trace interval of the testing session exhibited by different groups that received bilateral infusions of APV or aCSF into dorsal hippocampus. Infusions of APV prior to training or prior to both training and testing impaired conditioned freezing during the test session. *(p <0.05).

Figure 6

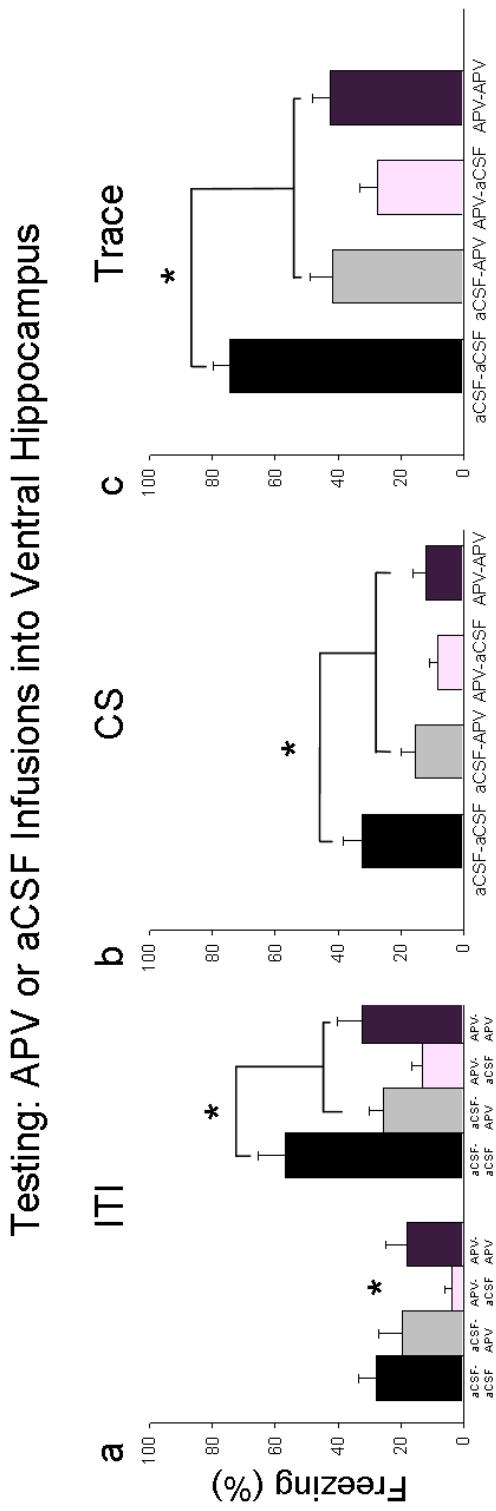

Figure 6. Mean (±*SEM*) percentage freezing during the a) ITI, b) CS, and c) trace interval of the testing session exhibited by different groups that received bilateral infusions of APV or aCSF into ventral hippocampus. Infusions of APV prior to training, testing, or prior to both training and testing impaired conditioned freezing during the test session. *(p <0.05).

Figure 7

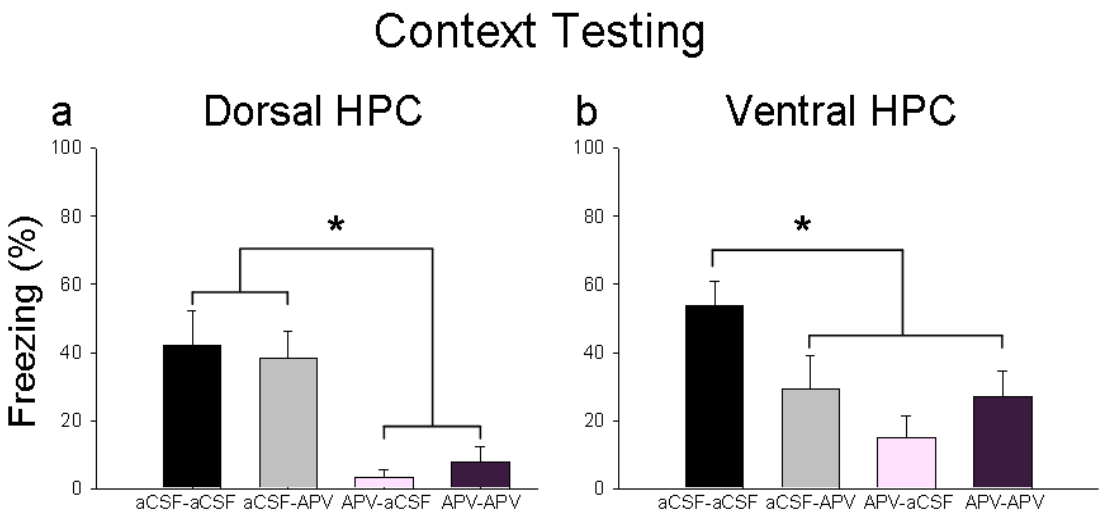

Figure 7. Mean *(±SEM)* percentage freezing during the context testing session exhibited by different groups that received bilateral infusions of APV or aCSF into a) dorsal or b) ventral hippocampus. Infusions of APV into dorsal hippocampus prior to training or prior to both training and testing impaired conditioned freezing during the context testing session. Infusions of APV into ventral hippocampus prior to training, prior to testing, or prior to both training and testing impaired conditioned freezing during the context testing session. *(p <0.05).

Figure 8

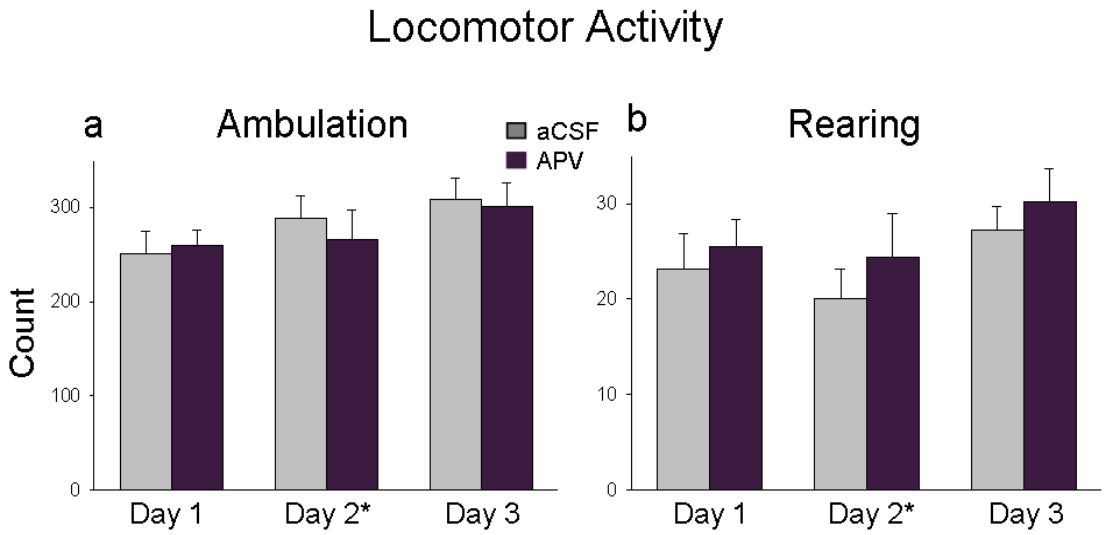

Figure 8. Mean *(±SEM)* number of a) ambulation and b) rearing counts during locomotor activity assessment for subjects that received APV or aCSF infusions into dorsal hippocampus.

Figure 9

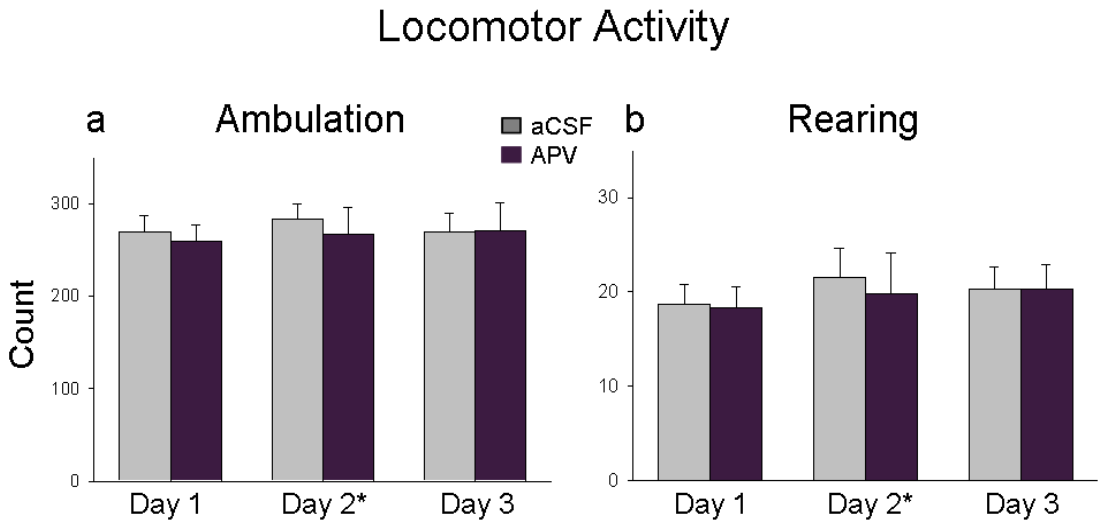

Figure 9. Mean *(±SEM)* number of a) ambulation and b) rearing counts during locomotor activity assessment for subjects that received APV or aCSF infusions into ventral hippocampus.

Figure 10

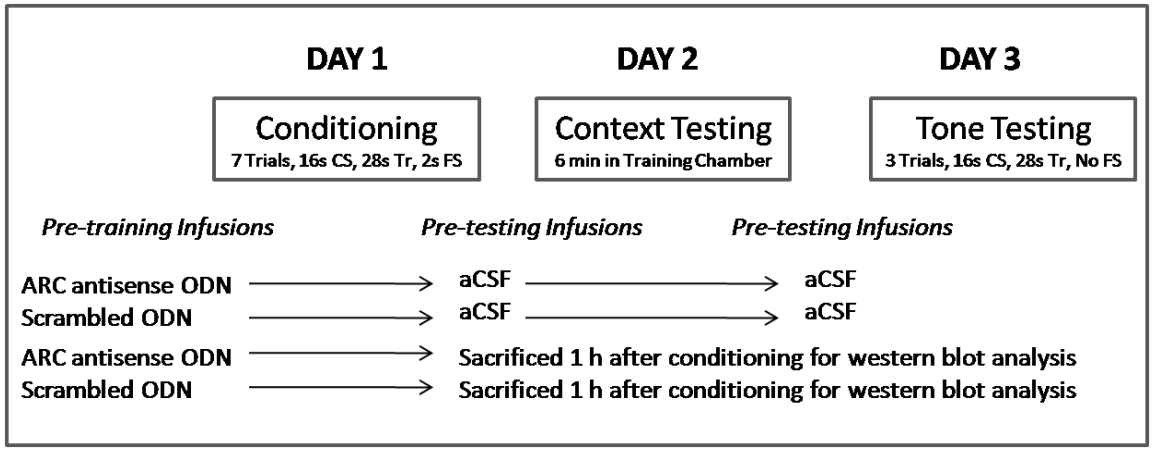
DAY 1
DAY 2
DAY 3
Conditioning
7 Trials, 16s CS, 28s Tr, 2s FS
Context Testing
6 min in Training Chamber
Tone Testing
3 Trials, 16s CS, 28s Tr, No FS
Pre-training Infusions
Pre-testing Infusions
Pre-testing Infusions
ARC antisense ODN
aCSF
aCSF
Scrambled ODN
aCSF
aCSF
ARC antisense ODN
Sacrificed 1 h after conditioning for western blot analysis
Scrambled ODN
Sacrificed 1 h after conditioning for western blot analysis

Figure 11

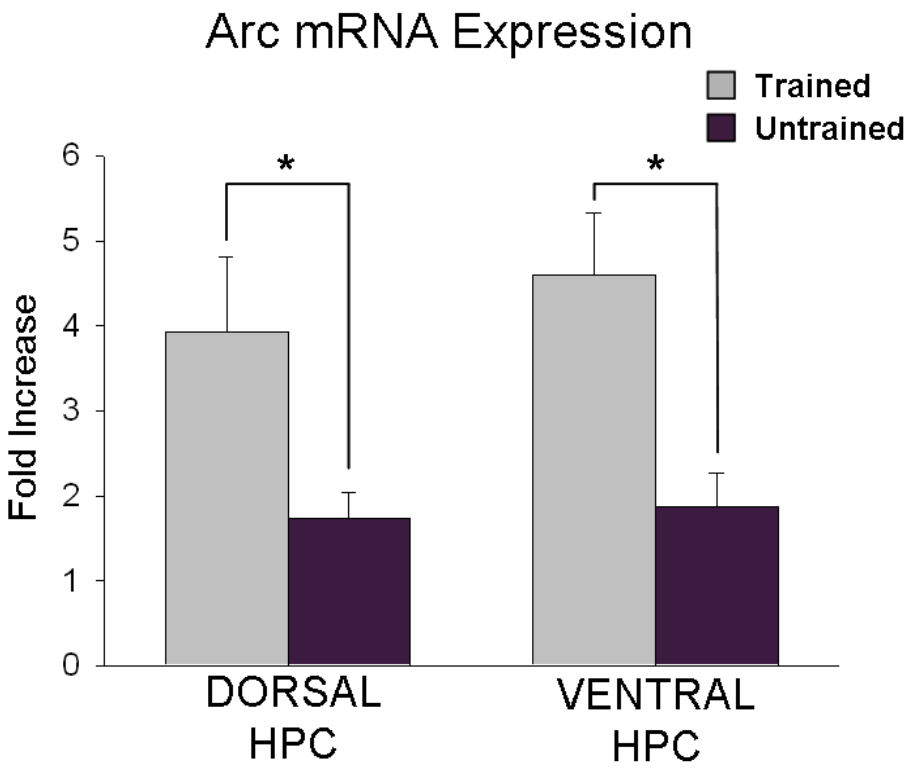

Figure 11. Mean (±SEM) fold change in Arc mRNA relative to both untrained home cage controls and GAPDH. Trace fear conditioning enhances Arc mRNA levels in dorsal and ventral hippocampus. *p<0.05

Figure 12

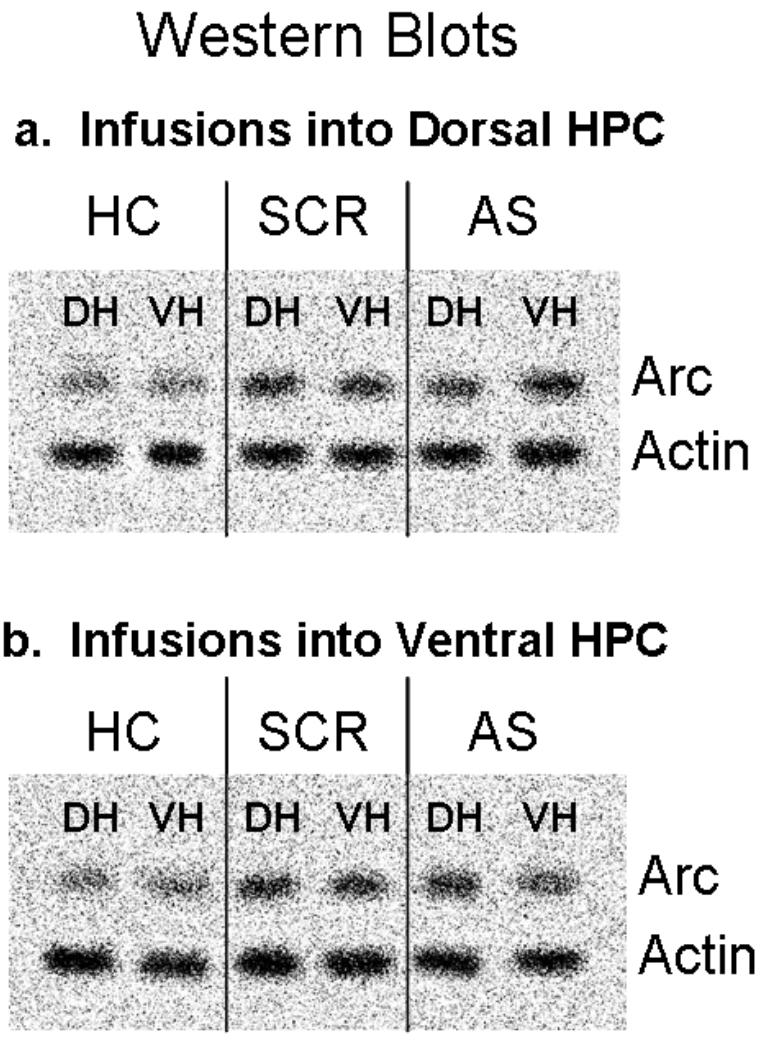

Figure 12. Infusions of Arc antisense oligodeoxynucleotides in a) dorsal and b) ventral hippocampus block Arc protein expression. *HC = home cage controls, SCR = scrambled ODN infusion, AS = Arc antisense ODN infusion, DH = dorsal hippocampus, VH = ventral hippocampus.*

Figure 13

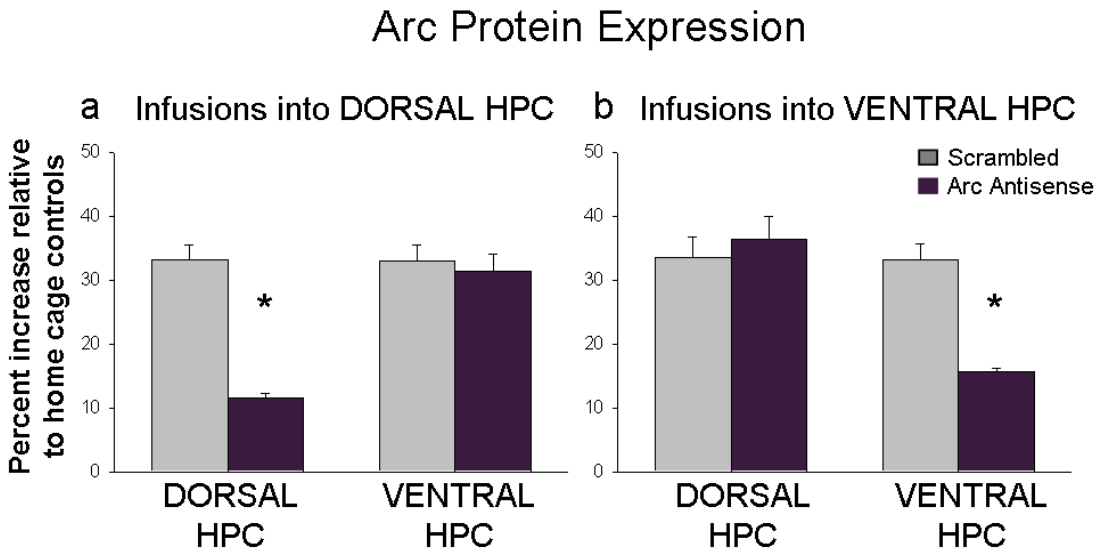

Figure 13. Mean (±SEM) percentage increase in Arc protein expression relative to untrained home cage controls and actin. Infusions of Arc antisense oligodeoxynucleotides into a) dorsal or b) ventral hippocampus attenuated the learning-induced enhancement of Arc protein without affecting Arc protein levels in the non-cannulated hippocampal subregion. *p <0.05.

Figure 14

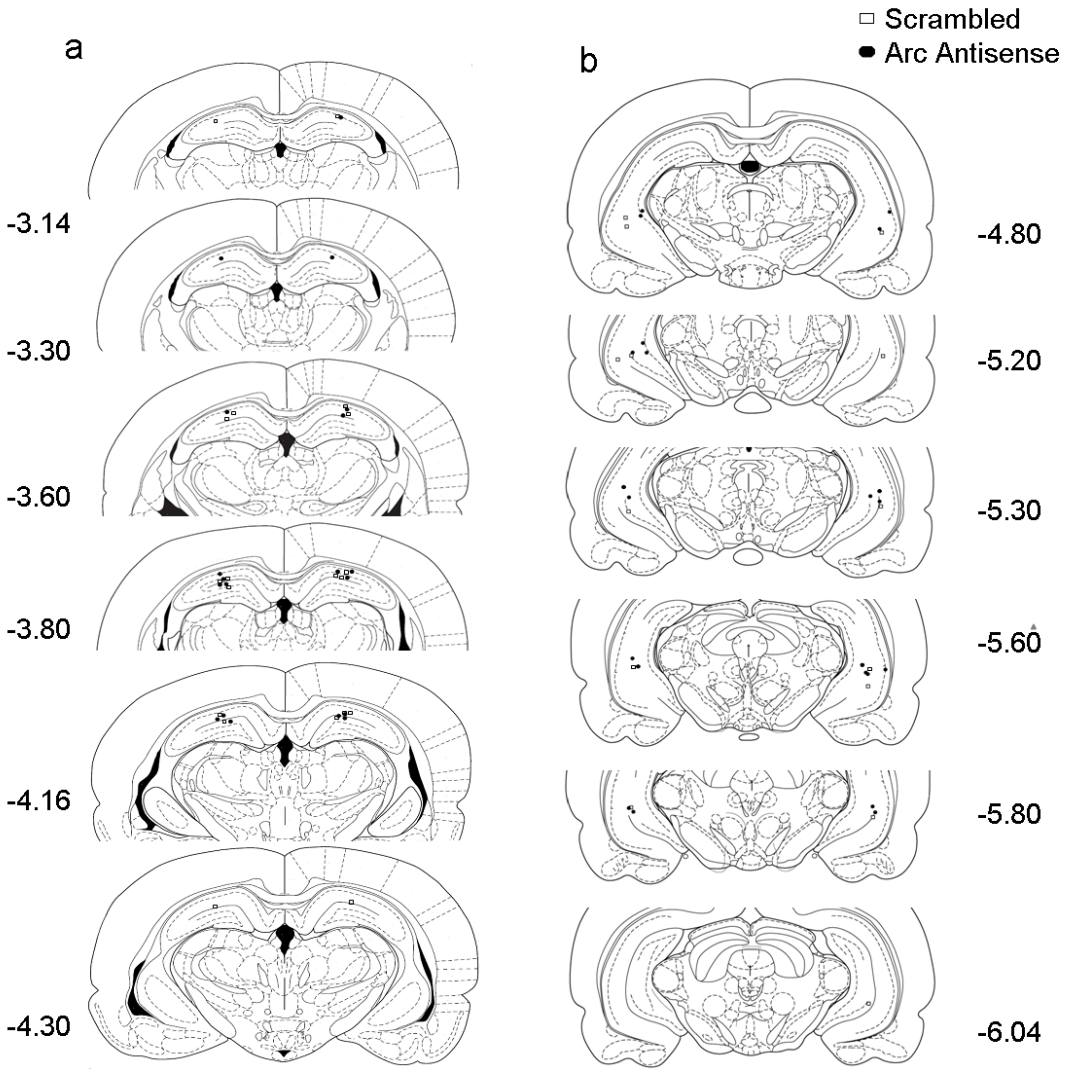

Figure 14. Schematic representation of cannula placement in coronal sections of a) dorsal or b) ventral hippocampus for Experiment 2.

Figure 15

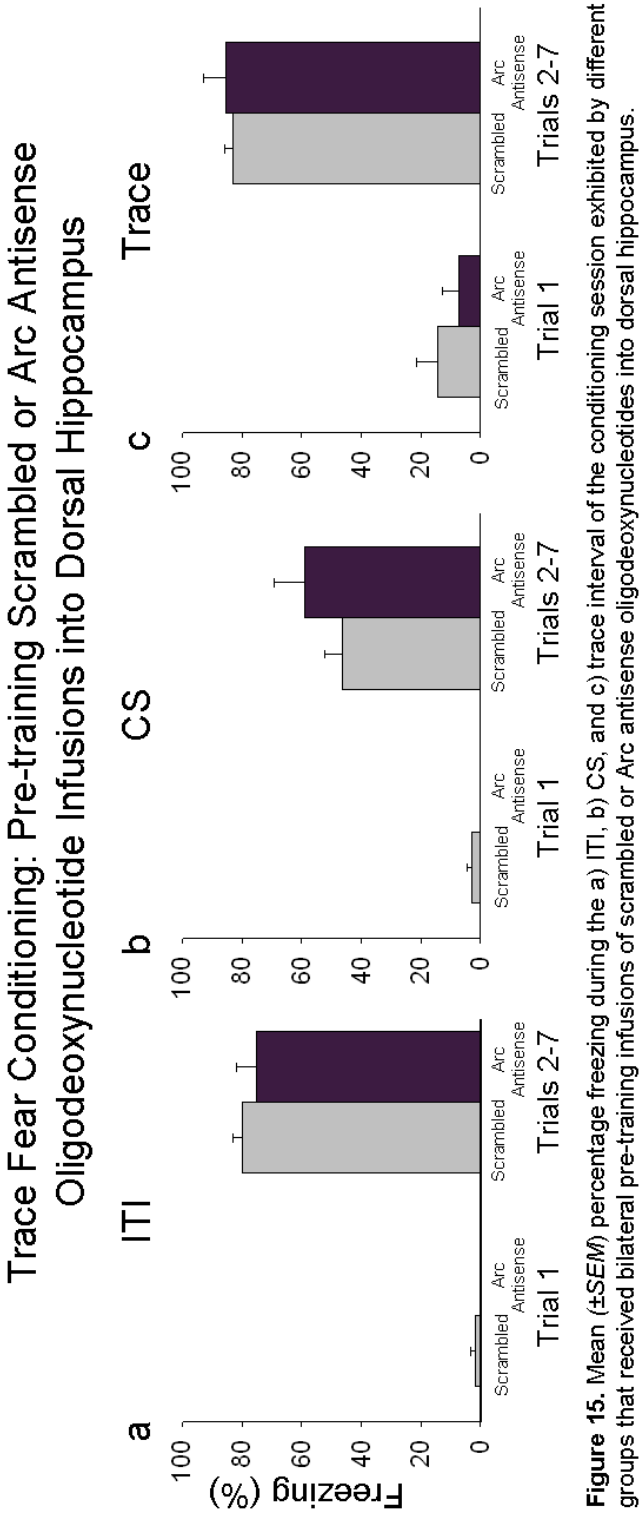

Figure 15. Mean (±*SEM*) percentage freezing during the a) ITI, b) CS, and c) trace interval of the conditioning session exhibited by different groups that received bilateral pre-training infusions of scrambled or Arc antisense oligodeoxynucleotides into dorsal hippocampus.

Figure 16

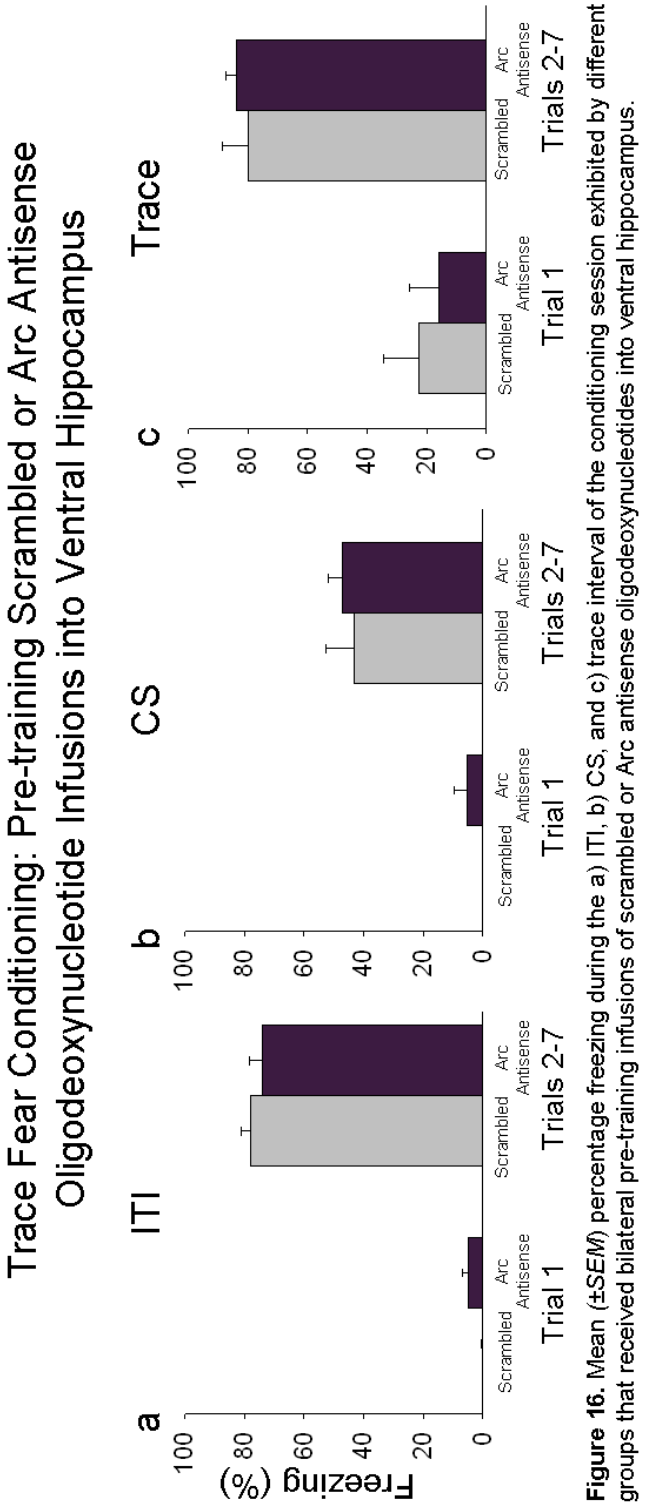

Figure 16. Mean (±*SEM*) percentage freezing during the a) ITI, b) CS, and c) trace interval of the conditioning session exhibited by different groups that received bilateral pre-training infusions of scrambled or Arc antisense oligodeoxynucleotides into ventral hippocampus.

Figure 17

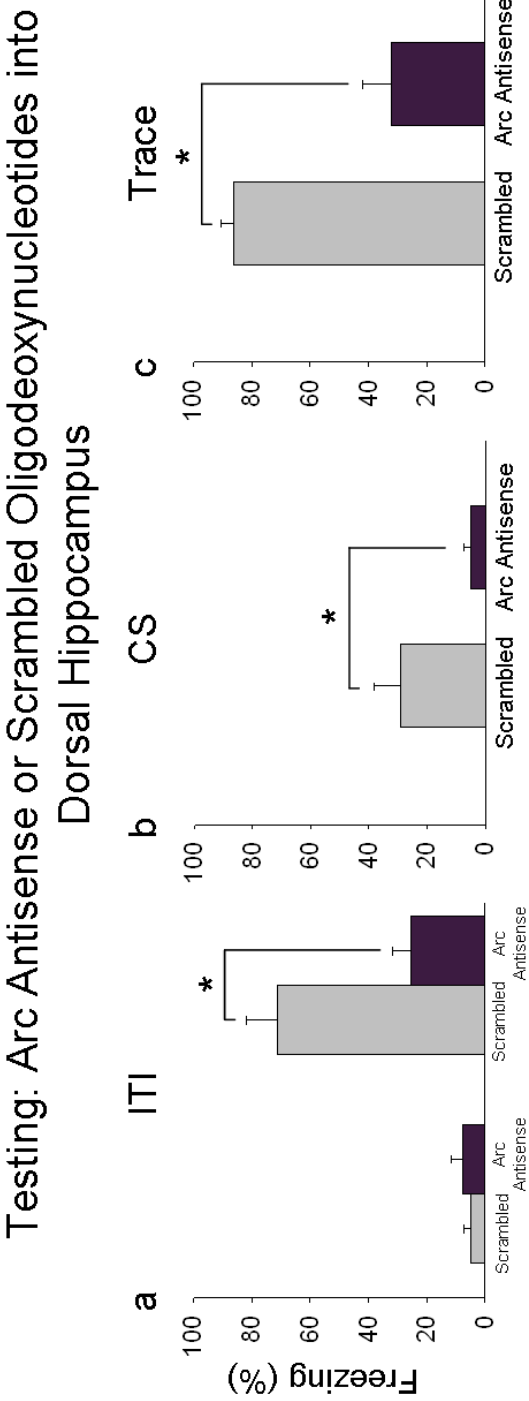

Figure 17. Mean (±*SEM*) percentage freezing during the a) ITI, b) CS, and c) trace interval of the testing session exhibited by different groups that received bilateral pre-training infusions of Arc antisense or scrambled oligodeoxynucleotides into dorsal hippocampus. Infusions of Arc antisense oligodeoxynucleotides prior to training impaired conditioned freezing during the test session 48 h later. *(p <0.05).

Figure 18

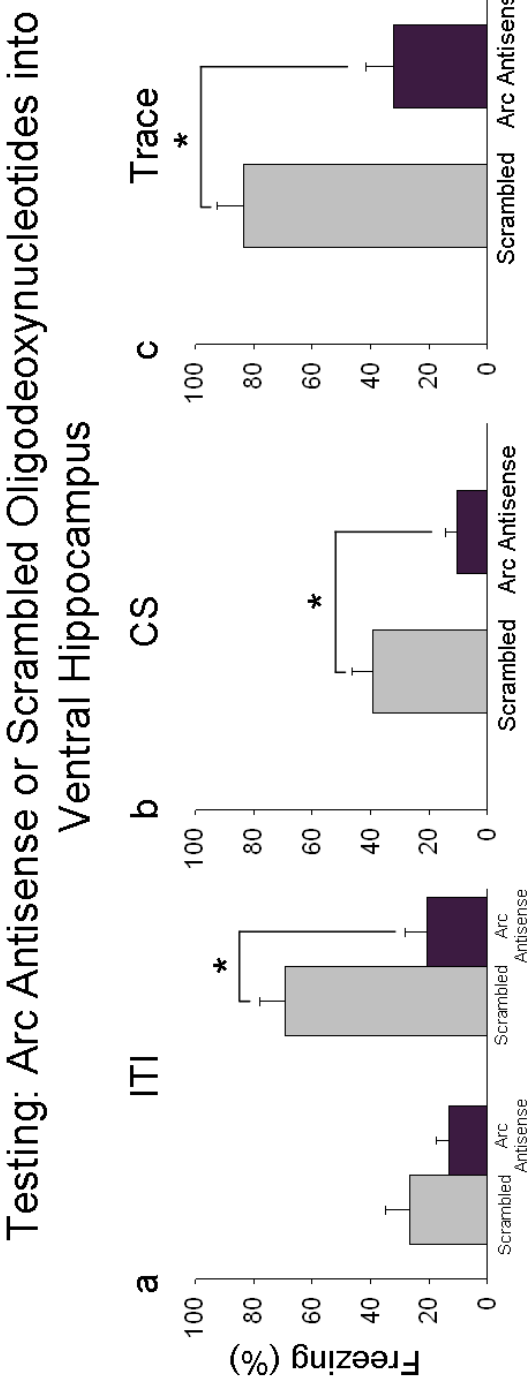

Figure 18. Mean (±*SEM*) percentage freezing during the a) ITI, b) CS, and c) trace interval of the testing session exhibited by different groups that received bilateral pre-training infusions of Arc antisense or scrambled oligodeoxynucleotides into ventral hippocampus. Infusions of Arc antisense oligodeoxynucleotides prior to training impaired conditioned freezing during the test session 48 h later. *($p < 0.05$).

Figure 19

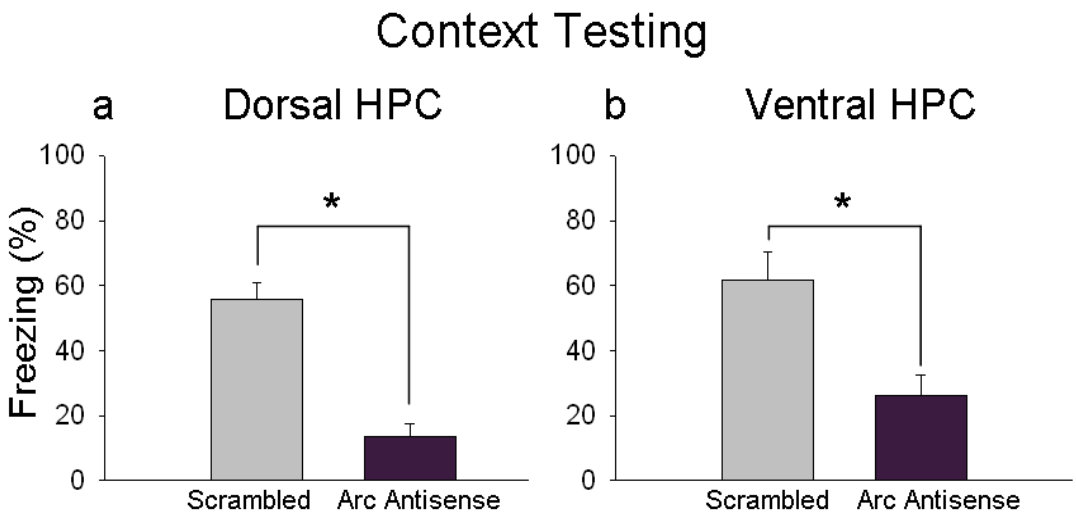

Figure 19. Mean *(±SEM)* percentage freezing during the context testing session exhibited by different groups that received bilateral pre-training infusions of Arc antisense or scrambled oligodeoxynucleotides into a) dorsal or b) ventral hippocampus. Infusions of Arc antisense oligodeoxynucleotides into dorsal or ventral hippocampus prior to training impaired conditioned freezing during the context test session 24 h later *(p <0.05).

Figure 20

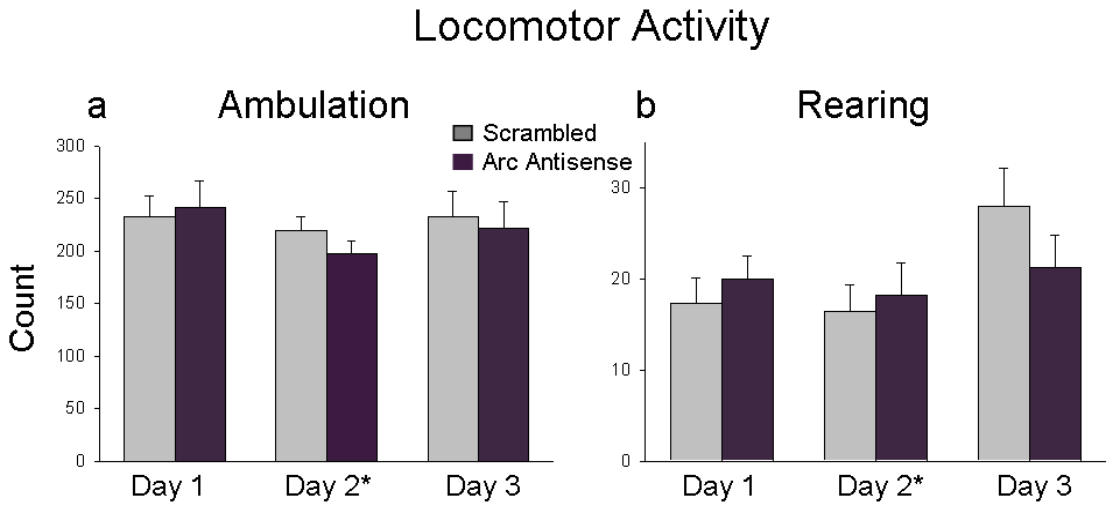

Figure 20. Mean *(±SEM)* number of a) ambulation and b) rearing counts during locomotor activity assessment for subjects that received Arc antisense or scrambled oligodeoxynucleotide infusions into dorsal hippocampus.

Figure 21

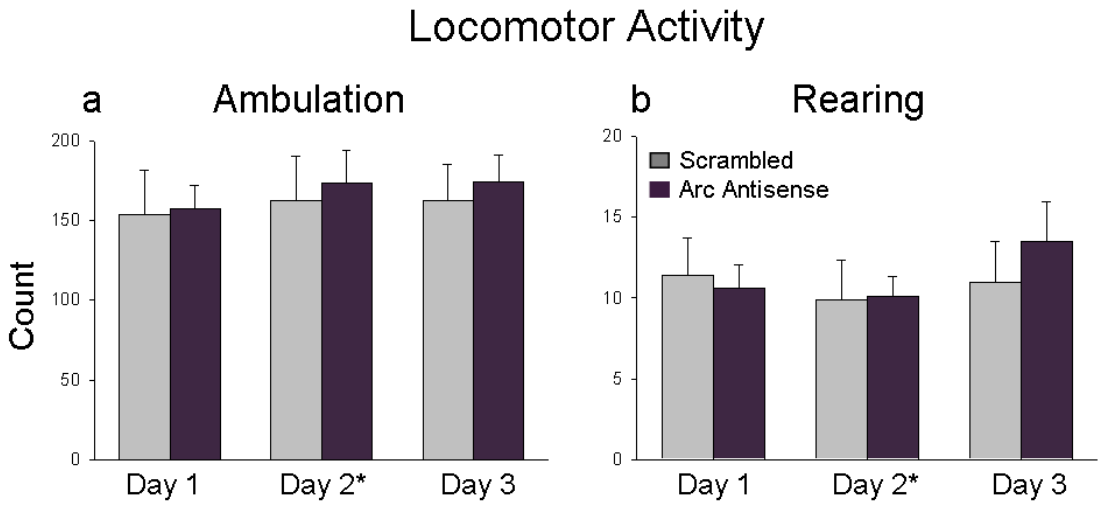

Figure 21. Mean *(±SEM)* number of a) ambulation and b) rearing counts during locomotor activity assessment for subjects that received Arc antisense or scrambled oligodeoxynucleotide infusions into ventral hippocampus.

Figure 22

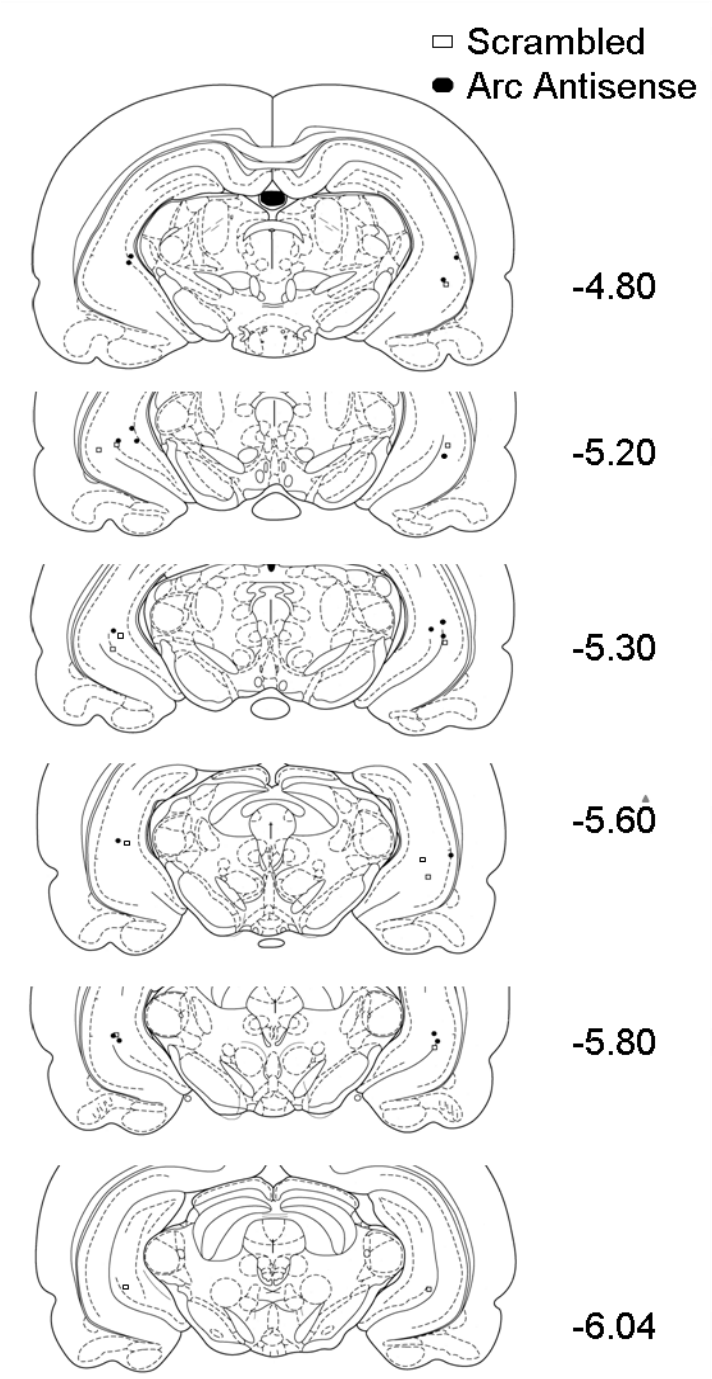

Figure 22. Schematic representation of cannula placement in coronal sections of a) dorsal or b) ventral hippocampus for Experiment 2.

Figure 23

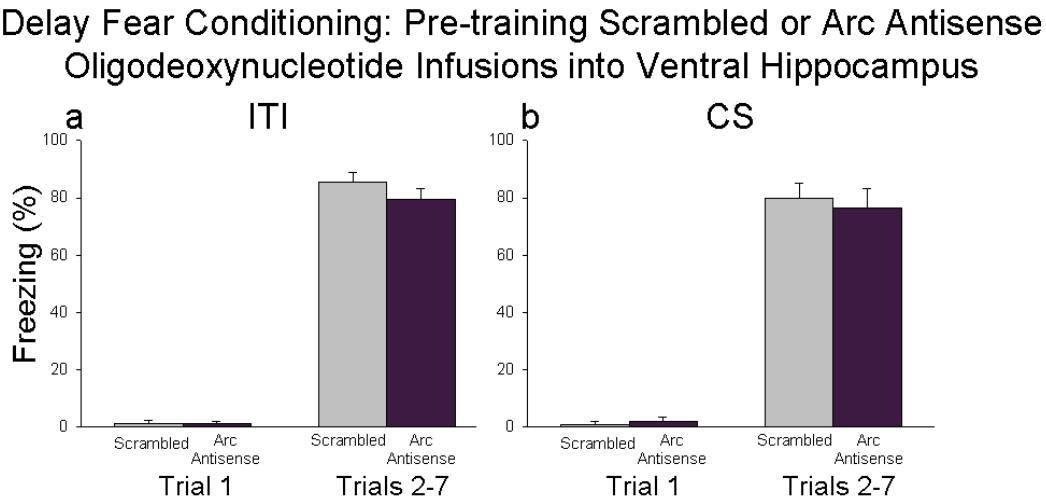

Figure 23. Mean *(±SEM)* percentage freezing during the a) ITI and b) CS of the delay fear conditioning session exhibited by different groups that received bilateral pre-training infusions of scrambled or Arc antisense oligodeoxynucleotides into ventral hippocampus.

Figure 24

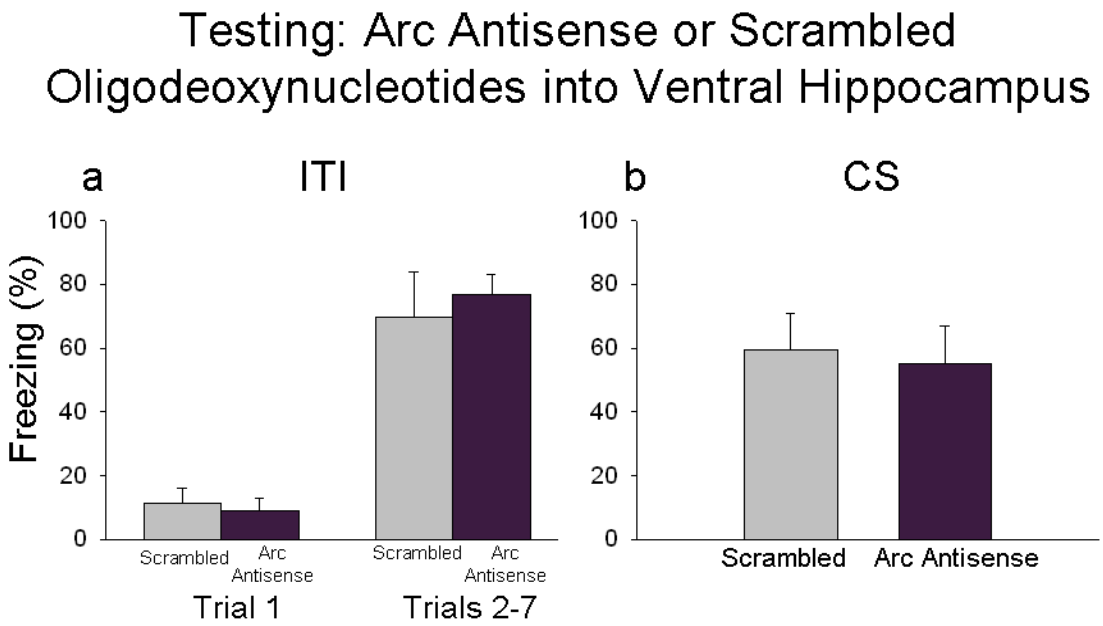

Figure 24. Mean *(±SEM)* percentage freezing during the a) ITI and b) CS of the testing session exhibited by different groups that received bilateral infusions of Arc antisense or scrambled oligodeoxynucleotides into ventral hippocampus prior to delay fear conditioning.

Figure 25

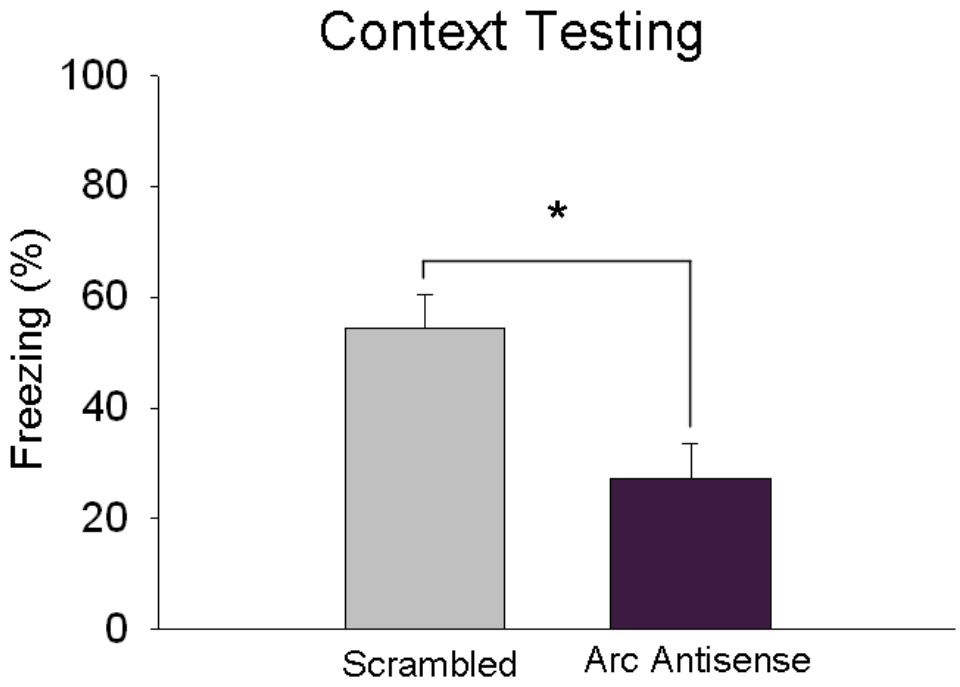

Figure 25. Mean *(±SEM)* percentage freezing during the context testing session. Infusions of Arc antisense oligodeoxynucleotides into ventral hippocampus prior to delay fear conditioning impaired conditioned freezing during the context test session 24 h later *(p <0.05).

Figure 26

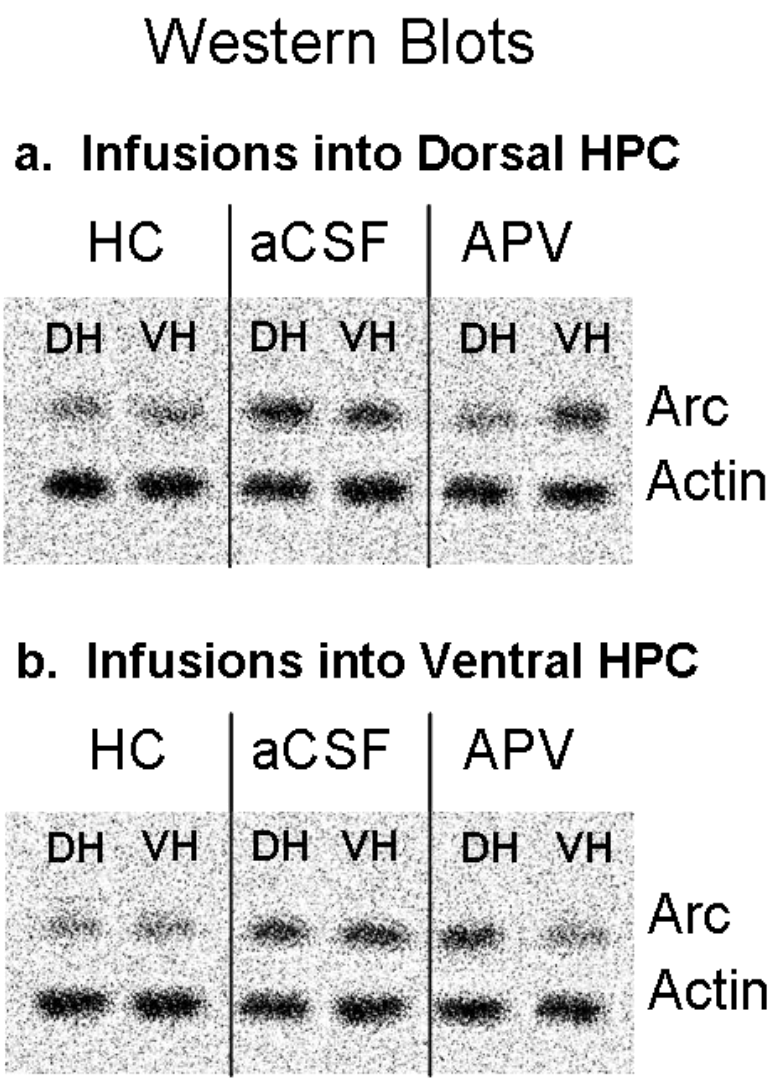

Figure 26. Infusions of APV in a) dorsal and b) ventral hippocampus block Arc protein expression.

Figure 27

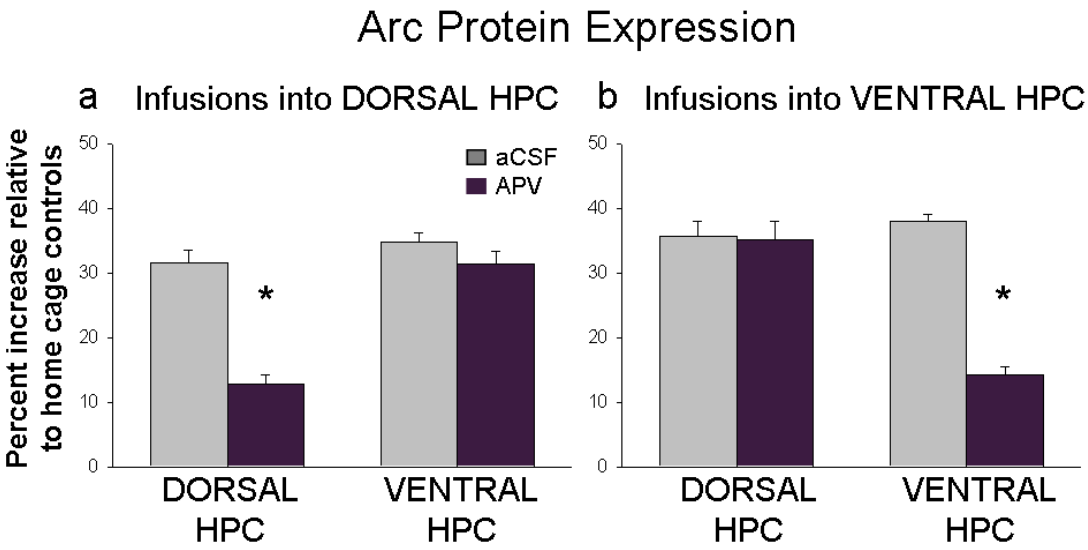

Figure 27. Mean (±SEM) percentage increase in Arc protein expression relative to untrained home cage controls and actin. Infusions of APV into a) dorsal or b) ventral hippocampus attenuated the learning-induced enhancement of Arc protein without affecting Arc protein levels in the non-cannulated hippocampal subregion. *p <0.05.

Figure 28

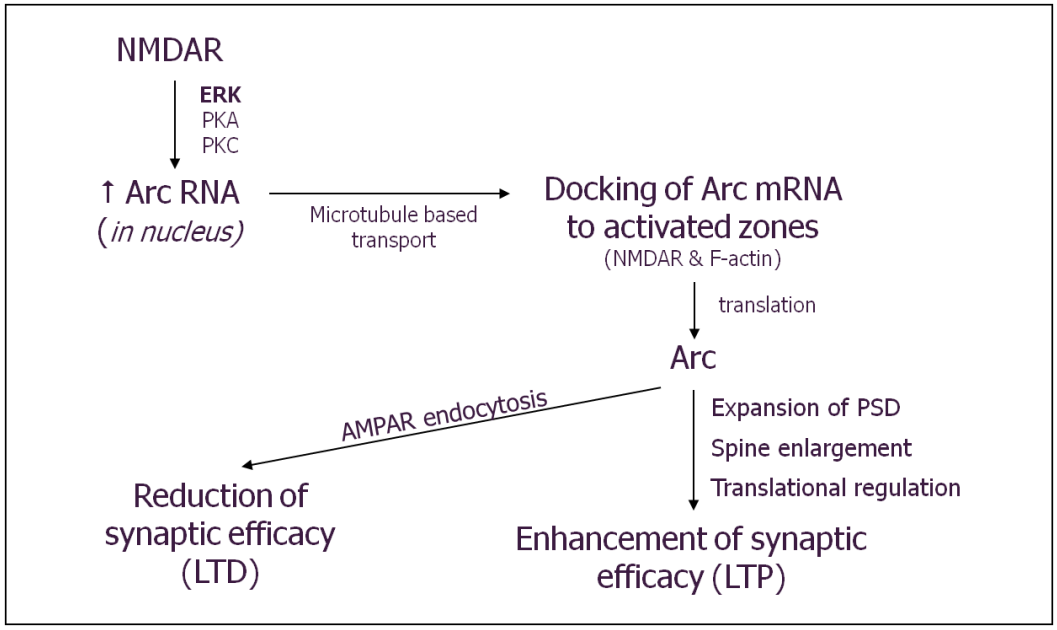

Figure 28. Speculative hypothesis of the role of Arc in plasticity. Arc is induced by NMDA receptor activation and transported to recently activated synapses where it undergoes local translation and can have bidirectional effects that may lead to an enhancement (LTP) or reduction (LTD) in synaptic efficacy.