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Molecular and Physiological Plasticity in the Ventral Hippocampus Following Associative Fear Learning

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MOLECULAR AND PHYSIOLGOCAL PLASTICITY IN THE VENTRAL HIPPOCAMPUS FOLLOWING ASSOCIATIVE FEAR LEARNING

by

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ABSTRACT

MOLECULAR AND PHYSIOLGOCAL PLASTICITY IN THE VENTRAL HIPPOCAMPUS FOLLOWING ASSOCIATIVE FEAR LEARNING

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Pavlovian fear conditioning is useful for understanding how various brain regions support associative fear memory. The hippocampus plays an especially important role in fear learning. Evidence suggests disrupted dorsal and/or ventral hippocampal activity leads to pronounced fear memory deficits, and the dorsal hippocampus displays distinct plasticity changes following fear learning, including altered intrinsic excitability and immediate early gene expression. These mechanisms support fear memory consolidation. However, the molecular and physiological plasticity of the ventral hippocampus following various forms of fear learning remains poorly understood. The current experiments examine the nature of associative fear learning-related plastic changes in the ventral hippocampus by determining the effect of trace and context fear conditioning on immediate early gene expression and intrinsic excitability in this brain region. Our data indicate that 1) the ventral hippocampus displays selective increases of Arc and pCREB protein expression following context, but not trace, fear retrieval; 2) ventral hippocampal CA1 regular-spiking neurons display increased excitability following context fear conditioning, but not following trace fear conditioning; and 3) ventral hippocampal CA1 late-spiking neurons demonstrate heterogeneity of intrinsic plasticity following trace fear conditioning, such that neurons from good learners display increased excitability relative to neurons from poor learners. Together, these data highlight the unique contribution of the ventral hippocampus to context and

trace fear conditioning, and suggest for the first time that distinct neuronal subpopulations within the ventral hippocampus can contribute to different types of associative fear memories.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

4-AP: 4-aminopyridine

aCSF: artificial cerebral spinal fluid

ADP: after-depolarization

AHP: afterhyperpolarization

AMPA: alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

AMPAR: alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor

AP: action potential

APV: (2R)-amino-5-phosphonovaleric acid

BLA: basolateral amygdala

BS: burst-spiking

CaMKII: Ca^{2+}/calmodulin-dependent protein kinase II

cAMP: cyclic AMP (3',5'-cyclic adenosine monophosphate)

CeM: medial portion of the central nucleus of the amygdala

CeL: lateral portion of the central nucleus of the amygdala

CNQX: cyanquixaline (6-cyano-7-nitroquinoxaline-2,3-dione)

CR: conditional response

CREB: cAMP response element-binding protein

CS: conditional stimulus

DG: dentate gyrus

DH: dorsal hippocampus

FS: fast-spiking

GABA: gamma-Aminobutyric acid

gRSC: granular retrosplenial cortex

IEG: immediate early gene

IL: infralimbic subregion of the mPFC

ISI: inter-spike interval

ITI: inter-trial interval

LA: lateral amygdala

LS: late-spiking

LTD: long-term depression

LTP: long-term potentiation

mPFC: medial prefrontal cortex

NMDA: N-methyl-D-aspartic acid

NMDAR: N-methyl-D-aspartic acid receptor

PL: prelimbic subregion of the mPFC

RMP: resting membrane potential

RS: regular-spiking

RSC: retrosplenial cortex

sCSF: sucrose cerebral spinal fluid

SK: small-conductance Ca^{2+}-activated K^{+} channel

TEA: tetraethylammonium

TI: trace interval

UR: unconditional response

US: unconditional stimulus

VH: ventral hippocampus

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Chapter One: General Introduction and Background Information

Pavlovian Fear Conditioning

Pavlovian fear conditioning paradigms are well-established as a means to study the neurobiology underlying associative fear memory (Fanselow & Poulos, 2005; LeDoux, 2000; Raybuck & Lattal, 2014; Rudy et al., 2004). Fear conditioning involves pairing a neutral cue called the conditional stimulus (CS) with an aversive cue called the unconditional stimulus (US) that naturally elicits an unconditional response (UR). Several CS-US pairings eventually lead to conditional responding (CR) in the presence of the CS alone. While several types of auditory and/or visual stimuli can be used for the CS, the US is often presented as a footshock, and the UR and eventual CR are reflected in the animal's defensive freezing behavior.

Some of the more commonly used variations of Pavlovian fear conditioning include delay and trace fear paradigms. In delay fear conditioning the CS and US overlap and are therefore contiguous. Delay fear learning primarily involves the amygdala (Johansen et al., 2011; LeDoux, 2000; Phillips & LeDoux, 1992) and the ventral hippocampus (Esclassan et al., 2009; Gresack et al., 2009; Hunsaker & Kesner, 2008; Maren & Holt, 2004; Richmond et al., 1999; Sierra-Mercado et al., 2011). In contrast, in trace fear conditioning the CS and US do not overlap, but instead are separated by a brief temporal gap called the trace interval (TI). Successful trace fear learning requires maintaining an association between the CS and US across the TI, and engages multiple higher-order brain regions including the medial prefrontal cortex (mPFC), dorsal hippocampus, and retrosplenial cortex (RSC), in addition to amygdala and ventral hippocampus (for review, see Raybuck & Lattal, 2014; Yousuf, Ehlers, et al., 2020).

A conditioned fear response to the surrounding context can also be acquired, either in the absence (foreground contextual fear conditioning) or presence (background contextual fear

conditioning) of a discrete auditory CS. Context fear conditioning recruits some of the same brain regions as auditory fear conditioning, including the dorsal hippocampus (Besnard et al., 2020; Debiec et al., 2002; Denny et al., 2014; Frankland et al., 1998; Goshen et al., 2011; Liu et al., 2012; Maren et al., 1997; McEchron et al., 1998; McNish et al., 2000), prelimbic subregion of the mPFC (PL; Corcoran & Quirk, 2007), amygdala (Helmstetter & Bellgowan, 1994; Muller et al., 1997), and ventral hippocampus (Bast et al., 2001; Besnard et al., 2020; Kim & Cho, 2017; Maren & Holt, 2004; Pentkowski et al., 2006; Rudy & Matus-Amat, 2005; Trivedi & Coover, 2004; Twining et al., 2020; Zhang et al., 2001; Zhu et al., 2014).

Contributions of Dorsal and Ventral Hippocampus to Associative Fear

The fear circuit is complex and requires coordination among various cortical and subcortical brain regions (see Figure 1). A considerable amount of work conducted over the past few decades highlights medial temporal lobe structures, especially the hippocampus, as crucial components of the fear network. This research has primarily focused on the role of the dorsal hippocampus in various associative fear learning tasks, with the ventral hippocampus receiving relatively less attention. However, more recent work has begun to address the disparate functions of each hippocampal subregion within the larger fear circuitry, and as a result we are beginning to see some of the unique contributions of the dorsal and ventral hippocampal subregions to associative fear memories.

There is a wealth of research that suggests that lesions or inactivation of the dorsal hippocampus disrupt context and/or trace fear learning. Lesions of the dorsal hippocampus either before or after training disrupt trace and context fear memory (Chowdhury et al., 2005; McEchron et al., 1998; Quinn et al., 2002). Dorsal hippocampal involvement in contextual fear retrieval is also time-dependent, as dorsal hippocampal lesions disrupt recent contextual fear

memory, but leave remote context fear intact (Anagnostaras et al., 1999; Kim & Fanselow, 1992; Maren et al., 1997). Dorsal hippocampal inactivation using either the $GABA_A$ agonist muscimol or the NMDAR antagonist APV disrupts trace and context fear memory (Guimarãis et al., 2011; Quinn et al., 2005; Raybuck & Lattal, 2011). Interestingly, AMPAR-dependent mechanisms that contribute to trace fear memory are likely subregion-specific, as trace fear retrieval is disrupted following CNQX infusions into the dentate gyrus (DG), but not CA1 (Pierson et al., 2015). Other evidence also suggests a role for the ventral hippocampus in context and trace fear learning. Lesions or optogenetic/chemogenetic silencing of ventral hippocampal neurons disrupt context fear memory retrieval (Kim & Cho, 2017; Maren & Holt, 2004; Zhu et al., 2014), while muscimol inactivation of the ventral hippocampus disrupts both trace and context fear memory (Gilmartin et al., 2012). Additionally, trace and context fear memory are disrupted when the entire hippocampus is lesioned, or when either dorsal or ventral hippocampus is inactivated, suggesting these memories are non-selective for either dorsal or ventral hippocampus (Esclassan et al., 2009). Thus, both dorsal and ventral hippocampus are critical for trace and context fear memory, but the relative contribution of these distinct subregions are not completely clear from these studies alone.

Other evidence suggests that the role of the ventral hippocampus in fear learning is more extensive than that of the dorsal hippocampus. Lesions of ventral but not dorsal hippocampus disrupt context fear retrieval (Pentkowski et al., 2006; Trivedi & Coover, 2004). Neurotoxic lesions of the ventral hippocampus prior to training disrupt trace fear acquisition and retrieval, while dorsal hippocampal lesions before training have no effect (Yoon & Otto, 2007). APV or muscimol infusion into ventral but not dorsal hippocampus impairs trace (Czerniawski et al., 2009) and context (Czerniawski et al., 2012) fear retrieval, suggesting NMDA-dependent activity

in the ventral hippocampus is critical for associative fear learning. While dorsal hippocampal lesions disrupt context fear retrieval only, ventral hippocampal lesions disrupt both context *and* trace fear retrieval (Rogers et al., 2006). The ventral hippocampus is also required for multiple temporal aspects of trace and context fear memories. Muscimol inactivation of the dorsal hippocampus disrupts recent trace fear retrieval, while muscimol inactivation of the ventral hippocampus disrupts both recent and remote trace fear memory retrieval (Cox et al., 2013). Similarly, while neurotoxic lesions of either dorsal or ventral hippocampus 1 d after conditioning disrupt both trace and context fear retrieval, lesions of ventral hippocampus 30 d after training disrupt context fear memory, while dorsal hippocampal lesions have no effect on remote context fear retrieval (Beeman et al., 2013). Thus, when the roles of dorsal and ventral hippocampus in associative fear learning are investigated simultaneously, evidence suggests that disrupted ventral hippocampal function leads to more consistent and persistent behavioral impairments when compared with disrupted dorsal hippocampal function.

Several ventral hippocampal circuits uniquely contribute to context fear learning. Ventral hippocampal neurons that double-project to both the mPFC and amygdala display greater context fear conditioning-induced activation of the immediate early gene (IEG) c-fos (cellular FBJ osteosarcoma oncogene) when compared to single-projecting neurons (Kim & Cho, 2017). Furthermore, optogenetic silencing of ventral hippocampal terminals in PL during trace fear conditioning trials disrupts context fear retrieval, but leaves trace fear retrieval intact (Twining et al., 2020). This suggests that ventral hippocampus-mPFC interactions are likely critical for context, but not trace, fear associations. Taken together, while dorsal and ventral hippocampus are clearly integral for associative fear learning, the specific role of each subregion is not well-defined.

Figure 1

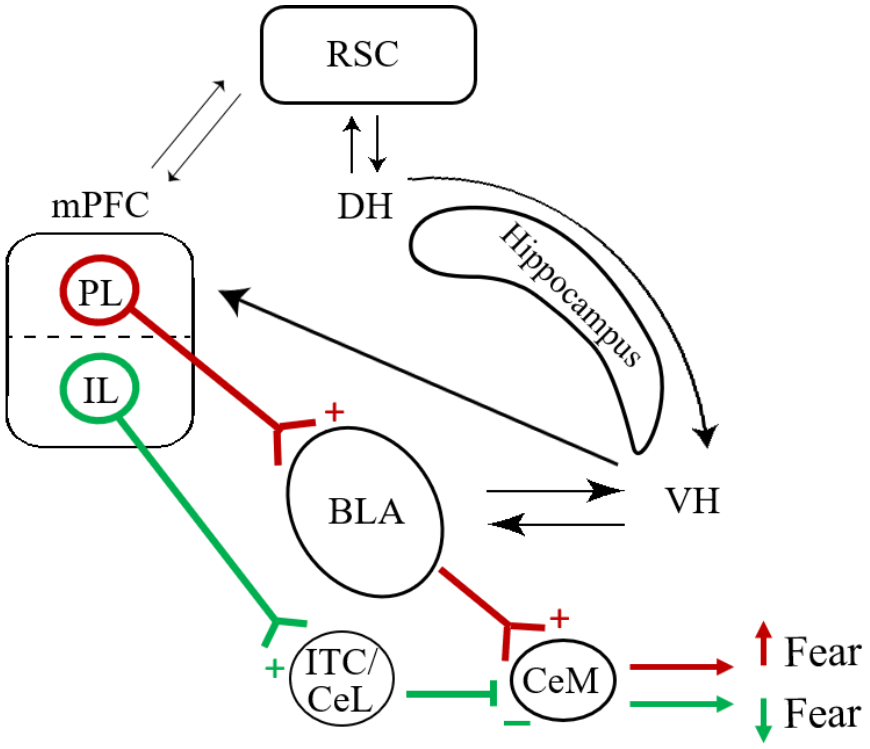

Figure 1. Neural circuitry involved in fear conditioning and extinction. Contextual and trace fear acquisition and extinction are supported by reciprocal connectivity between RSC and DH. Contextual information is routed from DH to VH, which then projects to the mPFC and shares bidirectional connectivity with the BLA. PL promotes fear expression via direct connectivity with the BLA (red lines), whereas IL promotes fear extinction via projections to the ITC/CeL (green lines). These distinct pathways converge on the CeM, and ultimately either promote fear expression (red) or suppress it (green). *Abbreviations:* retrosplenial cortex (RSC); dorsal hippocampus (DH); ventral hippocampus (VH); medial prefrontal cortex (mPFC); basolateral amygdala (BLA); prelimbic subregion of the mPFC (PL); infralimbic subregion of the mPFC (IL); intercalated cells (ITC); lateral subregion of the central nucleus of the amygdala (CeL); medial subregion of the central nucleus of the amygdala (CeM). Figure adapted from Yousuf, Ehlers et al., 2020 (Neurobiology of Learning and Memory).

Molecular Signaling Mechanisms Underlying Fear Conditioning

Fear conditioning can trigger neuronal signaling processes that ultimately activate cAMP-response element binding protein (CREB) to drive transcription of several downstream IEGs, including Arc (activity regulated cytoskeletal protein), Zif-268 (zinc finger binding protein clone 268), and c-fos. The IEG Arc is especially dynamic. An effector gene, Arc regulates many neuronal functions implicated in learning, including actin polymerization (Messaoudi et al., 2007), immature dendritic spine proliferation (Peebles et al., 2010), AMPAR internalization (Chowdhury et al., 2006), and long-term potentiation (LTP) as well as long-term depression

(LTD; for review, see Bramham et al., 2010). Arc expression is also critical for homeostatic plasticity. AMPAR endocytosis occurs in inactive dendritic spines that also display increased CaMKIIβ-mediated Arc expression. This process is known as inverse synaptic tagging, and serves to suppress plasticity at weakened synapses while simultaneously enhancing synaptic strength at active synapses in potentiated neurons (Okuno et al., 2012). In contrast to Arc, Zif-268 and c-fos both act as transcription factors and drive a subsequent wave of late-onset gene transcription to support memory stabilization (for review, see Jaworski et al., 2018; Minatohara et al., 2015; Veyrac et al., 2014). Zif-268 activity is specifically linked to several epigenetic modifications that underlie memory formation. For example, context fear learning precipitates histone methylation at the Zif-268 promoter, and genetic knock-out of histone methyltransferases disrupts context fear memory (Gupta et al., 2010). Thus, it comes as little surprise that CREB and downstream IEGs play a major role in learning and memory.

CREB expression in the amygdala and hippocampus supports various forms of fear learning. Overexpression of CREB in the lateral amygdala (LA) of CREB-deficient mice increases intrinsic excitability by simultaneously reducing the afterhyperpolarization (AHP) and increasing spiking activity of LA neurons (Zhou et al., 2009), and enhances delay fear learning (Han et al., 2007). Trace fear conditioning increases phosphorylated CREB (pCREB) protein expression in the hippocampus of C57BL/6 mice relative to unpaired controls (Hwang et al., 2010). Interestingly, both delay and unpaired fear conditioning induce elevated pCREB levels in dorsal hippocampal CA1 (Trifilieff et al., 2006), suggesting dorsal hippocampal pCREB induction may be non-selective for different forms of fear learning, including unpaired conditioning. Preventing CREB expression in the dorsal hippocampus disrupts trace and context fear memory (Peters et al., 2009), and impedes long-term spatial memory (Guzowski &

McGaugh, 1997). Interestingly, CREB blockade in the dorsal hippocampus impedes context fear memory retrieval in mice, while disrupted CREB expression in the ventral hippocampus enhances context fear retrieval (Fisher et al., 2017). In contrast, CREB overexpression in the forebrain leads to enhanced trace fear conditioning and retrieval, and increases apical dendrite complexity, and overall spine density in dorsal CA1 (Serita et al., 2017). Thus, abundant evidence supports a role for CREB activation in fear learning.

Similar to CREB, Arc also has a known role in various forms of fear learning. Delay fear conditioning induces Arc mRNA and protein expression in the amygdala (Ploski et al., 2008), and induces Arc protein in the dorsal hippocampus (Lonergan et al., 2010). More recent evidence suggests fear conditioning leads to differential patterns of Arc expression within dorsal and ventral hippocampal subregions. Trace fear conditioning induces Arc expression in dorsal CA3, ventral CA3 and ventral CA1, but not dorsal CA1, while context fear conditioning induces robust Arc expression only in ventral CA1, but not in ventral CA3, dorsal CA1 or dorsal CA3 (Hudgins & Otto, 2019). While these studies suggest Arc activity is induced by fear learning, other evidence indicates blockade of Arc expression disrupts normal fear learning. Pre-training blockade of Arc induction in either dorsal or ventral hippocampus disrupts trace and context fear memory (Czerniawski et al., 2011), while pre-testing blockade of Arc in dorsal or ventral hippocampus disrupts trace fear memory (Chia & Otto, 2013). Together, these studies highlight the importance of both CREB and Arc in associative fear learning.

Other IEGs, including Zif-268 and c-fos, are also implicated in various forms of learning. Induction of c-fos and/or Zif-268 mRNA occurs following two-way avoidance learning (Nikolaev et al., 1992), brightness discrimination (Grimm et al., 1997; Tischmeyer et al., 1990), alternation learning (Nagahara & Handa, 1995), odor discrimination (Hess et al., 1995a, 1995b),

and water maze learning (Guzowski et al., 2001). Transgenic animal models with targeted c-fos or Zif-268 deletion or knockdown further demonstrate that IEG expression is paramount for successful learning. Despite intact discrimination learning on a T-maze task, spatial and cued water maze memory is disrupted in c-fos-deficient mice (Paylor et al., 1994). Additionally, spatial memory, taste aversion, social transmission of food preference, and object recognition are impaired in Zif-268-deficient mice, and these mice further display attenuated late-phase LTP in the DG (Jones et al., 2001). Together, these studies highlight essential roles for c-fos and Zif-268 in various discrimination and spatial learning tasks.

Associative fear conditioning also induces c-fos and Zif-268 expression in brain regions involved in successful fear learning, such as the amygdala and hippocampus. Fear conditioning induces c-fos mRNA (Rosen et al., 1998) and Zif-268 mRNA (Hall et al., 2001) in the amygdala. The experience-dependent nature of IEG induction has also been used to demonstrate that fear acquisition and retrieval are supported by overlapping populations of neurons. Retrieval of a delay fear memory induces Zif-268 in the same basolateral amygdala (BLA) neurons that express c-fos-driven tau-LacZ (induced by delay fear acquisition), indicating neurons involved in delay fear acquisition also support delay fear retrieval (Reijmers et al., 2007). In the dorsal hippocampus, acquisition of delay fear conditioning induces Zif-268 protein (Lonergan et al., 2010), and context fear memory retrieval induces Zif-268 mRNA in CA1 (Hall et al., 2001). Extinction of learned fear has also been shown to reverse learning-induced IEG expression. While dorsal hippocampal levels of nuclear c-fos peak 1 h following context fear conditioning, five days of extinction brings c-fos back to pre-training levels (Tronson et al., 2009). Thus, similar to CREB and Arc, Zif-268 and c-fos are vital for conditioned fear.

Associative fear learning also drives IEG expression in several fear networks, and more recent advances in optogenetic technology have allowed for precise temporal manipulations of these networks to examine their role in fear. When c-fos-positive BLA neurons (i.e. those that represent fear encoding), and dorsal hippocampal CA1 c-fos positive neurons (i.e. those that represent encoding of a neutral context) are co-activated using optogenetic stimulation, fear memory can be generated in a neutral environment (Ohkawa et al., 2015). Other evidence suggests interaction between the dorsal hippocampus and RSC promotes context fear memory retrieval. While context fear memory is impaired following pharmacological inactivation of the dorsal hippocampus, animals successfully express context fear memory when c-fos positive RSC neurons are optogenetically activated, suggesting activity of RSC neurons can compensate for dorsal hippocampal dysfunction (Cowansage et al., 2014). In support, c-fos levels in the RSC and central amygdala are reduced specifically within neurons activated by context fear conditioning when dorsal CA1 is optogenetically silenced (Tanaka et al., 2014). Further, delay fear conditioning increases c-fos expression in neurons that project directly from the amygdala to the ventral hippocampus (Senn et al., 2014), whereas delay fear renewal increases c-fos in neurons projecting from the ventral hippocampus to the amygdala (Orsini et al., 2011), suggesting delay fear conditioning and renewal my differentially modify the activity of these circuits. These studies suggest that IEG induction is likely occurring in several brain regions that are part of a circuit to support different types of fear memory.

Learning-related changes of IEG expression are also closely linked with structural and synaptic plasticity that often accompanies learning. Fear conditioning simultaneously induces increased expression of calcium-permeable GluA1-containing AMPARs (which are linked to synaptic potentiation (Clem & Barth, 2006; Guire et al., 2008; Plant et al., 2006)) and c-fos in

cortical neurons (Descalzi et al., 2012). While c-fos positive CA1 neurons display reduced spine density following context fear conditioning (Sanders et al., 2012), c-fos expressing DG neurons demonstrate increased spine density (Ryan et al., 2015), suggesting changes in c-fos activity drive dynamic spine changes that vary between hippocampal subregions to support the same type of fear memory. Hippocampal LTP maintenance is disrupted following targeted deletion of Zif-268 (Jones et al., 2001), and inhibition of Arc protein expression (Guzowski et al., 2000). Taken together, several forms of learning and memory rely on CREB and IEG induction in numerous brain regions to support other forms of plasticity and memory consolidation.

Neurophysiological Plasticity Mechanisms Underlying Fear Conditioning

Learning is also accompanied by altered physiological function of individual neurons (i.e. intrinsic plasticity or altered intrinsic excitability (see Figure 2)). Learning-related increases of intrinsic excitability often emerge as reduced spike frequency adaptation (i.e. the degree of neuronal firing in response to sustained excitation), and reduced postburst AHP. Increased intrinsic excitability ostensibly drives subsequent changes in synaptic plasticity, and likely serves as a metaplasticity mechanism underlying future learning (Abraham, 2008; Sehgal et al., 2013). Thus, examining learning-related changes of intrinsic plasticity can provide insights into the role of a particular brain region or network in various learning tasks, including classical eyeblink conditioning, discrimination learning, spatial navigation, and acquisition as well as extinction of conditioned fear (for review, see Sehgal et al., 2013; Yousuf, Ehlers, et al., 2020).

Figure 2

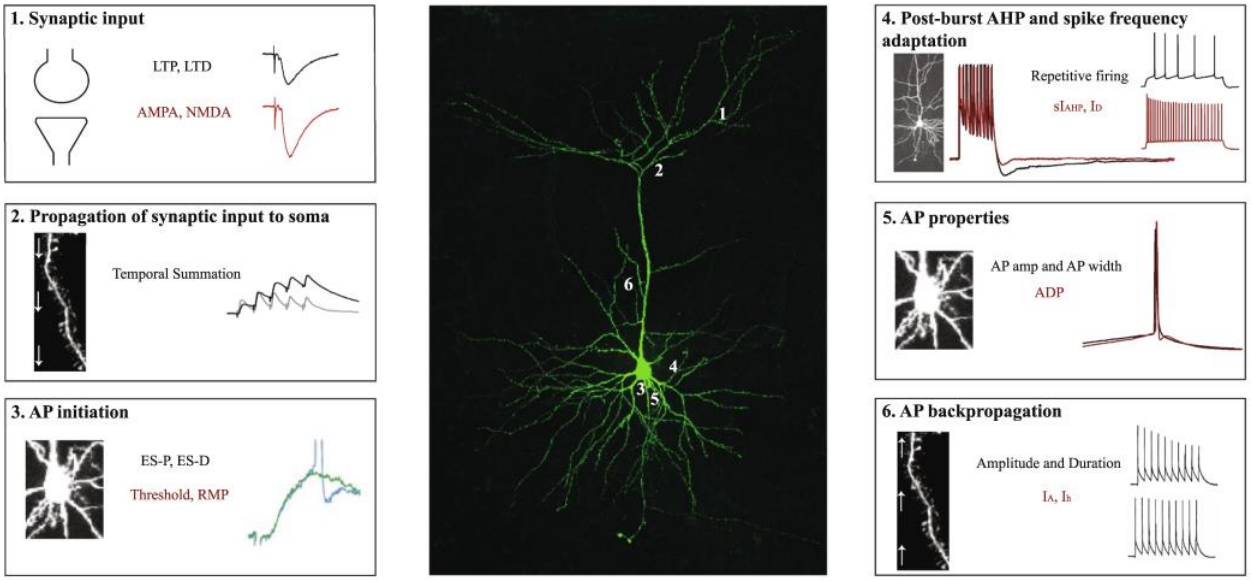

Figure 2. Neuronal information processing is regulated by synaptic and intrinsic properties. (1) Dendritic processes are the origin of most synaptic inputs. Synaptic plasticity can take the form of LTP or LTD, and is governed by AMPARs and NMDARs, in addition to intrinsic neuronal properties. (2) EPSP propagation toward the soma is regulated by dendritic cable and membrane properties. (3) E-S potentiation, or increased chance of EPSP-induced AP firing, is mediated by several factors, including AP threshold and RMP. (4) Neuronal output is influenced by excitability changes, including altered postburst AHP and/or spike frequency adaptation. (5) Further processing of synaptic input is influenced by other factors that are intrinsic to the neuron, including AP amplitude, AP duration, and whether or not there is the presence of an ADP. (6) Dendritic morphology as well as ionic conductance can modulate backpropagating APs, which in turn influence synaptic strength. *Abbreviations:* long-term potentiation (LTP); long-term depression (LTD); excitatory postsynaptic potential (EPSP); EPSP-spike (E-S); action potential (AP); resting membrane potential (RMP); afterhyperpolarization (AHP); afterdepolarization (ADP). Figure adapted from Yousuf, Ehlers et al., 2020 (Neurobiology of Learning and Memory). Electrophysiological traces in boxes 3 and 6 were adapted from Daoudal et al. (2002) (Copyright [2002] National Academy of Sciences, USA) and Tsubokawa, Offermanns, Simons, and Kano (2000) (Copyright [2000] Society for Neuroscience).

Distinct changes of hippocampal excitability are consistently observed following eyeblink conditioning (de Jonge et al., 1990; Disterhoft et al., 1986; Moyer et al., 1996; Oh et al., 2009; Thompson et al., 1996). For example, both the AHP and spike frequency adaptation are transiently reduced in dorsal hippocampal neurons following trace eyeblink learning (Moyer et al., 1996). In a separate study comparing dorsal and ventral hippocampal excitability, trace eyeblink conditioning induced greater overall firing rates in dorsal neurons when compared to ventral neurons (Weible et al., 2006). However, dorsal and ventral hippocampal neuronal populations also demonstrated heterogeneity in that some neurons fired *more*, while other fired

less after learning. When the authors examined this phenomenon more closely, they found that regardless of whether neurons were located in dorsal or ventral hippocampus, the proportion of neurons firing more action potentials (APs) was greater in animals that underwent trace eyeblink conditioning compared to a pseudo conditioned control group (Weible et al., 2006). This suggests that despite increased excitability in dorsal neurons relative to ventral hippocampal neurons, both dorsal and ventral neurons displayed increased plasticity specifically following trace and not pseudo conditioning. It is crucial, therefore, to examine changes in excitability that could be occurring within individual populations of neurons to better understand the contribution of specific brain regions to learning.

Within the fear literature, learning-related changes of intrinsic excitability in amygdala neurons occur following olfactory and auditory delay fear conditioning. For instance, olfactory fear conditioning enhances neuronal excitability in LA neurons (Rosenkranz & Grace, 2002). In contrast, BLA neurons display increased spike frequency adaptation following olfactory fear conditioning (indicating reduced excitability), but also display reduced adaptation and postburst AHP following reward-based olfactory discrimination learning (suggesting increased excitability (Motanis et al., 2014)). Thus, the direction of excitability changes (i.e. increased or decreased excitability) induced by olfactory conditioning could vary dramatically between LA and BLA neurons, or could depend on the positive (i.e. reward) or negative (i.e. aversive US) valuation of the stimuli used during conditioning. In addition to olfactory conditioning, auditory delay fear conditioning also induces distinct changes of excitability in amygdala neurons. Delay fear conditioning reduces both spike frequency adaptation and the AHP in roughly one-third of LA neurons (Sehgal et al., 2014). Consistent with this, delay fear conditioning preferentially increases spiking activity within Arc-positive LA neurons (Gouty-Colomer et al., 2016). These

studies not only support a role for amygdala excitability in fear learning, but also suggest distinct neuronal populations support delay fear memory.

In contrast to intrinsic plasticity of amygdala neurons, hippocampal intrinsic plasticity is known to occur following inhibitory avoidance learning, as well as trace fear conditioning. In inhibitory avoidance training, rodents are exposed to a context consisting of a dark compartment and a lit compartment. A shock is presented when rodents move to the dark compartment, and successful learning is usually observed as an increase in escape latency (i.e. an increase in the length of time it takes a rodent to "escape" to the dark, but also shock-associated, compartment). Inhibitory avoidance learning reduces the postburst AHP and suppresses spike frequency adaptation in both dorsal and ventral hippocampal CA1 neurons, and this learning-related increase of intrinsic excitability is prevented by inactivation of BLA (Farmer & Thompson, 2012). This suggests inhibitory avoidance learning is regulated via amygdala-hippocampus networks. Trace fear conditioning also induces distinct changes of excitability within dorsal hippocampal neurons. Reduced spike frequency adaptation and/or reduced postburst AHP are evident in dorsal hippocampal CA1 neurons following trace fear conditioning (Kaczorowski & Disterhoft, 2009; McKay et al., 2009; Song et al., 2012). Enhanced dorsal hippocampal CA1 intrinsic excitability is also thought to be learning-specific, such that neurons from good learners demonstrate the greatest increase of excitability following trace fear conditioning, whereas neurons from poor learners display excitability changes that are comparable with that of neurons from control animals (Kaczorowski & Disterhoft, 2009; Song et al., 2012). To our knowledge, no work has yet investigated the effect of hippocampus-dependent trace or context fear conditioning on intrinsic excitability within ventral hippocampal CA1 neurons.

Fear extinction engages distinct intrinsic plasticity mechanisms in the infralimbic (IL) and PL subregions of the mPFC. While delay fear acquisition suppresses IL intrinsic excitability, delay fear extinction enhances it (Santini et al., 2008). In contrast, trace fear conditioning simultaneously increases excitability of IL-BLA projection neurons while reducing excitability of PL-BLA projections, but trace fear extinction reverses these effects such that IL-BLA projections demonstrate suppressed excitability but PL-BLA projections show enhanced excitability (Song et al., 2015). Thus, fear conditioning-induced changes of intrinsic excitability are reversible following extinction learning.

While the above studies strongly suggest learning alters intrinsic excitability, others demonstrate that direct pharmacological manipulations of intrinsic excitability mechanisms can also modify memory strength. Treatment with the L-type Ca^{2+} channel blocker nimodipine, which reduces the postburst AHP and suppresses spike frequency adaptation (Moyer et al., 1992), enhances trace eyeblink learning in aged rabbits (Deyo et al., 1989). In contrast, activation of small conductance Ca^{2+}-activated K^+ (SK2) channels, which normally underlie the medium component of the postburst AHP (Bond et al., 2004; Faber & Sah, 2007; but see Gu et al., 2008; 2005; Kaczorowski et al., 2007; Kramár et al., 2004; Oh et al., 2000; Stocker et al., 1999; but see Gu et al., 2005; 2008), impedes trace eyeblink conditioning (McKay et al., 2012). Manipulations of CREB expression also influence excitability and learning. Overexpressing CREB in LA neurons enhances fear learning in CREB-deficient mice (Han et al., 2007), and reduces the AHP while increasing spiking activity (Zhou et al., 2009). Further, overexpressing CREB in dorsal hippocampal CA1 neurons of aged rats reduces the AHP in these neurons, and facilitates spatial memory (Yu et al., 2017). Taken together, these studies strongly implicate

intrinsic excitability as a vital plasticity mechanism underlying various forms of learning and memory.

Heterogeneity of Firing Properties Within Fear Networks

Examining the contribution of distinct subpopulations of neurons to learning and memory processes is crucial if we want to examine the contribution of specific brain regions and/or networks to Pavlovian fear conditioning. One way to do this is by dividing neurons into distinct firing type classifications on the basis of their physiological profiles. Distinct firing types are evident in several brain regions involved in fear learning, including the RSC, perirhinal cortex, mPFC, dorsal hippocampal CA1, central amygdala, and LA (Beggs et al., 2000; Faulkner & Brown, 1999; Kurotani et al., 2013; Martina et al., 1999; McGann & Brown, 2000; Moyer et al., 2002; Song et al., 2015; Storm, 1988; Yousuf, Nye, et al., 2020). The predominant firing types observed in these regions include regular-spiking (RS), burst-spiking (BS), late-spiking (LS), and fast-spiking (FS) neurons. Each firing type is proposed to encode distinct types of inputs, and collectively they promote efficient information processing.

The relative proportion of these firing types depends on location, with many recorded neurons in the fear circuit being classified as RS or LS firing types. Within central amygdala, the central medial nucleus (CeM) consists of 95% LS, while the central lateral nucleus (CeL) contains 56% LS and 38% RS neurons (Martina et al., 1999). In the perirhinal cortex, firing type proportions vary between cortical layers. The majority (86%) of layer VI neurons are classified as LS (McGann & Brown, 2000), while layer V consists of only 14% LS neurons (Moyer et al., 2002). Similarly, firing type proportions vary between layers within the RSC. In layer V, the majority of granular RSC (gRSC) neurons are classified as RS, while layer II/III consists primarily of LS neurons (Kurotani et al., 2013; Yousuf, Nye, et al., 2020). Additionally, the

relative proportions of BS and FS neurons in layers II/III and V of gRSC are much lower than the population of RS neurons in either layer II/III or V (Yousuf, Nye, et al., 2020).

RS neurons tend to fire early during current injections close to rheobase (i.e. minimum current required to elicit a spike), display pronounced adaptation in response to a prolonged current, and often demonstrate prominent hyperpolarizing sag (Faulkner & Brown, 1999; Kurotani et al., 2013; Moyer et al., 2002; Yousuf, Nye, et al., 2020; but see Martina et al., 1999). RS neurons can be further subdivided into two distinct types: RS and RS_{ADP}. RS_{ADP} neurons have a pronounced after-depolarization (ADP) after firing a single AP, and are known to trigger bursting in the hippocampus (Azouz et al., 1996; Jensen et al., 1996; Schwartzkroin, 1975). Interestingly, a subpopulation of RS neurons in gRSC display an ADP, and some of these RS_{ADP} neurons occasionally display burst firing (Yousuf, Nye, et al., 2020). Similar patterns have been observed in the subthalamic nucleus, where neurons commonly oscillate between single AP firing and burst firing (Beurrier et al., 1999). Given the propensity for bursting among the RS_{ADP} firing type, and the evidence that Schaffer collateral-CA1 synaptic plasticity requires bursting activity (Pike et al., 1999; Thomas et al., 1998), the ADP is likely an important component of learning-related plasticity.

In contrast to RS neurons, LS neurons are primarily defined by their tendency to display a prolonged depolarizing ramp followed by a spike toward the end of near-rheobase current steps. LS neurons also display distinct subthreshold membrane characteristics, including little to no sag, high input resistance, and large time constants (Beggs et al., 2000; Chu et al., 2003; Faulkner & Brown, 1999; Kurotani et al., 2013; Martina et al., 1999; McGann & Brown, 2000; Moyer et al., 2002). Suprathreshold properties of LS neurons include little to no spike frequency adaptation (Kurotani et al., 2013; Storm, 1988) and enlarged AHPs (Chu et al., 2003; Moyer et

al., 2002). Morphologically, LS neurons can be heterogenous, displaying somatic and dendritic characteristics that vary between locations. For instance, LS neurons in layer II/III of gRSC tend to have small somas and reduced dendritic complexity and branching when compared to RS neurons (Kurotani et al., 2013; Yousuf, Nye, et al., 2020). In contrast, LS neurons in CeM and CeL display no consistent morphological differences when compared with other firing types (Martina et al., 1999). LS neurons in layers V and VI of perirhinal cortex also display heterogenous morphologies (McGann & Brown, 2000; Moyer et al., 2002). Thus, the mechanisms governing the LS firing property may depend more on morphological properties like dendritic arborization in the RSC, whereas in other brain regions like the amygdala and perirhinal cortex LS function may be less reliant on so-called "fixed" properties like morphology. Instead, LS firing may rely on more dynamic mechanisms, such as ionic conductance, in those regions.

Indeed, several studies indicate K^+ currents underlie the delayed firing that is so prevalent in LS neurons. Kv1.1 and Kv1.2 underlie the LS firing property in rat striatal medium spiny neurons (Shen et al., 2004), and in the superior colliculus (Saito & Isa, 2000). Layer II of gRSC expresses elevated levels of Kv1.1, Kv1.4 and Kv4.3, and blockade of these channels reduces latency to onset of first spike (Kurotani et al., 2013). Similarly, application of 4-aminopyridine (4-AP) to block slowly inactivating K^+ currents (e.g. I_D) reduces spike latency but also increases input resistance in LS neurons of the CeM (Martina et al., 1999). Dorsal hippocampal CA1 LS neurons are also sensitive to voltage-gated K^+ currents. Application of 4-AP minimizes spike onset latency, while application of either muscarine (used to block M current, or I_M) or TEA (used to block slow delayed rectifier current, or I_K) does not reduce time to first spike (Storm, 1988). Functionally, LS neurons are poised to play a role in information encoding over extended

periods of time (McGann & Brown, 2000; Storm, 1988; Tieu et al., 1999). A series of identical direct current injections in dorsal hippocampal CA1 LS neurons gradually increased responding until a spike was fired, and application of 4-AP minimized this effect, again suggesting this pattern of responding in dorsal CA1 LS neurons relies on I_D (Storm, 1988). This indicates depolarizing synaptic stimulation can likely be integrated over a long period of time among LS neurons, and suggests the LS firing type could be particularly suitable for prolonged information encoding.

Significance and Summary

The ventral hippocampus plays a critical role in associative fear learning. Lesions and/or inactivation techniques targeted to this brain region lead to dramatic impairments in trace and contextual fear memory. However, unlike the dorsal hippocampus, little is known about the role of fear conditioning in ventral hippocampal plasticity. Learning-related plasticity is a critical component of various forms of learning and memory, and such plasticity can emerge as changes in molecular signaling and/or physiological function. Thus, the overall goal of this dissertation is to gain a better understanding of the role of the ventral hippocampus in associative fear learning by examining how distinct forms of fear conditioning, specifically context and trace fear conditioning, modify molecular and physiological plasticity in the ventral hippocampus. In chapter two, we examine the expression patterns of CREB and Arc proteins in the dorsal and ventral hippocampus following context and trace fear memory retrieval. In chapter three, we examine how trace and context fear learning affect intrinsic excitability in ventral hippocampal CA1 neurons, and how this excitability varies between firing types. Our data suggest that context fear memory retrieval increases Arc and pCREB protein expression in the ventral hippocampus, and reduces Arc levels in the dorsal hippocampus. In contrast, trace fear retrieval has no effect

Arc or pCREB protein expression in either dorsal or ventral hippocampus. Further, patch-clamp recordings in ventral hippocampal CA1 neurons reveal context fear conditioning increases intrinsic excitability in RS neurons, while trace fear conditioning bidirectionally modifies intrinsic excitability in LS neurons. Increased intrinsic excitability is evident in ventral hippocampal LS neurons from good learners, while LS neurons from poor learners demonstrate suppressed excitability. Together, these experiments provide a greater understanding of the role of the ventral hippocampus in two distinct forms of associative fear learning, and raise interesting questions about the underlying mechanisms supporting these changes in plasticity. Ultimately, the ventral hippocampus likely plays a nuanced role in fear conditioning by recruiting distinct subpopulations of neurons to support the fear memory trace.

Chapter Two: Context Fear Induces Arc and pCREB Expression in the Ventral Hippocampus

Introduction

Associative fear learning can trigger molecular signaling cascades, inducing expression of specific proteins that are essential for learning and memory. These include the transcription factor CREB and its downstream target Arc. CREB is known to play a vital role in several forms of fear conditioning, including trace and context fear. Trace fear conditioning increases hippocampal pCREB protein expression (Hwang et al., 2010), and trace as well as context fear memory deficits arise when dorsal hippocampal CREB expression is disrupted (Fisher et al., 2017; Peters et al., 2009). Both trace and context fear conditioning induce Arc expression in dorsal and ventral hippocampus (Czerniawski et al., 2011), as does trace fear retrieval (Chia & Otto, 2013). Immunohistochemical analysis of Arc expression within hippocampal subregions reveals distinct expression patterns following trace and context fear conditioning. Context fear conditioning increases Arc in ventral CA1 but not in ventral CA3, dorsal CA1, or dorsal CA3, while trace fear conditioning increases Arc in dorsal CA3, ventral CA1, and ventral CA3 but not in dorsal CA1 (Hudgins & Otto, 2019). Though these studies together clearly support a role for both CREB and Arc in hippocampal-dependent fear learning, no work to our knowledge has examined the effect of trace or context fear retrieval on Arc and pCREB protein levels in dorsal and ventral hippocampus from the same animals.

Thus, the current experiments investigate the expression of Arc and pCREB in the dorsal and ventral hippocampus following foreground context or trace fear memory retrieval. Overall, our data indicate that Arc and pCREB expression is elevated in the ventral hippocampus following context fear retrieval, but not following trace fear retrieval. We also observed reduced

Arc expression in the dorsal hippocampus following context fear retrieval, but no other changes in dorsal hippocampal IEG expression were evident. Thus, the ventral hippocampus demonstrates pronounced activation following retrieval of unsignaled context-shock associations.

Methods

Subjects

Subjects were 49 adult male F344 rats (3-6 mo.) maintained on a 14 h light/10 h dark cycle and housed individually with free access to food and water in an AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care) accredited facility. All procedures followed guidelines from the University of Wisconsin – Milwaukee Animal Care and Use Committee and NIH.

Apparatus

Plexiglas and stainless-steel rectangular chambers (30.5 × 25.4 × 30.5 cm; Coulbourn Instruments) were used to conduct fear conditioning. Each chamber had a grid floor consisting of 26 parallel steel rods (each 5 mm in diameter, with 6 mm spacing) that delivered a scrambled footshock US via a precision adjustable shock generator (Coulbourn Instruments). Each training chamber was located within a separate sound-attenuating box, where a ventilation fan produced a constant background noise of ~58dB (measured by a sound meter, Radioshack), and miniature incandescent white lamps (28 V, type 1819) provided illumination. Before each conditioning session, training chambers were wiped with 5% ammonium hydroxide. Room lights were left on during conditioning.

Auditory cued fear tests were conducted in separate Plexiglas chambers (contained within their own sound-attenuating boxes) located in the same room as the conditioning chambers.

These chambers were octagonal and contained black-painted Plexiglas floors with an array of drilled holes, and included a tray underneath the floor that contained clean bedding. Infrared light was used for illumination (room lights were off during cue tests), and the chambers were wiped with 2% acetic acid. These chambers were thus distinct from the conditioning chambers in terms of visual, tactile and olfactory cues. Context tests were conducted in the same chambers as fear conditioning. Chambers were wiped with 5% ammonium hydroxide and room lights were left on during context testing.

Freezing was recorded using a remote CCTV video camera (model STC-MB33USB; Sentech) mounted to the top of each chamber. Video data were viewed and acquired using a computer running FreezeFrame 4.01 (Actimetrics, Coulbourn Instruments). Freezing data were analyzed using FreezeView 4.01. A 1 s bout of immobility was scored as freezing.

Fear Conditioning

Rats were handled for at least 1 week before being randomly assigned to one of five experimental conditions: Naïve, Pseudo, Trace, Chamber-exposed, or Context (see Figure 3A for experimental setup). Naïve rats remained in the home cage throughout the experiment. Trace fear conditioned rats received one 10-trial session of CS-US pairings, where each trial consisted of a 15 s, 80 dB white noise CS followed by a 30 s stimulus-free TI, ending with a 1 s, 1 mA footshock US. Each trial was separated by a 5.2 min (±20%) inter-trial interval (ITI) to minimize background context and maximize freezing to the CS (Detert et al., 2008). Pseudo conditioned rats received 10 CS and 10 US presentations identical to those used for trace fear conditioning, except they were explicitly unpaired. The training session for both groups lasted 60 min. Percent freezing during the TI was used to assess fear acquisition.

Context conditioned rats received one 10-trial session of 1 s, 1 mA unsignaled footshock US presentations over 60 min. Chamber-exposed rats were exposed to the training context for 60 min in the absence of the US. The training session was divided into 10 bins (6 min each), and average percent freezing during each bin was used to assess fear acquisition. Rats were returned to their home cages immediately after the training session.

Retrieval Test

Testing occurred 24 h after conditioning. Pseudo and Trace rats received a brief cue test in a novel context. Following a 2 min baseline, these rats received two CS-alone presentations (15 s, 80 dB white noise) separated by a 2.9 min ITI. Rats were removed from the test chamber ~2 min after the second trial and placed in their home cages. Since several Trace rats displayed near-ceiling levels of average TI freezing during the cue test, baseline freezing was subtracted from average CS (ΔCS) and average TI (ΔTI) freezing for each rat to better observe individual differences in freezing. To test context fear memory, Chamber-exposed and Context rats were exposed to the original training context in the absence of the US for 10 min, then returned to their home cages. Average percent freezing during the entire 10 min context test was used to assess context fear memory. Rats were sacrificed 60 min after the test session.

Western Blot

To determine the role of the hippocampus in fear memory, western blot analysis was used to assess protein levels of the IEG Arc and the transcription factor CREB (both total and phosphorylated). Rats were anesthetized with an overdose of isoflurane 60 min following behavioral testing. Brains were rapidly removed, frozen on dry ice, and stored at -80°C. Bilateral dorsal and ventral hippocampal tissue was dissected from each rat and homogenized separately.

Tissue samples were centrifuged at 4000 rpm for 20 min at 4°C. The supernatant was removed, and protein levels were assessed using a Lowry protein assay.

Samples were normalized (50µg/lane) and loaded on 10% TGX gels (Bio-Rad). Proteins were then transferred to PVDF membranes using a Turbo Transfer System (Bio-Rad). Membranes were rinsed for 5 min in dH20, incubated in blocking buffer (3% nonfat dry milk in TBS) for 1 h at room temperature, then incubated in antibody buffer (3% nonfat dry milk in TBS with 0.05% Tween-20) with primary antibody overnight at 4°C (mouse anti-Arc (1:500), mouse anti-pCREB-1 (1:1000), mouse anti-CREB-1 (1:1000), and mouse anti-β-actin (1:1000)). All primary antibodies were obtained from Santa Cruz Biotechnology.

The following day, membranes were rinsed with antibody buffer, then incubated with secondary antibody (anti-mouse (1:1000, R&D Systems)) for 90 min at room temperature. Membranes were washed with buffer containing 0.05% Tween-20 in TBS then incubated with chemiluminescence solution (SuperSignal West Dura, Thermo) for 5 min. A G-Box Chemi XT4 camera (Syngene) was used to develop and take images. Optical density for each sample was analyzed using GeneSYS software (Syngene). Arc, p-CREB, and CREB protein levels were normalized to β-actin by calculating an optical density ratio (e.g. Arc protein expression was calculated by dividing Arc optical density by β-actin optical density). This optical density ratio was then divided by the average optical density ratio of the Naïve group, and the resulting quotient was multiplied by 100 to obtain a percent of Naïve score for each sample.

Statistical Analyses

Microsoft Excel or SPSS (IBM SPSS Statistics 26) were used to analyze overall treatment effects via Student's *t*-test, one-way ANOVA or repeated-measures ANOVA, where

appropriate. Fisher's least significant difference (LSD) was used for *post hoc* comparisons. Data are expressed as mean ± 1 SEM.

Results

Behavioral Performance During Fear Conditioning and Testing

Percent freezing during the TI of each trial was used to analyze behavioral performance during the conditioning session for Pseudo and Trace rats (Figure 3B). Both groups displayed comparable freezing during the first half of the conditioning session, while during the second half Trace rats displayed consistently elevated freezing relative to Pseudo rats. Repeated-measures ANOVA using trial as a within-subjects factor and behavior group as a between-subjects factor revealed a significant main effect of trial ($F_{(9, 144)} = 9.6$, $p < .001$), a significant main effect of group ($F_{(1, 16)} = 9.5$, $p < .01$), and a significant trial by group interaction ($F_{(9, 144)} = 3.7$, $p < .001$).

During the cue test we analyzed the effect of trace and pseudo conditioning on percent ΔCS freezing and percent ΔTI freezing. Since several Trace conditioned rats displayed near-ceiling levels of TI freezing during the cue test, we chose to subtract baseline freezing from CS and TI freezing to better observe individual differences in behavioral performance. Results of a two-tailed Student's *t*-test comparing ΔCS freezing between Pseudo and Trace groups revealed no significant effect of training condition ($t_{(16)} = -1.3$, $p = 0.2$). In contrast, ΔTI freezing was significantly higher for Trace rats relative to Pseudo rats ($t_{(10.4)} = -2.4$, $p < .05$), indicating successful trace fear memory retrieval for Trace rats.

Figure 3

A.

Group	Day 1	Day 2
Naive	—	—
Pseudo	10 CS & 10 US (unpaired)	Cue test
Trace	10 CS-US (paired)	Cue test
Chamber-exposed	60 min chamber exposure	Context test
Context	10 US	Context test

brains removed 60 min post-test

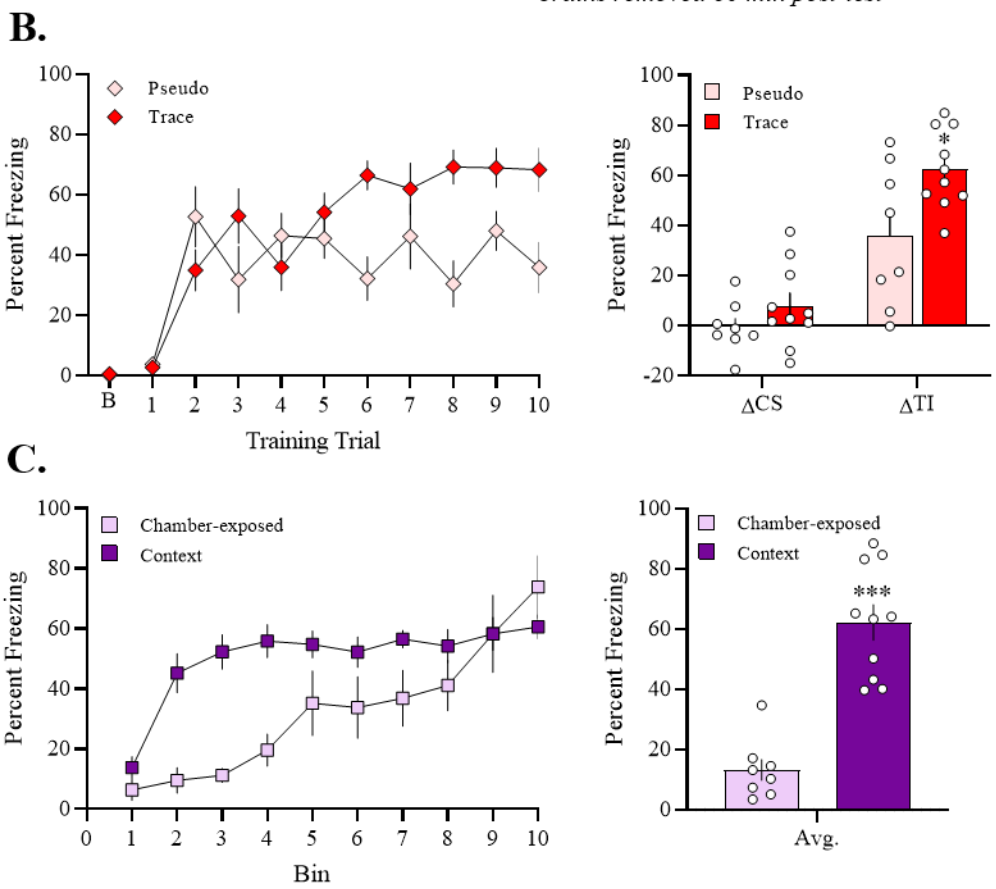

Figure 3. Experimental design, and behavioral performance for trace and context fear conditioned rats. ***A,*** Rats were randomly assigned to one of five experimental conditions. Naïve rats remained in the home cage throughout the experiment. Pseudo rats received 10 unpaired CS and US presentations, while Trace rats received 10 CS-US pairings with a 30 s trace interval separating CS and US. Pseudo and Trace groups received a brief CS test session in a novel context the following day. Context conditioned rats received 10 US presentations over 60 min, while Chamber-exposed rats were exposed to the training context for 60 min without the US. Chamber-exposed and Context rats received a 10 min test session in the original training context the next day. All behaviorally trained groups were sacrificed 60 min following the test session. ***B,*** *Left,* During the training session, Trace rats displayed increased freezing over the first five trials, and maintained consistently elevated freezing over the second half of the training session. Pseudo rats maintained freezing levels that were consistently below that of Trace rats for the later part of the training session. *Right,* During the cue test, Trace rats displayed significantly greater ΔTI freezing when compared to Pseudo rats. ***C,*** *Left,* Context rats displayed greater freezing relative to Chamber-exposed rats for most of the context conditioning session. *Right,* During the context test, Context rats froze significantly more than Chamber-exposed rats. *Abbreviations:* baseline (B); conditional stimulus (CS); trace interval (TI); **p* < .05; ****p* < .001.

For Chamber-exposed and Context groups the training session was divided into ten 6 min bins, and percent freezing was analyzed separately for each bin (Figure 3C). Context rats displayed consistently elevated freezing levels compared to Chamber-exposed rats for the majority of the training session. A repeated-measures ANOVA using bin as a within-subjects factor and behavior group as a between-subject factor revealed a significant effect of bin ($F_{(3.1, 49.7)} = 17.0$, $p < .001$), a significant main effect of behavior group ($F_{(1, 16)} = 9.4$, $p < .01$), and a significant bin by group interaction ($F_{(3.1, 49.7)} = 4.8$, $p < .01$). Analysis of percent freezing during the entire 10 min context test was conducted using a two-tailed Student's *t*-test. Context rats demonstrated significantly elevated freezing during this test compared with Chamber-exposed rats ($t_{(16)} = 6.7$, $p < .001$), thus demonstrating successful context fear memory retrieval.

Arc, pCREB Protein Expression is Increased in the Ventral Hippocampus Following Context Fear Retrieval

To determine the roles of the dorsal and ventral hippocampus in trace and context fear learning, protein expression of Arc, pCREB and total CREB was examined 1 h following testing. All protein levels were normalized to the loading control (β-actin) and were expressed as a percent of Naïve control. No differences in β-actin were observed either between behavior conditions or between brain regions.

Arc protein expression was altered in both a region- and learning-specific manner (Figure 4). In the dorsal hippocampus, Arc expression remained unchanged following trace fear retrieval, but was reduced following context fear retrieval. A one-way ANOVA comparing Naïve, Chamber-exposed, and Context groups revealed a trending effect of behavior group ($F_{(2, 27)} = 2.9$, $p = .075$), and multiple comparisons indicated dorsal hippocampal Arc expression was

significantly reduced following context fear retrieval relative to memory retrieval for a neutral context ($p < .05$; Figure 4A).

Figure 4

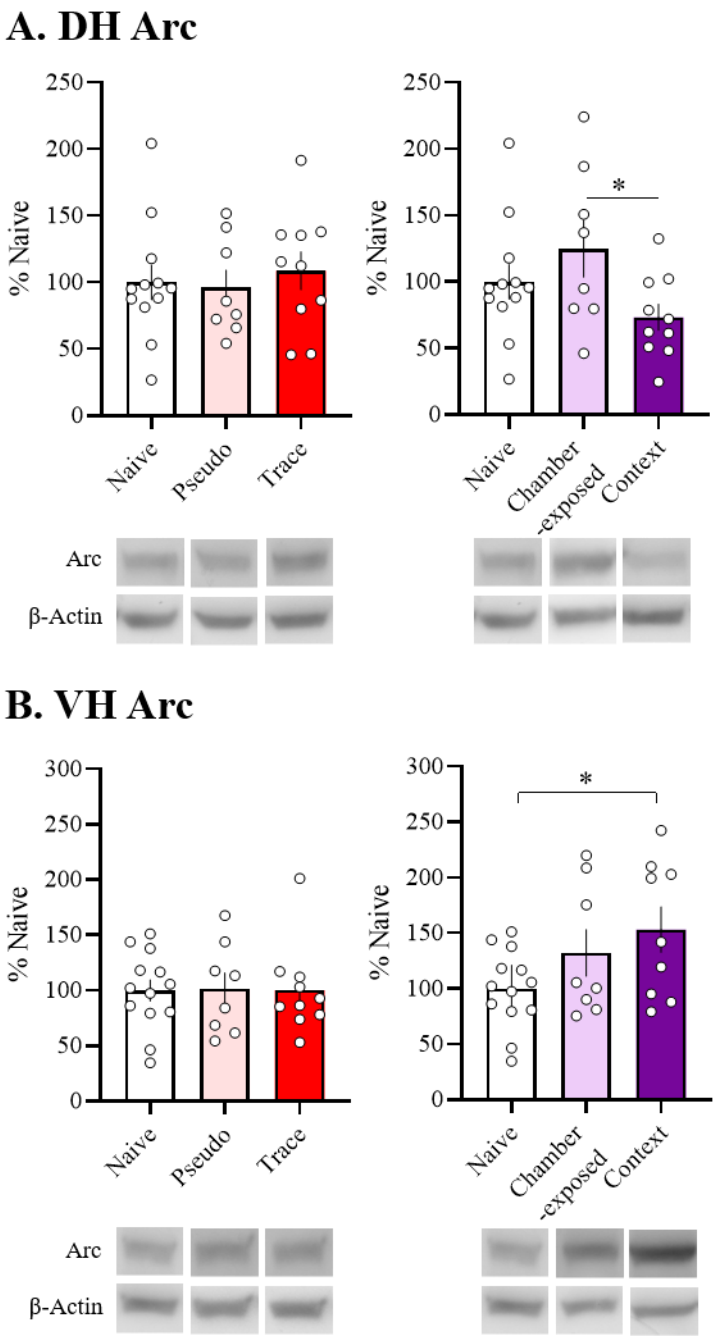

Figure 4. **Bidirectional change of Arc expression in dorsal and ventral hippocampus following context fear retrieval. *A,*** Dorsal hippocampal Arc expression as a percent of Naive. *Left,* Arc protein expression remains unchanged in dorsal hippocampus following retrieval of a trace fear memory. *Right,* Context fear retrieval significantly reduces dorsal hippocampal Arc protein relative to chamber-exposed controls. ***B,*** Ventral hippocampal Arc expression as a percent of Naive. *Left,* Arc protein expression remains unchanged in the ventral hippocampus following trace fear retrieval. *Right,* Context fear retrieval significantly increases ventral hippocampal Arc expression relative to Naïve levels. *Abbreviations:* dorsal hippocampus (DH); ventral hippocampus (VH); *$p < .05$.

Similar to Arc expression in the dorsal hippocampus, Arc expression in the ventral hippocampus following trace fear retrieval remained unchanged. However, context fear retrieval increased ventral hippocampal Arc protein expression relative to Naïve controls (Figure 4B). One-way ANOVA comparing Naïve, Chamber-exposed, and Context groups indicated a trending effect of behavior group ($F_{(2, 27)} = 3.0$, $p = .065$), and multiple comparisons further revealed a significant difference between Context and Naïve groups ($p < .05$). These data suggest that while trace fear memory retrieval has no effect on Arc protein expression in either dorsal or ventral hippocampus, context fear memory retrieval leads to bidirectional effects on hippocampal Arc – increasing Arc protein in ventral hippocampus while reducing it in dorsal hippocampus.

Hippocampal pCREB protein expression was also examined following trace and context fear retrieval (Figure 5). Overall, no effects of either trace or context fear memory retrieval on pCREB expression in the dorsal hippocampus were observed (Figure 5A). In the ventral hippocampus, pCREB expression remained unchanged following trace fear retrieval, but was significantly increased following context fear retrieval (Figure 5B). A one-way ANOVA comparing Naïve, Chamber-exposed, and Context groups revealed a significant effect of group ($F_{(2, 28)} = 3.4$, $p < .05$). Multiple comparisons further revealed ventral hippocampal pCREB protein levels were significantly increased for Context rats relative to Naïve controls ($p < .05$). Furthermore, analysis of total CREB protein expression revealed no significant effects of either trace or context fear memory retrieval in either the dorsal or ventral hippocampus (Figure 6). Thus, pCREB expression is selectively increased in the ventral hippocampus following context fear memory retrieval.

Relationship Between Behavior and Arc, pCREB Expression

To determine the relationship between memory retrieval and hippocampal plasticity we analyzed the correlation between percent freezing during the retrieval tests and protein expression of Arc and pCREB (Figure 7). Percent freezing during the context test for Chamber-exposed rats was positively correlated with Arc expression in the ventral hippocampus ($r = .850$, $p < .01$; Figure 7B). However, no other group demonstrated a significant relationship between fear retrieval and Arc expression in the dorsal (Figure 7A) or ventral hippocampus (Figure 7B).

Similarly, there was a weak relationship between pCREB levels and fear retrieval for most groups. The exception was the Trace group, which paradoxically demonstrated a negative relationship between freezing levels and dorsal hippocampal pCREB expression ($r = -.65$, $p < .05$; Figure 7C). This suggests that better trace fear retrieval is linked to reduced pCREB. Although this result is inconsistent with previous research that suggests trace fear conditioning increases dorsal hippocampal IEG expression, it is consistent with our current results that suggest trace fear memory retrieval leads to no change in Arc or pCREB levels in the dorsal hippocampus. Overall, these data suggest that percent freezing during a retrieval test is not closely linked with hippocampal Arc or pCREB induction.

Figure 5

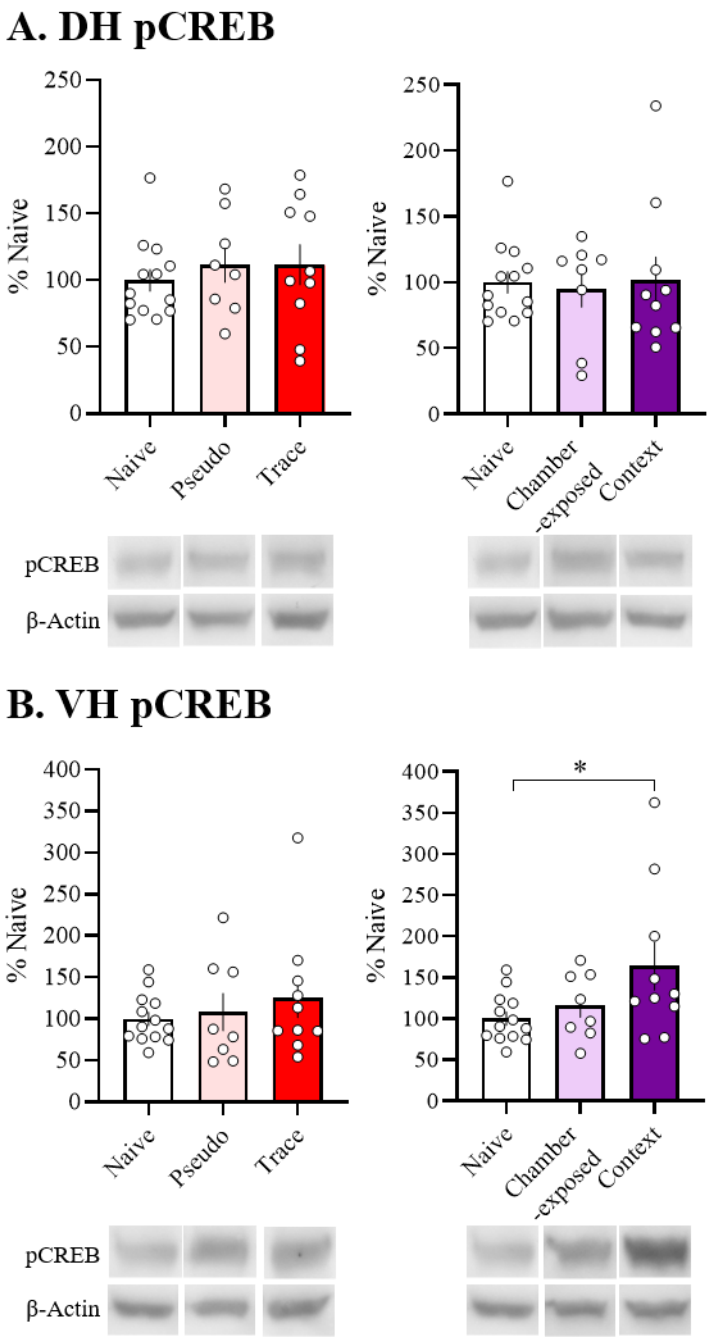

Figure 5. Context fear retrieval increases pCREB expression in ventral hippocampus. ***A,*** Dorsal hippocampal pCREB expression as a percent of Naive. Dorsal hippocampal pCREB levels remain unchanged following either trace (*left*) or context (*right*) fear memory retrieval. ***B,*** Ventral hippocampal pCREB expression as a percent of Naive. *Left,* pCREB protein expression remains unchanged in the ventral hippocampus following trace fear retrieval. *Right,* Context fear retrieval significantly increases ventral hippocampal pCREB expression relative to Naïve levels. *Abbreviations:* dorsal hippocampus (DH); ventral hippocampus (VH); **p* < .05.

Figure 6

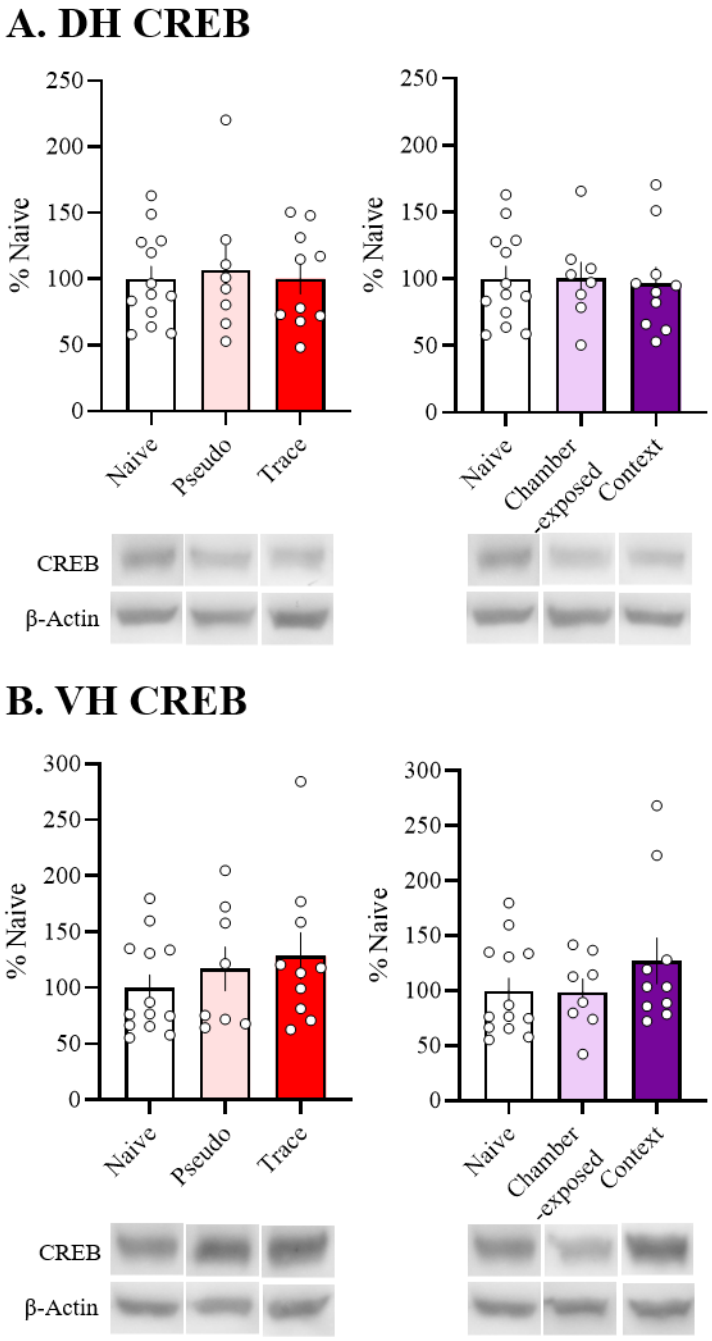

Figure 6. No change in total CREB expression in dorsal or ventral hippocampus following trace or context fear retrieval. ***A, B,*** Total CREB expression as a percent of dorsal hippocampal Naïve (***A***) or ventral hippocampal Naïve (***B***). *Abbreviations*: dorsal hippocampus (DH); ventral hippocampus (VH).

Positive Relationship Between Arc and pCREB Following Trace and Context Fear Retrieval

We also examined whether Arc levels were correlated with pCREB levels to determine the degree of co-activation of these two plasticity proteins, and whether these patterns varied between behavior conditions (Figure 8). In the dorsal and ventral hippocampus, Arc and pCREB expression demonstrated a significant positive correlation within the Trace group (dorsal hippocampus: $r = .692$, $p < .05$; ventral hippocampus: $r = .868$, $p < .01$), while Arc and pCREB correlation was significant for the Context group in the dorsal hippocampus only ($r = .813$, $p < .01$). When all behavior groups were combined, pCREB was significantly correlated with Arc in both dorsal ($r = .409$, $p < .01$) and ventral hippocampus ($r = .413$, $p < .01$). Further, behavioral controls failed to display significant Arc and pCREB correlations. This suggests that trace and context fear learning may lead to stronger co-activation of these proteins relative to controls that do not learn. The lack of a significant relationship between pCREB and Arc expression in the ventral hippocampus following context fear retrieval is somewhat puzzling due to our concurrent findings that indicate context fear induces both pCREB and Arc expression selectively in the ventral hippocampus. This suggests that context fear memory retrieval may recruit slightly different signaling mechanisms in different animals, or perhaps that distinct populations of neurons support context fear memory. In the present study, we can only conclude that Context rats that display elevated ventral hippocampal levels of pCREB may not also display elevated ventral hippocampal Arc expression, and vice versa. Overall, these data suggest that elevated expression of one plasticity protein is closely tied to increased expression of others, and this pattern is learning-specific (i.e. it occurs primarily within Trace and Context groups) and is also possibly region-specific (i.e. following context fear retrieval, there is a positive correlation

between dorsal hippocampal levels of pCREB and Arc, but there is no such correlation in ventral hippocampus).

Figure 7

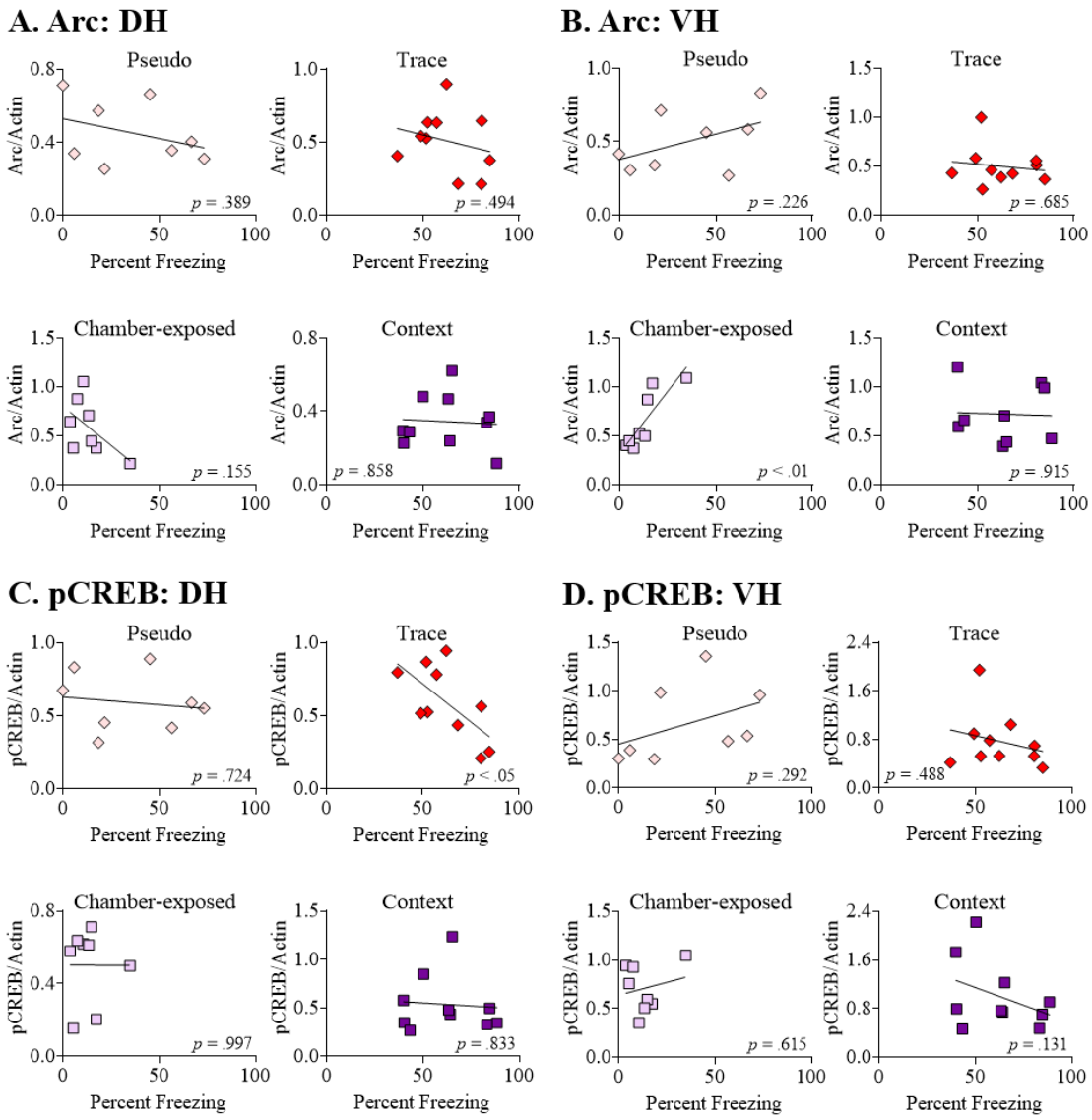

Figure 7. Correlations between fear memory and IEG expression. ***A & B,*** Correlation between percent freezing during the test and Arc expression in dorsal (***A***) and ventral hippocampus (***B***). Arc expression in ventral hippocampus of Chamber-exposed rats is significantly positively correlated with freezing percent during the context test ($p < .01$). There is no significant correlation between Arc expression and freezing percent in dorsal or ventral hippocampus in any other group. ***C & D,*** Correlation between percent freezing during the test and pCREB expression in dorsal (***C***) and ventral hippocampus (***D***). In dorsal hippocampus of Trace rats, pCREB is significantly negatively correlated with percent freezing during the cue test ($p < .05$). There is no significant correlation between pCREB and freezing for any other group in dorsal or ventral hippocampus. *Abbreviations:* dorsal hippocampus (DH); ventral hippocampus (VH).

Figure 8

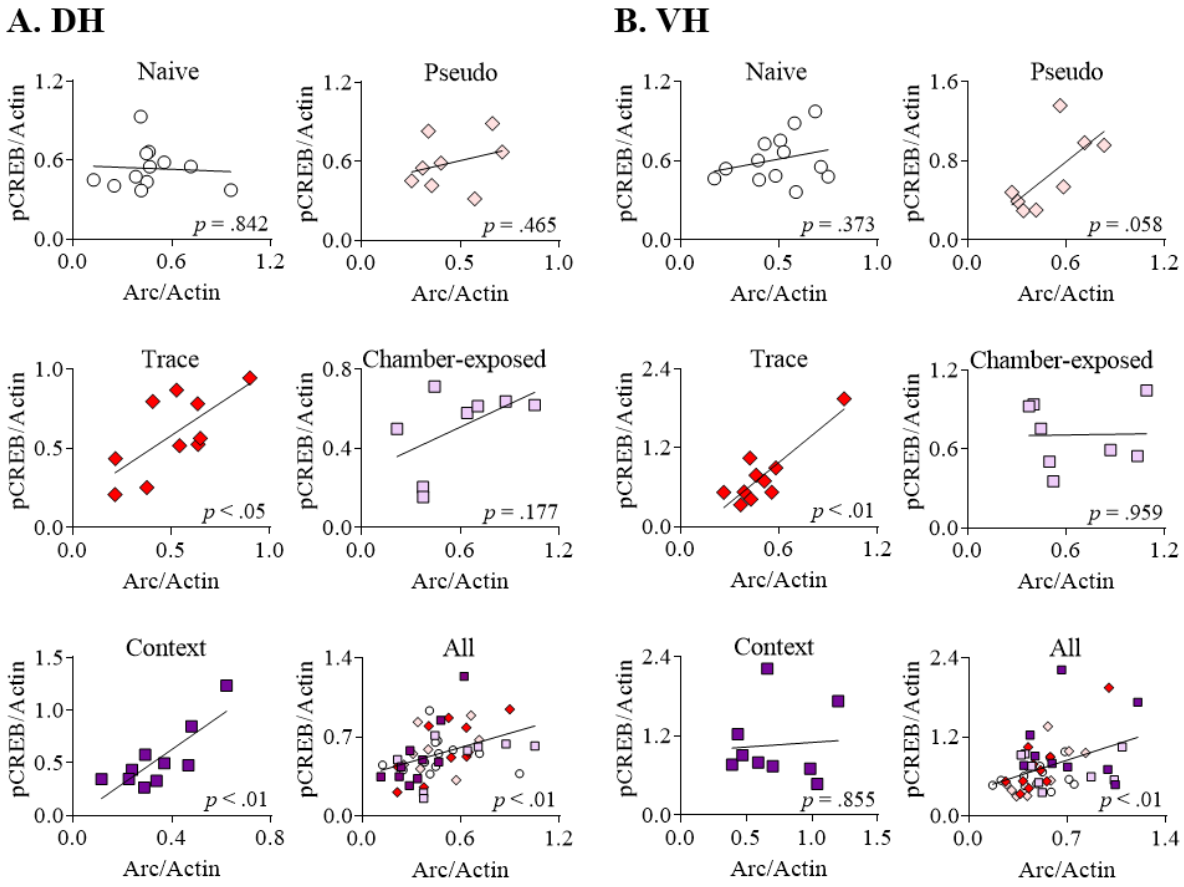

Figure 8. Correlations between Arc and pCREB protein expression. ***A,*** In the dorsal hippocampus, Arc is positively correlated with pCREB expression following trace fear conditioning ($p < .05$), context fear conditioning ($p < .01$), and in all behavior groups combined ($p < .01$). ***B,*** In the ventral hippocampus, Arc is positively correlated with pCREB expression following trace fear conditioning ($p < .01$) but not context fear conditioning. Additionally, Arc and pCREB levels are positively correlated when all behavior groups are combined ($p < .01$). *Abbreviations:* dorsal hippocampus (DH); ventral hippocampus (VH).

Discussion

In the current experiments we examined a role for two forms of Pavlovian fear learning, context and trace fear, on differential activation of dorsal and ventral hippocampus during the retrieval of these memories. Our data suggest that foreground context fear memory retrieval induces ventral hippocampal activity, as indicated by increased Arc and pCREB expression. In contrast, no significant changes in pCREB or Arc protein expression were observed following trace fear retrieval in either dorsal or ventral hippocampus. These data suggest that context fear memory retrieval selectively induces ventral hippocampal activity that likely supports context fear consolidation.

Subregion-specific analyses of fear conditioning-induced Arc expression suggest trace fear conditioning increases Arc levels in ventral CA1 and CA3, and in dorsal CA3 but not dorsal CA1 (Hudgins & Otto, 2019). Trace fear memory is also disrupted when Arc expression is blocked in either dorsal or ventral hippocampus (Chia & Otto, 2013; Czerniawski et al., 2011). In contrast, our data suggest no change in Arc or pCREB expression in either dorsal or ventral hippocampus following trace fear retrieval. These discrepant findings could be due differential IEG induction at different points in the memory process. For instance, the current experiments examined Arc and pCREB induction following retrieval, whereas Hudgins & Otto (2019) looked at Arc expression following conditioning (but see Chia & Otto, 2013). Another possibility is that fear learning-induced IEG expression is subregion-specific, and there are in fact changes in Arc and/or pCREB activity in dorsal and ventral hippocampus following trace fear retrieval, but these changes are obscured by the fact that we were unable to detect subregion-selective changes using western blotting. Therefore, a critical next step would be to examine Arc and pCREB expression

within distinct hippocampal subregions (e.g. CA1 and CA3) to determine more specific effects of trace fear retrieval on IEG induction in dorsal and ventral hippocampus.

In contrast to trace fear, our data suggest that context fear retrieval increases ventral hippocampal Arc and pCREB expression, while dorsal hippocampus demonstrates a reduction in Arc and no change in pCREB. This suggests that ventral hippocampal Arc and pCREB activity supports reconsolidation of contextual fear memory. These data are consistent with previous findings demonstrating context fear conditioning selectively increases Arc expression in ventral CA1 (Hudgins & Otto, 2019), and suggests that ventral hippocampal activity supports context fear memory. Indeed, other evidence supports a role for the ventral hippocampus *rather than* the dorsal hippocampus in context fear. Lesions or inactivation of ventral, but not dorsal hippocampus, disrupt context fear retrieval (Czerniawski et al., 2012; Czerniawski et al., 2009; Pentkowski et al., 2006; Trivedi & Coover, 2004; Zhu et al., 2014). Further, the reduction of dorsal hippocampal Arc levels following foreground context fear retrieval observed in the current experiments suggests dorsal hippocampal Arc activation may actually be detrimental for consolidation of contextual fear memory. In support, previous research indicates dorsal hippocampal lesions disrupt memory for background context fear associations, but not foreground context fear (Phillips & LeDoux, 1994). An important next step would be to examine the likely divergent effects of foreground vs. background context fear retrieval on IEG induction in distinct regions of the hippocampus. Taken together, the evidence from the current experiments and previous research suggests an essential role for ventral over dorsal hippocampus in foreground context fear memory.

The current experiments also suggest Arc and pCREB protein levels are selectively correlated following trace and context fear retrieval. Other work demonstrates similar patterns of

hippocampal IEG co-activation following various forms of learning. Arc and Homer1a ensembles demonstrate substantial overlap following re-exposure to a familiar context, in contrast to exposure to a novel environment (Vazdarjanova & Guzowski, 2004). Hippocampal-dependent spatial navigation tasks similarly induce co-activation of Arc and Zif-268 mRNA in the dorsal hippocampus (Guzowski et al., 1999; Guzowski et al., 2001). Further, dorsal hippocampal Arc and Zif-268 protein levels demonstrate some overlap following delay fear conditioning (Lonergan et al., 2010). Interestingly, we observed significant correlations between Arc and pCREB in the dorsal hippocampus following trace and context fear retrieval, and in the ventral hippocampus following trace fear retrieval, but no such relationship was evident in the ventral hippocampus following context fear retrieval. This is inconsistent with our concurrent findings suggesting ventral hippocampal levels of Arc and pCREB are selectively increased following context fear retrieval. A further caveat is that the current experiments are unable to determine whether Arc and pCREB co-activation is occurring in the same neurons. Nevertheless, these results suggest that context fear retrieval-induced ventral hippocampal Arc and pCREB expression occurs in different rats, and that perhaps there are individual differences in the way in which these signaling mechanisms support the same fear memory.

Overall, the data from the current experiments suggest foreground context fear memory retrieval selectively increases IEG expression in the ventral hippocampus. Arc and pCREB protein expression are increased in the ventral hippocampus, but not in the dorsal hippocampus, following context, but not trace, fear retrieval. Further, Arc and pCREB levels are correlated following trace fear retrieval in both the dorsal and ventral hippocampus, and following context fear retrieval in the dorsal hippocampus but not in the ventral hippocampus. This suggests that individual differences in the retrieval-induced activation of these proteins support context fear

consolidation in ventral hippocampal neurons. These findings support differential contribution of dorsal and ventral hippocampal signaling to consolidation of contextual and trace fear memories, and further establish specific roles of these hippocampal subregions in fear learning.

Chapter Three: Fear Conditioning Alters Ventral Hippocampal CA1 Intrinsic Excitability in a Learning- and Cell-Type-Specific Manner

Introduction

Learning-related changes of intrinsic excitability are observed in several brain regions following various learning paradigms. These excitability changes include altered spike frequency adaptation and postburst AHP, and are considered by many to underlie successful memory formation (for review, see Sehgal et al., 2013; Yousuf, Ehlers, et al., 2020). Hippocampal neurons display distinct changes of intrinsic excitability following trace fear and eyeblink conditioning. For example, dorsal hippocampal CA1 neurons demonstrate reduced AHP and spike frequency adaptation following trace fear conditioning (Kaczorowski & Disterhoft, 2009; McKay et al., 2009; Song et al., 2012). While trace eyeblink conditioning increases dorsal hippocampal firing rate relative to ventral hippocampal firing rate, a larger proportion of dorsal and ventral hippocampal neurons displayed greater firing rates following trace fear conditioning than pseudo conditioning (Weible et al., 2006). These studies suggest that trace conditioning increases intrinsic excitability in hippocampal neurons, and these changes are not observed in neurons from control animals.

Although the ventral hippocampus has a prominent role in many anxiety- and fear-based learning paradigms (Ferbinteanu & McDonald, 2001; Henke, 1990; Kjelstrup et al., 2002; Ritov et al., 2014), including Pavlovian fear conditioning (Beeman et al., 2013; Cox et al., 2013; Czerniawski et al., 2012; Czerniawski et al., 2009; Esclassan et al., 2009; Gilmartin et al., 2012; Gresack et al., 2009; Hunsaker & Kesner, 2008; Maren & Holt, 2004; Richmond et al., 1999; Rudy & Matus-Amat, 2005; Sierra-Mercado et al., 2011; Yoon & Otto, 2007; Zhang et al., 2001), little is known about the relationship between associative fear learning and intrinsic

excitability in ventral hippocampal neurons. The present experiment therefore examines how trace and context fear conditioning distinctly affect ventral hippocampal CA1 neuronal function using patch-clamp electrophysiological recordings. Our data suggest that context fear conditioning increases intrinsic excitability of ventral hippocampal CA1 RS neurons. In contrast, trace fear conditioning increases intrinsic excitability of ventral hippocampal CA1 LS neurons, but only for LS neurons from rats that displayed exceptionally good trace fear memory. Thus, fear learning-related changes of ventral hippocampal CA1 intrinsic excitability vary as a function of paradigm and firing type.

Methods

Subjects

Subjects were 50 male adult F344 rats (3-6 mo.) that were given free access to food and water and maintained on a 14 h light/10 h dark cycle in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) facility. All procedures were conducted in accordance with the University of Wisconsin-Milwaukee Animal Care and Use Committee (ACUC) and NIH guidelines.

Apparatus

Cued and context fear conditioning were conducted using Plexiglas and stainless-steel rectangular chambers (30.5 × 25.4 × 30.5), which were located in separate sound-attenuating boxes. Grid floors consisting of 26 parallel steel rods (5 mm diameter and 6 mm spacing) were connected to precision adjustable shock generators (Coulbourn Instruments) to deliver a scrambled footshock US. Chambers were illuminated with a miniature incandescent white lamp (28 V, type 1819), and a ventilation fan produced a constant background noise of ~58 dB. Chambers were wiped with 5% ammonium hydroxide before each training session, and room lights were left on. In addition to conditioning, context fear tests were conducted in these

chambers. Similar to the conditioning session, chamber walls were wiped with 5% ammonium hydroxide, and room lights were left on.

Separate Plexiglas chambers, located within separate sound-attenuating boxes, were used to conduct cued fear tests. These had black painted Plexiglas floors with an array of drilled holes, and were octagonal instead of rectangular. A tray beneath the floor contained clean bedding. Chambers were wiped with 2% acetic acid before each test session. Infrared light was used to illuminate each chamber, and room lights were off.

A remote CCTV camera (model STC-MB33USB; Sentech) mounted to the top of each chamber was used to record activity during conditioning and testing. Video data were acquired and viewed using a computer running FreezeFrame 4.01, while freezing data were analyzed using FreezeView 4.01 (Actimetrics, Coulbourn Instruments). Freezing was defined using a 1 s bout of immobility.

Fear Conditioning

Handling occurred for at least 1 week before behavioral training and testing. Rats were randomly assigned to one of five behavioral groups: Naïve, Pseudo, Trace, Chamber-exposed, or Context (see Figure 9A for experimental setup). Naïve rats remained in the home cage throughout the experiment. On day 1, Trace rats received a single 10 trial session of auditory trace fear conditioning, where each CS was a 15 s, 80 dB white noise, and each US was a 1 s, 1 mA footshock. The CS and US were separated by a 30 s TI, and each trial was separated by a 5.2 min (±20%) ITI for maximal CS and minimal background context freezing (Detert et al., 2008). Pseudo rats received 10 CS and 10 US presentations that were explicitly unpaired. Context rats received a single 10 trial conditioning session of 1 s, 1 mA unsignaled US presentations over 60 min. Chamber-exposed rats were exposed to the training chamber in the absence of the US for

the same length of time as the Context group. All behaviorally trained rats were returned to their home cages immediately following the conditioning session.

Figure 9

A.

Group	Day 1	Day 2
Naive	—	— ↓
Pseudo	10 CS & 10 US (unpaired)	Cue test ↓
Trace	10 CS-US (paired)	Cue test ↓
Chamber-exposed	60 min chamber exposure	Context test ↓
Context	10 US	Context test ↓

↓ 400µm coronal brain slices prepared

B.

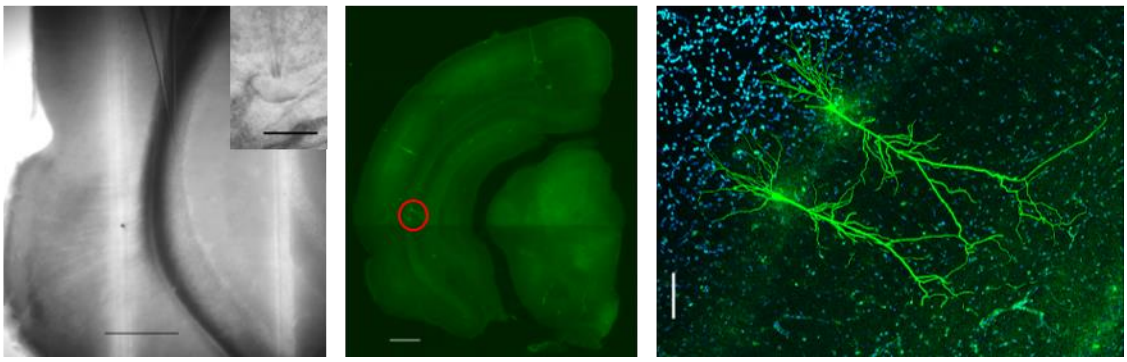

Figure 9. Experimental design and ventral hippocampal CA1 electrophysiological recording. *A,* Rats were randomly assigned to one of five experimental conditions. Trace conditioned rats received one 10-trial session of CS-US pairings, while Pseudo conditioned rats were exposed to 10 unpaired CS and US presentations. Both Trace and Pseudo rats were given a brief cue test the following day in a novel context. Context fear conditioned rats received 10 unsignaled US presentations over a 60 min training session, while Chamber-exposed rats were exposed to the training chamber without the US for 60 min. Both Context and Chamber-exposed rats were placed into the original training chamber for a 10 min context test the following day. Naïve rats remained in the home cage throughout the experiment. ***B,*** *Left,* Representative 2x image of a patch pipette in ventral hippocampal CA1 (scale bar, 500 µm). *Inset,* 60x infrared differential interference contrast image of a representative ventral hippocampal CA1 neuron and patch electrode (scale bar, 10µm). *Middle,* Fluorescent 10x stitched image showing representative biocytin-filled neurons in ventral hippocampal CA1 (circled in red; scale bar, 1000µm). *Right,* Confocal z-stack stitched image taken at 20x showing biocytin-filled ventral hippocampal CA1 neurons with DAPI counterstain (scale bar, 100µm). *Abbreviations:* conditional stimulus (CS); unconditional stimulus (US).

Retrieval Test

Retrieval tests occurred on day 2 (24 h following conditioning). Trace and Pseudo rats received a two-trial auditory CS test session in a novel context. Following a 2 min baseline, rats received two CS-alone presentations (15 s, 80 dB). Trials were separated by a 2.9 min ITI. Rats were removed immediately following testing and returned to their home cages. Average percent freezing during the two trials (CS and TI freezing were calculated separately) was used to assess trace fear memory. Context and Chamber-exposed rats received a single 10 min context test in the original training context. No US presentations occurred during the context test. Rats were immediately returned to their home cages following context testing Average percent freezing over the entire 10 min session was used to assess context fear memory.

Brain Slice Preparation

Immediately after behavioral testing, rats were anesthetized and perfused with ice-cold sucrose cerebral spinal fluid (sCSF; in mM: 2.8 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$, 2 $MgSO_4$, 2 $MgCl_2$, 26 $NaHCO_3$, 206 sucrose, and 10 glucose), bubbled with 95% O_2/5% CO_2. Coronal brain slices (400 µm) containing ventral hippocampus (Bregma -4.8 to -6.0 mm) were cut in ice-cold sCSF using a vibrating tissue slicer (VT1200; Leica). Slices recovered for 30 min in a slice incubation chamber (Moyer & Brown, 1998) containing 32-36°C oxygenated artificial CSF (aCSF; in mM: 124 NaCl, 2.8 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$, 2 $MgSO_4$, 26 $NaHCO_3$, and 20 glucose). Slices were then transferred to a separate incubation chamber containing room temperature (~21-23°C) aCSF for a minimum of 30 additional min before beginning electrophysiological recording.

Electrophysiological Recordings

Slices were transferred to a submerged recording chamber that was continuously perfused with oxygenated aCSF (~2 ml/min). Slices were maintained at ~32°C throughout recording using an in-line temperature controller (Warner Instruments). An Olympus BX51WI upright microscope equipped with a camera (Hamamatsu Camera Ltd.) was used to visualize and photograph neurons and brain slices. Infrared-differential interference contrast (IR-DIC) was used to identify individual neurons suitable for patch-clamp recordings.

A HEKA EPC10 amplifier and ITC-16 digital-to-analog converter (HEKA instruments) were used to obtain and transfer current-clamp recording to a PC. Signals were filtered at 2.9 kHz and digitized at 20 kHz using Patchmaster software (HEKA). Data analysis was conducted offline using Patchmaster and Igor Pro (version 8.0.4.2; Wavemetrics). Recordings were made from both right and left VH hemispheres. Thin-walled capillary glass recording pipettes (A-M systems) were pulled using a Sutter Instruments P97 puller. Voltages were not corrected for liquid-liquid junction potentials (~13 mV; Moyer & Brown, 2007).

Internal solution (in mM: 110 K-gluconate, 20 KCl, 10 di-Tris-P-creatine, 10 HEPES, 2 $MgCl_2$, 2 Na_2ATP, 0.3 Na_2GTP, and 0.2% biocytin) was used to fill patch pipettes and obtain recordings from CA1 neurons. Only neurons with a resting membrane potential (RMP) $\leq$ -50 mV, an input resistance (R_N) $\geq$ 40 MΩ, and an initial series resistance $\leq$ 30 MΩ were included in the analysis. All neurons were held at -67 mV during recordings. Series resistance was fully compensated and continuously monitored throughout recording.

Intrinsic Excitability Measurements

The following protocols were used to record measures of intrinsic excitability under current clamp: (1) Voltage-current (*V-I*) relations were obtained using a series of 500 ms current

injections (from -200 to +190pA) and plotting plateau voltage deflection against current amplitude. Input resistance was calculated from the slope of the linear portion of the *V-I* plot where voltage sweeps did not exhibit sags or active conductance. (2) AP properties were examined using a series of ascending 500 ms depolarizing current pulses. AP threshold (AP_{thresh}) was defined as the voltage when dV/dt first exceeded 28 mV/ms (Kaczorowski et al., 2012). AP amplitude (AP_{amp}) was measured relative to AP_{thresh}. Width of the AP (AP_{width}) was measured as the width at half of AP_{amp}. Fast AHP (fAHP) was measured as the negative-going peak voltage (within 2-5 ms) relative to AP threshold. (3) Since the amplitude of the postburst AHP can be influenced by variations in AP timing, the AHP was evoked by applying a brief (2 ms) series of 10 regularly-spaced somatic current injections at 50 Hz (3 × at 20 s intervals). The peak AHP amplitude during the first 150 ms after current offset was used to analyze the medium AHP (mAHP), while the AHP amplitude at 1 s after current offset was used to analyze the slow AHP (sAHP). The AHP amplitude was also measured at various timepoints after the last AP. (4) Spike frequency adaptation was measured using a series of 1 s depolarizing current steps (50-450pA, 50pA increments, 20 s intervals). The number of APs elicited during each sweep was counted. To aid in firing type classification (see below), adaptation index was calculated for each neuron by dividing steady-state firing frequency (firing frequency of the last two APs) by the initial firing frequency (firing frequency of the first two APs) during a 1 s, 450 pA current step. The quotient was then subtracted from 1 and multiplied by 100 to obtain adaptation index as a percentage (e.g. Hönigsperger et al., 2015).

The majority of recorded ventral hippocampal CA1 neurons were classified as either RS or LS based on time to first spike at rheobase and pattern of spike frequency adaptation. At rheobase, RS neurons were defined as those that fired a single AP within ~350 ms of current

onset during a 500 ms injection, whereas LS neurons were defined as those that delayed firing until the end of a near-threshold current injection (~350-500 ms of a 500 ms current injection). Relative to RS neurons, LS neurons also displayed little adaptation or anti-adaptation (i.e. successively shorter inter-spike intervals (ISIs)) in response to a 1 s depolarizing current step (Beggs et al., 2000; Faulkner & Brown, 1999; Kurotani et al., 2013; McGann & Brown, 2000; Moyer et al., 2002; Storm, 1988; Yousuf, Nye, et al., 2020). A smaller proportion of neurons displayed properties of both RS and LS firing types (e.g. delayed firing at rheobase and significant adaptation, or firing early at rheobase and little to no adaptation); these were separated from excitability analyses and classified as RS/LS. Burst-spiking (BS) neurons were defined as those that fired two or more APs in short succession at near-rheobase current steps, and we observed one fast-spiking (FS) neuron that fired short duration APs and did not display bursting activity (Yousuf, Nye, et al., 2020).

Biocytin Staining

Following patch-clamp recordings, slices were fixed in formalin and stored at 4°C for up to 4 weeks. Slices were incubated in 3% H_2O_2/10% methanol for 45 min, washed with 0.1 M phosphate-buffered saline, incubated in 0.4% Triton-X-100/2% BSA for 45 min, then incubated in 1:500 streptavidin Alexa Fluor 488 (Invitrogen) overnight. Slices were then washed with phosphate-buffered saline and mounted onto unsubbed slides, coverslipped with Ultra Cruz Mounting Medium with DAPI (Santa Cruz Biotechnology) and sealed with nail polish. Location of recorded neurons was confirmed using a fluorescence microscope (BX51WI), and z-stack images of recorded neurons were obtained using an Olympus Fluoview confocal system.

Statistical Analyses

Microsoft Excel or SPSS (IBM SPSS Statistics 26) were used to analyze overall treatment effects via Student's *t*-test, one-way ANOVA, repeated-measures ANOVA, or chi-square statistic, where appropriate. Fisher's least significant difference (LSD) was used for *post hoc* comparisons. Data are expressed as mean ± 1 SEM.

Results

Behavioral Performance During Fear Conditioning and Testing

Percent freezing during each TI of the conditioning session was analyzed for Trace and Pseudo rats. Trace rats displayed a rapid increase in TI freezing over the first three conditioning trials, and freezing levels were consistently elevated throughout the rest of the session. Pseudo rats displayed TI freezing that was consistently less than that of Trace rats after the first two trials (Figure 10A). Repeated-measures ANOVA indicated a significant main effect of training trial ($F_{(9, 162)} = 11.5$, $p < .001$), a significant main effect of group ($F_{(1, 18)} = 4.9$, $p < .05$), and a significant trial by group interaction ($F_{(9, 162)} = 4.1$, $p < .001$).

Behavioral performance during the cue test was analyzed by calculating average baseline (B), CS and TI freezing (Figure 10A). A median split of average TI freezing for the Trace group was done to further explore the relationship between neuronal excitability and good (Trace-Good) or poor (Trace-Poor) trace fear memory. To analyze behavioral performance, average TI freezing was collapsed across Trace-Good and Trace-Poor groups, however, these groups were separated for excitability analyses. A repeated-measures ANOVA revealed a significant effect of stimulus (B, CS, and TI; $F_{(2, 36)} = 81.1$, $p < .001$), a significant main effect of group ($F_{(1, 18)} = 7.5$, $p < .05$), and a significant interaction between stimulus and group ($F_{(2, 36)} = 3.8$, $p < .05$). Pairwise comparisons of revealed that Trace rats displayed significantly greater TI freezing than

Pseudo rats ($p < .01$), but there were no significant group differences for either CS or baseline freezing.

For Context and Chamber-exposed groups, the conditioning session was divided into 10 bins (6 min/bin). During conditioning, Context rats displayed increased freezing over the first four bins and maintained elevated freezing thereafter. Freezing for Chamber-exposed rats was consistently below that of Context rats for the majority of the conditioning session (Figure 10B). Repeated-measures ANOVA indicated a significant effect of bin ($F_{(3.5, 59.5)} = 12.6$, $p < .001$), a significant effect of group ($F_{(1, 17)} = 19.8$, $p < .001$), and a significant interaction ($F_{(3.5, 59.5)} = 5.7$, $p < .001$).

Average percent freezing over the 10 min context test was analyzed to assess context fear memory. Context rats displayed significantly elevated freezing when compared to Chamber-exposed rats ($t_{(17)} = 5.3$, $p < .001$; Figure 10B). Taken together, these data suggest Trace and Context fear conditioning result in distinct behavioral performance relative to controls (Pseudo and Chamber-exposed, respectively).

Physiological and Morphological Heterogeneity of Ventral Hippocampal CA1 Naïve Neurons

The most predominant firing type observed in ventral hippocampal CA1 was RS (Naïve: 52%, 15 of 29; overall: 44%, 71 of 163), with LS neurons being the second most highly expressed firing type (Naïve: 38%, 11 of 29; overall: 40%, 66 of 163). We also observed a small proportion of neurons that either oscillated between RS and LS firing types, or displayed properties of both. These were referred to as RS/LS (Naïve: 7%, 2 of 29; overall: 12%, 20 of 163). An even smaller proportion of neurons were classified as BS (Naïve: 3%, 1 of 29; overall: 3%, 5 of 163) or FS (Naïve: 0%; overall: 1%, 1 of 163; Table 1). Our analyses focused primarily

on the RS and LS firing types, since they were the most prevalent neuronal classifications we observed in the ventral hippocampal CA1 region.

Naïve ventral hippocampal CA1 RS and LS neurons demonstrated a number of distinguishing physiological properties (Figure 11).

Figure 10

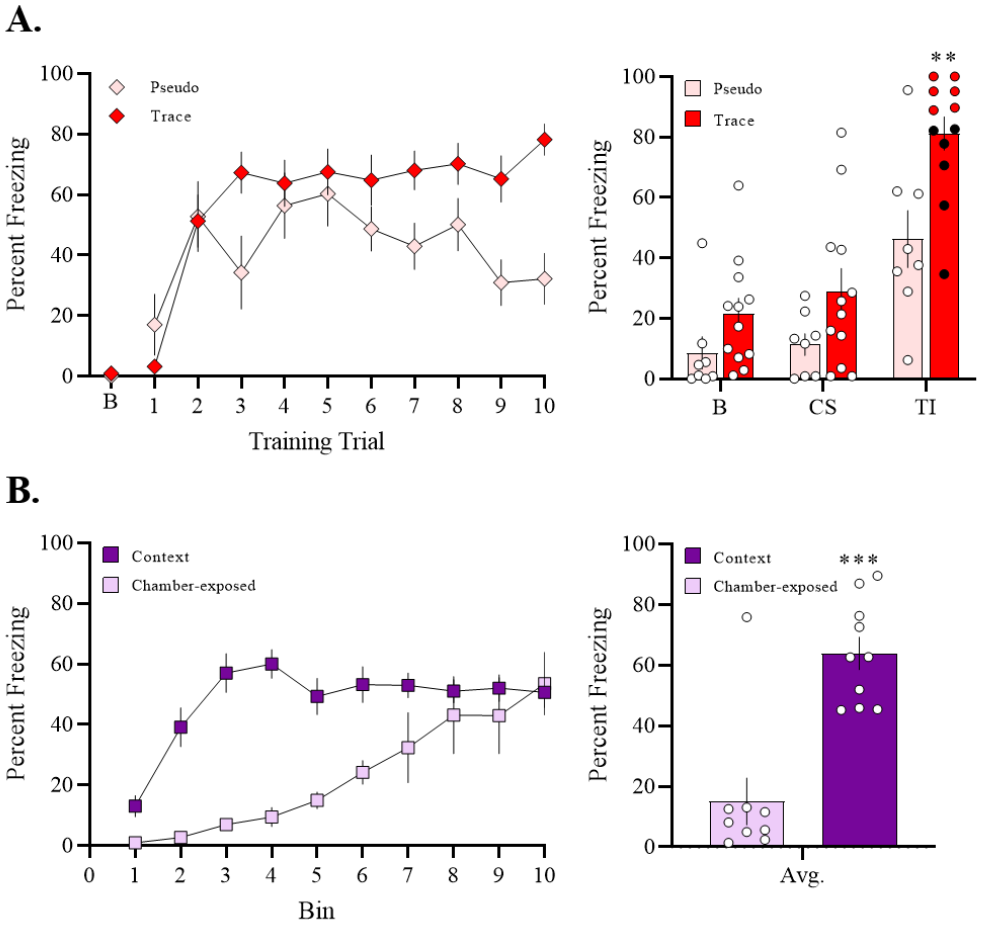

Figure 10. Behavioral performance during trace, context fear conditioning and testing. ***A,*** *Left,* Individual data points show percent freezing during the TI of each trial. Trace rats displayed a rapid increase in freezing over the first three conditioning trials and maintained elevated freezing levels thereafter. Pseudo rats displayed freezing levels that were consistently below that of Trace rats after the first two trials. *Right,* During the cue test, Trace rats displayed significantly increased freezing compared to Pseudo rats during the TI. Black filled circles represent poor learners (Trace-Poor), while red filled circles represent good learners (Trace-Good). This median split was used for subsequent electrophysiological analyses. ***B,*** *Left,* Context rats displayed increased freezing over the first four bins (1 bin = 6 min) and maintained consistent freezing levels throughout the remainder of the training session. Chamber-exposed rats displayed freezing levels that were consistently below that of the Context conditioned rats for the majority of the training session. *Right,* Analysis of average freezing over the 10 min context test revealed that Context rats froze significantly more than Chamber-exposed rats. *Abbreviations:* baseline freezing (B); freezing during the conditional stimulus (CS); freezing during the trace interval (TI); $^{**}p < .01$; $^{***}p < .001$.

Table 1. Ventral hippocampal CA1 firing type distribution as a function of behavior

Group	RS	LS	RS/LS	BS	FS
Naïve	52% (15)	38% (11)	7% (2)	3% (1)	0% (0)
Pseudo	46% (15)	46% (15)	6% (2)	3% (1)	0% (0)
Trace	33% (12)	44% (16)	19% (7)	3% (1)	0% (0)
Chamber-exposed	40% (12)	47% (14)	10% (3)	3% (1)	0% (0)
Context	49% (17)	29% (10)	17% (6)	3% (1)	3% (1)
All	44% (71)	40% (66)	12% (20)	3% (5)	1% (1)

Data indicate the percentage of each firing type observed in ventral hippocampal CA1 as a function of behavioral condition. Numbers in parentheses indicate number of cells. *Abbreviations:* regular-spiking (RS); late-spiking (LS); burst-spiking (BS); fast-spiking (FS).

LS neurons displayed a reduction in adaptation index ($t_{(24)} = 5.2$, $p < .001$; Figure 11A), and when firing frequency was plotted as a function of spike interval, LS neurons displayed a relatively linear change in frequency compared to RS neurons (Figure 11C, D). This suggests LS neurons in the ventral hippocampal CA1 region tend to display mild spike frequency adaptation.

Another prominent characteristic we observed in LS neurons was a significant delay in spike onset relative to RS neurons ($t_{(23.6)} = -2.8$, $p < .05$; Figure 11B). In contrast to previous findings (Beggs et al., 2000; Chu et al., 2003; Faulkner & Brown, 1999; Kurotani et al., 2013; Martina et al., 1999; McGann & Brown, 2000; Moyer et al., 2002; Yousuf, Nye, et al., 2020), the current experiments failed to reveal significant differences between RS and LS firing types for several subthreshold membrane properties, including input resistance ($t_{(24)} = 0.5$, $p = 0.6$), and sag ($t_{(24)} = 1.4$, $p = 0.2$).

Morphologically, ventral hippocampal CA1 LS neurons displayed reduced dendritic complexity compared to RS neurons (Figure 12). The total number of apical dendritic branches was significantly reduced for LS compared to RS neurons ($t_{(15)} = 2.4$, $p < .05$), while the total number of basilar dendrites was not different ($t_{(15)} = -0.4$, $p = 0.7$). There was no effect of firing type on total apical branch length ($t_{(15)} = 1.1$, $p = 0.3$) or basilar branch length ($t_{(15)} = -1.5$, $p =$

0.2). Repeated-measures ANOVA of apical Sholl data revealed a significant interaction between distance from soma and firing type ($F_{(84, 1260)} = 1.8$, $p < .001$), and follow-up pairwise comparisons further revealed significantly reduced numbers of intersections for LS neurons relative to RS neurons at 140µm-160µm, and 220µm from the soma (all p-values, $p < .05$). There were no differences between RS and LS neurons for soma volume ($t_{(15)} = -0.8$, $p = .4$), or soma surface area ($t_{(15)} = -0.9$, $p = 0.4$). Overall, ventral hippocampal CA1 LS neurons have several features that distinguish them from RS neurons, including reduced spike frequency adaptation, increased spike onset latency, and reduced apical dendritic complexity.

Context Fear Conditioning Enhances Intrinsic Excitability in Ventral Hippocampal CA1 RS Neurons

A total of 71 RS neurons were obtained, representing 44% of all ventral hippocampal CA1 neurons recorded from in the current study (Table 1). Analysis of spike frequency adaptation revealed RS neurons from Context rats were more excitable than RS neurons from Chamber-exposed or Naïve rats (Figure 13B). Repeated-measures ANOVA comparing Naïve, Chamber-exposed, and Context groups indicated a significant effect of current intensity ($F_{(1.3, 47.8)} = 134.5$, $p < .001$), a trending effect of group ($F_{(2, 36)} = 3.0$, $p = .06$), and a non-significant interaction ($F_{(2.7, 47.8)} = 2.3$, $p = .1$). One-way ANOVA of number of spikes elicited using a 250 pA current step revealed a significant overall effect ($F_{(2, 36)} = 3.4$, $p < .05$), with *post hoc* analysis revealing RS neurons from Context rats fired more APs when compared to RS neurons from Naïve and Chamber-exposed groups (both p-values, $p < .05$). Due to lack of differences in RS neuronal excitability between Trace-Good and Trace-Poor neurons (data not shown), these groups were collapsed into a single group (Trace) for the purposes of analyzing intrinsic excitability changes. Although Trace rats displayed elevated freezing during the retrieval test

relative to Pseudo rats, spike frequency adaptation was unaffected by behavioral condition (Figure 13A).

Figure 11

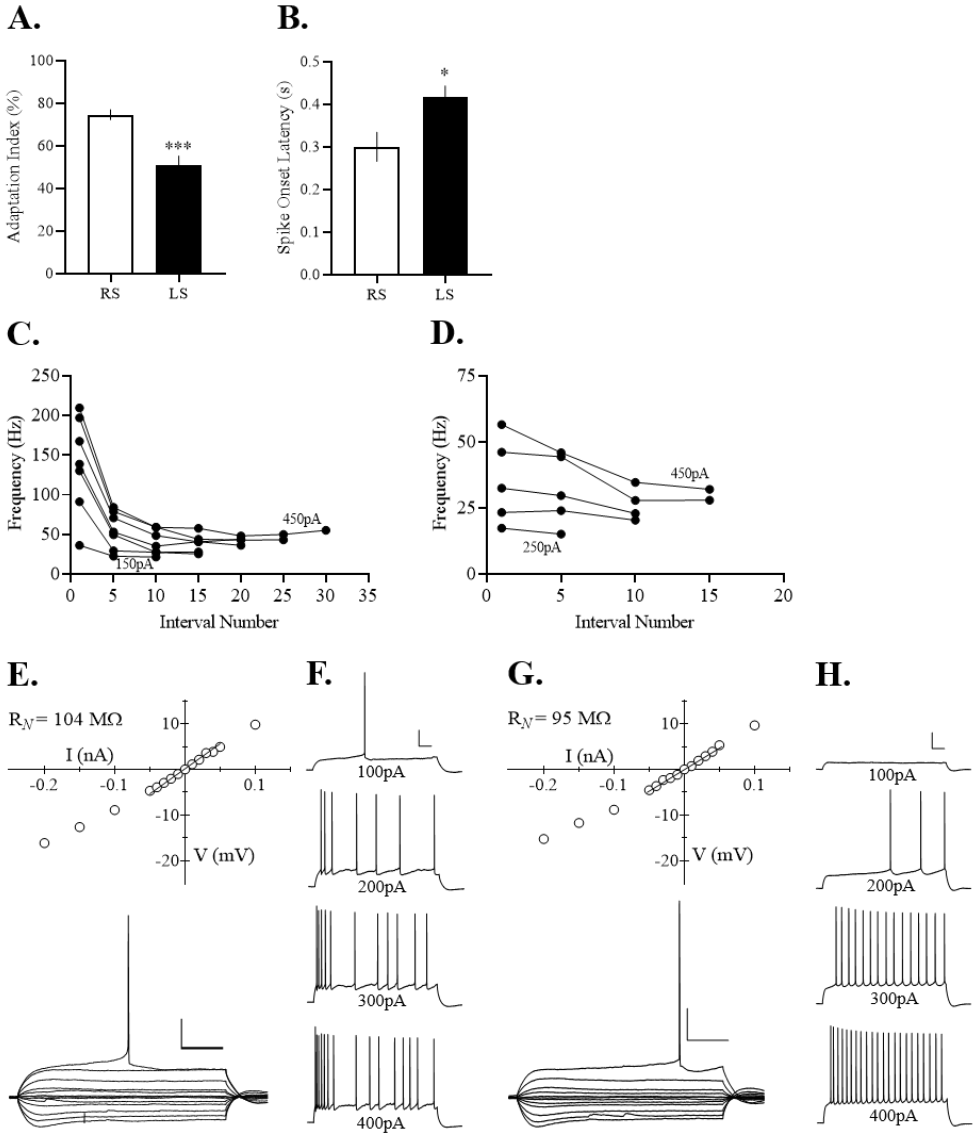

Figure 11. Ventral hippocampal CA1 neurons are broadly divided into regular-spiking and late-spiking neurons. ***A,*** Adaptation index is significantly reduced for LS neurons relative to RS neurons. ***B,*** LS neurons display increased spike onset latency relative to RS neurons. ***C,*** Plot showing spike frequency as a function of spike interval for RS neurons. The steep downward curve on the left side of the plot indicates substantial adaptation. ***D,*** Plot showing spike frequency as a function of spike interval number for LS neurons. Although these neurons display some reduction in firing frequency early on, particularly for larger current injections, they display relatively little adaptation. ***E, G,*** *V-I* plot used to measure input resistance, and representative waveform below for RS neurons (***E***) and LS neurons (***G***). ***F, H,*** A series of depolarizing currents were injected to measure neuronal excitability for RS neurons (***F***) and LS neurons (***H***). *Calibration:* ***E-H,*** 20mV, 100ms. *Abbreviations:* regular-spiking (RS); late-spiking (LS). **p* < .05; ****p* < .001.

Figure 12

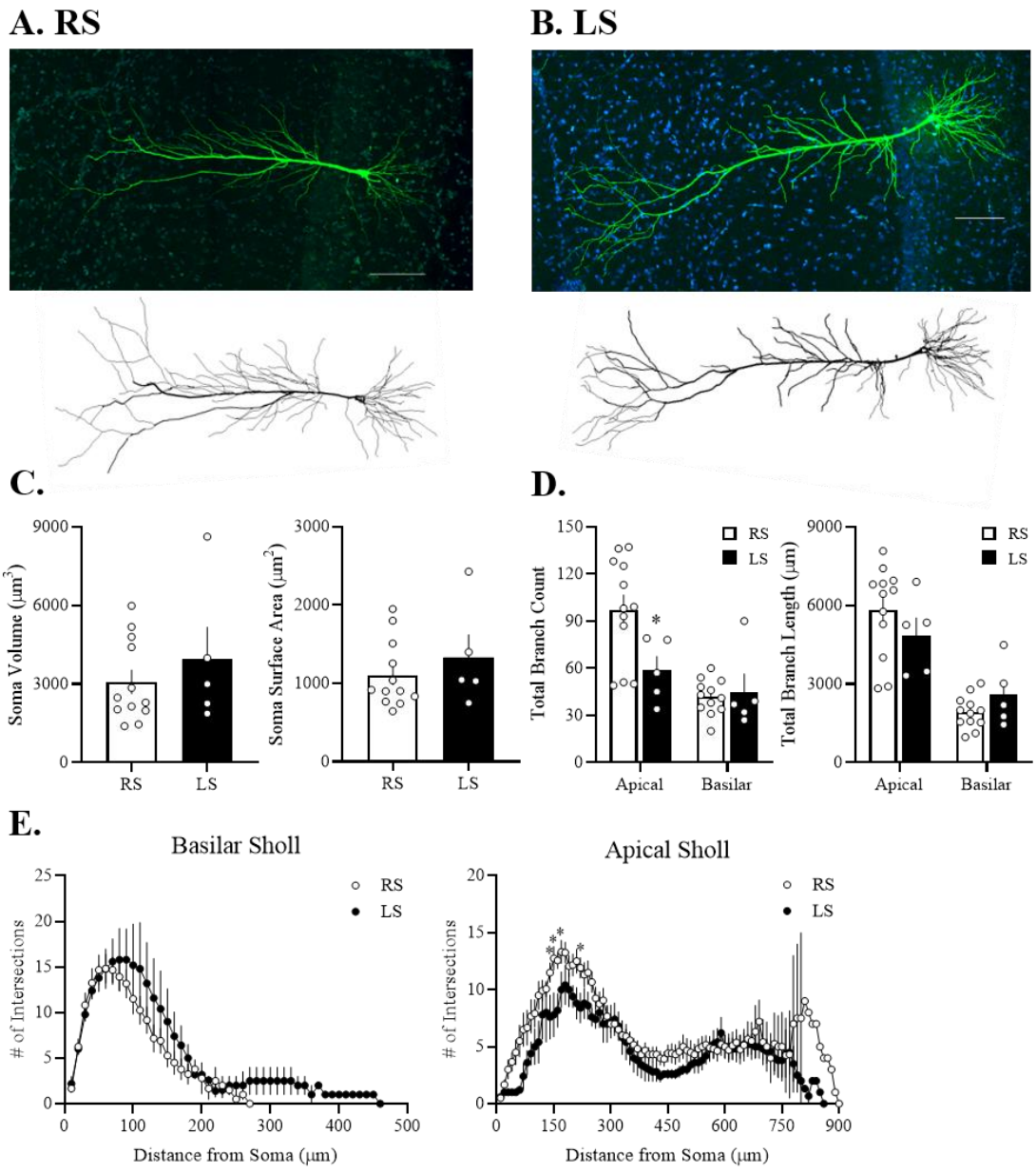

Figure 12. Morphological differences between ventral hippocampal CA1 regular-spiking and late-spiking neurons. ***A & B,*** Representative 20X confocal images of RS (***A***) and LS (***B***) neurons, with reconstructions shown below. ***C,*** Somatic measurements reveal no differences of soma surface area or volume between RS and LS neurons. ***D,*** *Left,* Dendritic branch analysis reveals LS neurons display a significant reduction in the total number of apical dendritic branches relative to that of RS neurons, but no change in the total number of basilar dendritic branches. *Right,* RS and LS neurons display similar apical and basilar total branch length. ***E,*** *Left,* Sholl analysis reveals no difference between RS and LS basilar dendritic complexity. *Right,* Sholl analysis of apical dendrites reveals LS apical branches make significantly fewer intersections than that of RS neurons at 140-160μm and 220μm from the soma. *Scale bars:* ***A, B,*** 100μm. *Abbreviations:* regular-spiking (RS); late-spiking (LS); $*p \leq .05$.

Repeated-measures ANOVA indicated a significant effect of current intensity ($F_{(1.4, 49.9)} = 151.1$, $p < .001$), but no effect of group ($F_{(2, 36)} = .03$, $p = 1.0$), and no interaction effect ($F_{(2.8, 49.9)} = .3$, $p = .9$). These data suggest context fear conditioning reduces spike frequency adaptation in ventral hippocampal CA1 RS neurons, while trace fear conditioning has no effect on RS neuronal spike frequency adaptation.

Previous research suggests behavioral performance is correlated with intrinsic excitability, both in the dorsal hippocampal CA1 region (Song et al., 2012) and in the mPFC (Song et al., 2015). We therefore analyzed the current data to determine whether behavioral performance was correlated with spiking activity in ventral hippocampal CA1 RS neurons. A Pearson correlation analysis revealed a significant positive correlation between number of spikes elicited at 450pA and percent freezing during the context test for the Context group ($r = 0.71$, $p < .05$; Figure 14). No other correlations were significant. Together, these data suggest that context, but not trace, fear conditioning increases intrinsic excitability in ventral hippocampal CA1 RS neurons, and that better context fear memory is linked with greater increases of excitability.

Postburst AHP in Ventral Hippocampal CA1 RS Neurons Remains Unchanged Following Fear Conditioning

Learning-related reduction of spike frequency adaptation is usually accompanied by reduced postburst AHP (see Oh et al., 2010). However, postburst AHP amplitude for RS neurons remained unchanged following either trace or context fear conditioning. Repeated-measures ANOVA comparing Naïve, Pseudo, and Trace groups revealed a significant effect of time ($F_{(2.4, 89.3)} = 34.0$, $p < .001$), but no significant effect of group ($F_{(2, 37)} = 1.2$, $p = .3$), and no significant interaction between time and group ($F_{(4.8, 89.3)} = .9$, $p = .5$; Figure 15A).

Figure 13

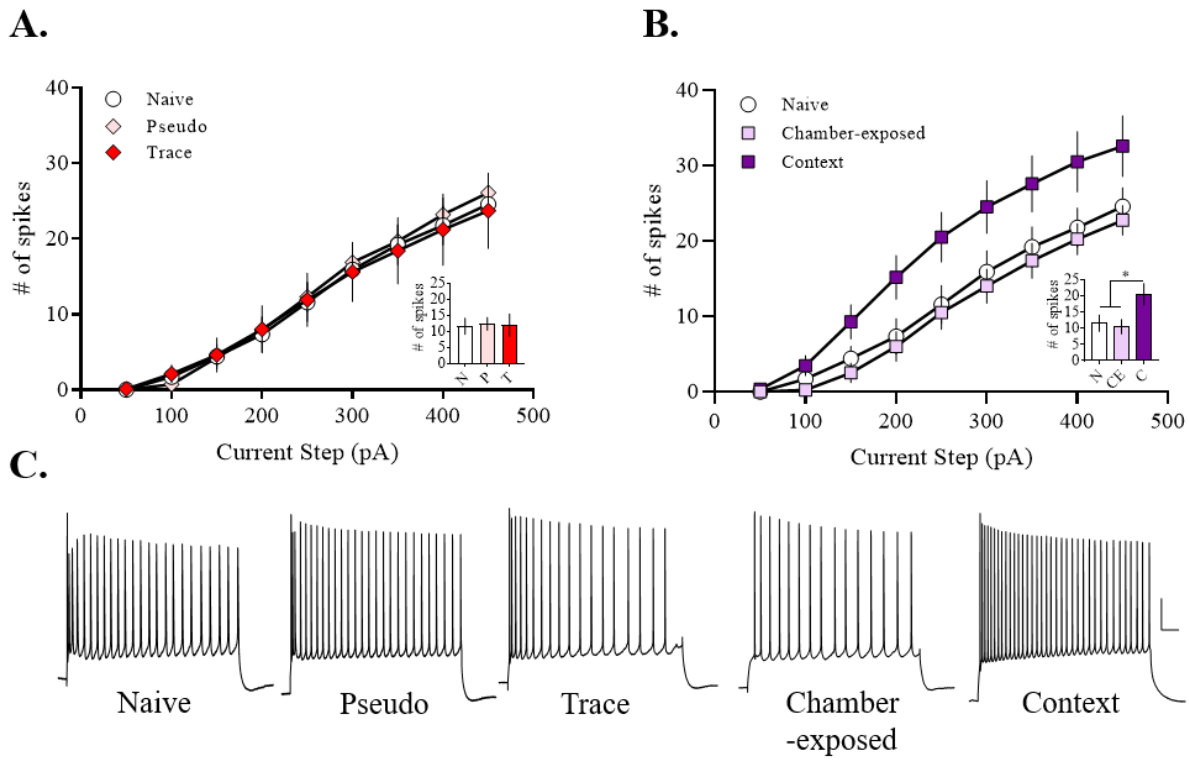

Figure 13. Context fear conditioning reduces spike frequency adaptation in ventral hippocampal CA1 regular-spiking neurons. ***A,*** RS neurons from Trace rats fired similar numbers of spikes to that of RS neurons from Pseudo and Naïve rats. *Inset*, average number of spikes evoked by a 250pA current injection is not different between groups. ***B,*** RS neurons from Context rats fired significantly more action potentials than RS neurons from Chamber-exposed and Naïve rats. *Inset*, average number of spikes evoked at 250pA is significantly greater for neurons from Context rats compared to neurons from Chamber-exposed and Naïve rats. ***C,*** Representative traces showing number of spikes elicited by a 450pA current injection. *Calibration:* 20mV, 100ms; *Abbreviation:* regular-spiking (RS); **p* < .05.

Trace-Poor and Trace-Good groups were not different from each other (data not shown), and were collapsed as one Trace group for this analysis. We did however observe a trending overall effect of group (Naïve, Pseudo, and Trace) on time to reach peak AHP ($F_{(2, 33)} = 2.8$, $p = .075$). Follow up *post hoc* analysis indicated RS neurons from Trace rats took significantly longer to reach peak postburst AHP voltage than RS neurons from either Naïve or Pseudo groups (both *p*-values, $p < .05$; Figure 15C). This suggests that differences in AP repolarization could be obscuring possible learning-related effects on postburst AHP changes over time. However, there

was no effect of trace fear conditioning on peak AHP voltage (Figure 15C) or on mAHP or sAHP measures (Table 2).

Figure 14

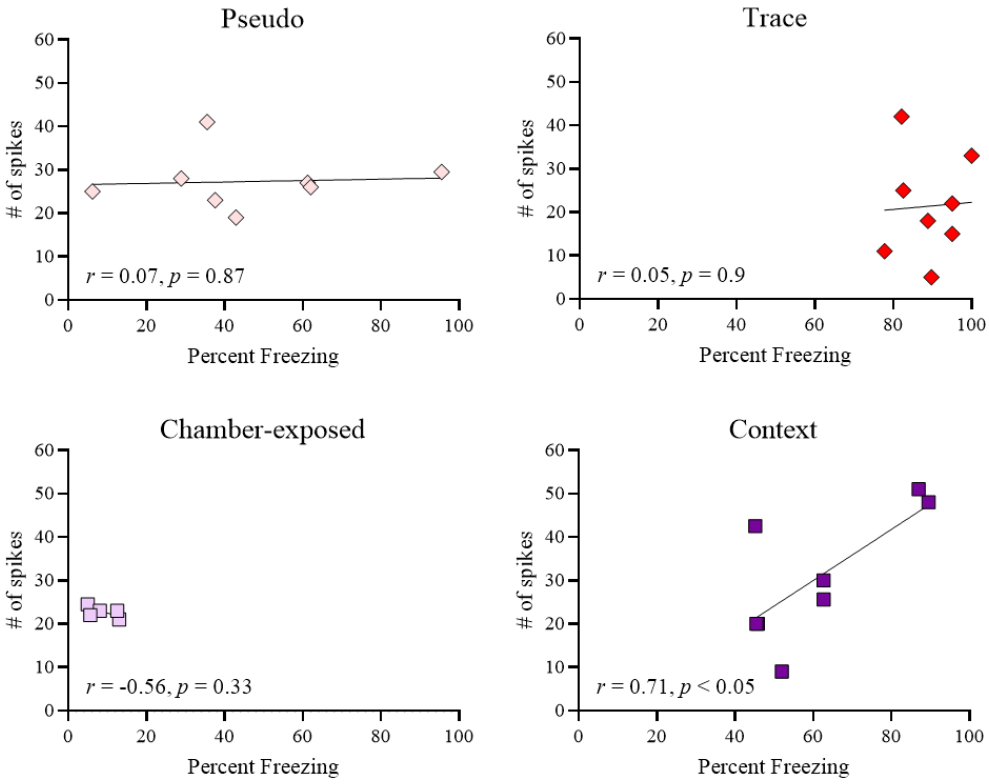

Figure 14. Positive correlation between context fear memory and excitability in ventral hippocampal CA1 regular-spiking neurons. There is a significant positive correlation between percent freezing during the context test and spiking activity of ventral hippocampal CA1 RS neurons from Context rats ($p < .05$). No significant correlation is observed between ventral hippocampal CA1 RS spiking activity and percent freezing during the test session for Pseudo, Trace, or Chamber-exposed groups. Spiking activity in response to a 450pA current step was used for analysis. *Abbreviation:* regular-spiking (RS).

Similarly, repeated-measures ANOVA comparing RS neurons from Naïve, Chamber-exposed, and Context groups revealed a significant effect of time ($F_{(1.8, 75.1)} = 37.5$, $p < .001$), but no significant effect of group ($F_{(2, 41)} = .5$, $p = .6$), and no significant interaction between time and group ($F_{(3.7, 75.1)} = .4$, $p = .8$; Figure 15B). There was no effect of context fear conditioning on time to peak AHP, peak AHP voltage (Figure 15D) or on mAHP or sAHP (Table 2). Overall, these data suggest neither trace nor context fear conditioning significantly affect postburst AHP in ventral hippocampal CA1 RS neurons.

Figure 15

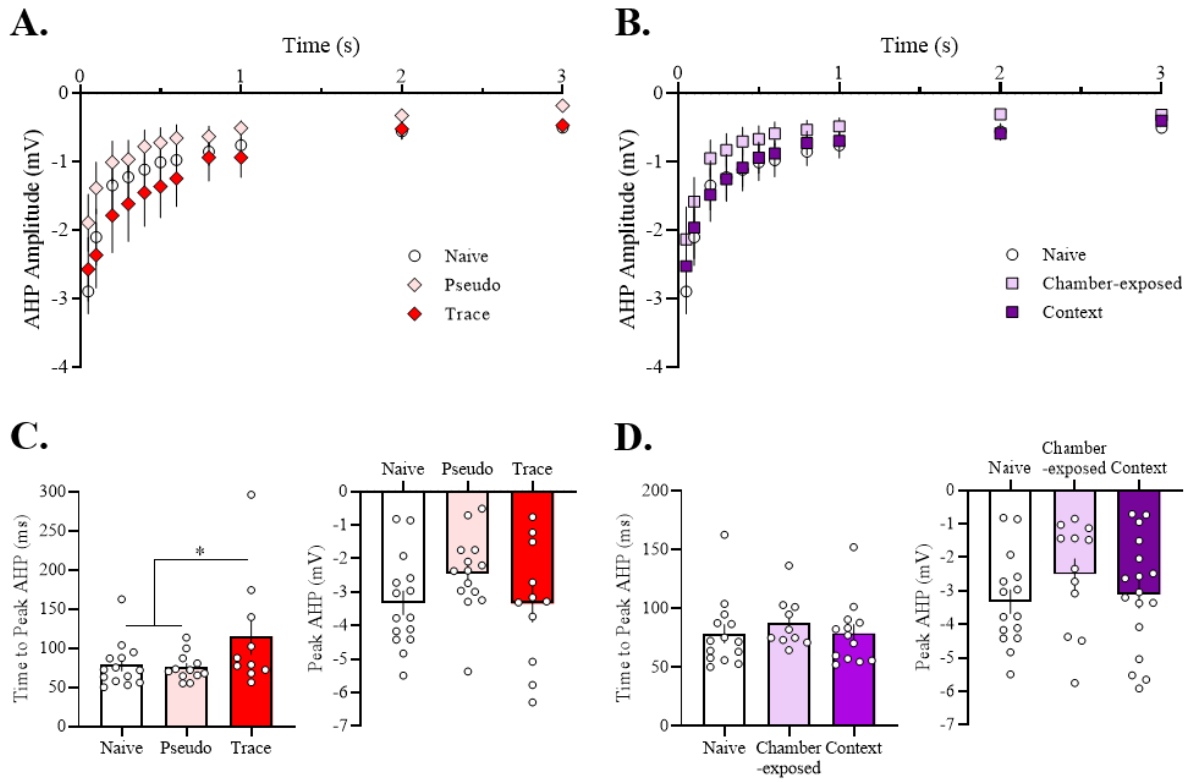

Figure 15. Postburst AHP of ventral hippocampal CA1 regular-spiking neurons remains unchanged following fear conditioning. ***A & B,*** Time course of the postburst AHP amplitude remains unchanged following trace fear conditioning (***A***), and following context fear conditioning (***B***). ***C,*** *Left,* Time to peak AHP is increased following trace fear conditioning, suggesting action potential repolarization is differentially affected by trace fear learning. *Right,* Peak AHP amplitude remains unchanged following trace fear conditioning. ***D,*** Time to peak AHP (*left*) and peak AHP amplitude (*right*) remain unchanged following context fear conditioning. **p* < .05.

Table 2. Effect of fear conditioning on mAHP and sAHP of ventral hippocampal CA1 regular-spiking neurons

Group (# of cells)	mAHP (mV)	sAHP (mV)
Naïve (15)	-3.6 ± 0.4	-0.8 ± 0.2
Pseudo (14)	-2.7 ± 0.4	-0.5 ± 0.1
Trace (11)	-3.4 ± 0.5	-0.9 ± 0.3
Chamber-exposed (12)	-2.9 ± 0.5	-0.5 ± 0.2
Context (17)	-3.2 ± 0.4	-0.7 ± 0.1

Data are mean ± SE. The mAHP was measured as the peak of the AHP during the first 150 ms after current offset. The sAHP amplitude was measured 1 s after current offset.

Differential Effect of Trace and Context Fear Conditioning on AP Amplitude and Half-Width of Ventral Hippocampal CA1 RS Neurons

Analysis of membrane properties revealed RS neurons from Chamber-exposed rats displayed altered AP_{amp}. One-way ANOVA revealed a significant effect of group ($F_{(2, 41)} = 3.9$, $p < .05$), and *post hoc* analysis further indicated that neurons from Chamber-exposed rats fired APs that were significantly shorter compared to neurons from Naïve and Context rats (both p-values, $p < .05$; Table 3). AP_{amp} was unchanged following trace fear conditioning ($F_{(2, 39)} = .7$, $p = .5$).

Additionally, AP_{width} of RS neurons was bi-directionally altered following trace and context fear conditioning (Table 3). Trace fear conditioning significantly increased AP_{width} ($F_{(2, 39)} = 3.7$, $p < .05$). *Post hoc* analysis further revealed neurons from Trace rats displayed significantly greater AP_{width} relative to that of neurons from Pseudo rats ($p < .05$). In contrast, context fear conditioning reduced AP_{width} ($F_{(2, 41)} = 4.1$, $p < .05$). *Post hoc* analysis indicated AP_{width} of neurons from Context rats was significantly reduced relative to that of neurons from Chamber-exposed rats ($p < .01$). All other AP and membrane properties (Table 3) of RS neurons remained unchanged following trace or context fear conditioning.

A Subset of Ventral Hippocampal CA1 RS Neurons Demonstrate Increased Excitability Following Context Fear Conditioning

Previous research suggests increased learning-related changes of intrinsic excitability are driven by a subset of neurons, and that these neurons likely contribute to some aspect of the memory trace (Moyer et al., 1996; Rumpel et al., 2005; Sehgal et al., 2014; Sehgal et al., 2013; Song et al., 2015).

Table 3. Effect of fear conditioning on membrane properties of ventral hippocampal CA1 regular-spiking neurons

Group (# of cells)	RMP (mV)	R_N (MΩ)	Sag (mV)	$I_{threshold}$ (pA)	AP_{thresh} (mV)	AP_{amp} (mV)	AP_{width} (µs)	fAHP (mV)
Naïve (15)	-57.9 ± 1.2	103.9 ± 12.9	4.8 ± 0.4	152.3 ± 18.6	-43.7 ± 1.6	99.2 ± 2.6	818 ± 47	-6.3 ± 0.9
Pseudo (15)	-57.0 ± 1.4	92.0 ± 4.7	3.4 ± 0.3	160.0 ± 15.8	-44.0 ± 1.3	94.8 ± 2.1	778 ± 27	-6.1 ± 1.1
Trace (12)	-60.2 ± 1.6	96.6 ± 10.6	4.4 ± 0.6	142.5 ± 19.5	-44.2 ± 2.2	95.0 ± 3.3	917 ± 37$^{\&}$	-6.7 ± 1.6
Chamber-exposed (12)	-59.3 ± 1.8	88.1 ± 6.7	3.5 ± 0.4	200.8 ± 17.0	-39.4 ± 1.8	90.4 ± 2.6*	937 ± 38	-5.3 ± 1.2
Context (17)	-60.0 ± 1.0	105.2 ± 14.1	4.0 ± 0.4	153.8 ± 18.7	-43.2 ± 1.5	99.3 ± 2.1†	769 ± 36‡	-8.5 ± 1.2

Data are mean ± SE. *Abbreviations:* resting membrane potential (RMP); neuronal input resistance (R_N); threshold current required to elicit an action potential ($I_{threshold}$); action potential threshold (AP_{thresh}); action potential amplitude (AP_{amp}); action potential half-width (AP_{width}); fast afterhyperpolarization (fAHP). Statistically different from Naïve: *, $p < .05$; statistically different from Pseudo: &, $p < .05$; statistically different from Chamber-exposed: †, $p < .05$; ‡ $p < .01$.

To determine whether a subset of ventral hippocampal CA1 RS neurons contributed to the observed reduction in spike frequency adaptation following context fear conditioning, we plotted a frequency histogram and cumulative probability plot (Figure 16A) showing the number of APs elicited by a 450pA current injection. Approximately one third of ventral hippocampal CA1 RS neurons (5 of 17 or 29%) from Context rats fired more APs than the most excitable Naïve neuron. Furthermore, plotting cumulative firing probability against number of spikes elicited by a 450pA current step revealed a noticeable rightward shift for neurons from the Context group, further supporting our finding that these neurons display altered intrinsic excitability.

Neurons within each behavior group were further classified as *Changed* or *Unchanged* (Figure 16B). *Changed* neurons were defined as those that fired more than 2 SEMs above the mean of the Naïve group in response to a 450pA current injection. Despite a non-significant chi-square statistic ($\chi^2_{(4)} = 7.2$, $p = .126$), the percentage of *Changed* neurons from Context rats (59%) was greater than all other groups (Figure 16B). Overall, these data suggest the likelihood that a subset of Context neurons are driving the observed reduction of spike frequency adaptation, effectively increasing intrinsic excitability in this group.

Ventral Hippocampal CA1 Spike Frequency Adaptation in LS Neurons is Bidirectionally Altered by Trace Fear Conditioning

A total of 66 LS neurons were obtained, representing 40% of all ventral hippocampal CA1 neurons recorded from in the current study (Table 1). Unlike RS neurons, LS neuronal excitability varied considerably with trace fear memory. Thus, physiological analyses of the effect of trace fear conditioning on intrinsic excitability took this into account by separating neurons from good learners (Trace-Good) from neurons from poor learners (Trace-Poor).

Figure 16

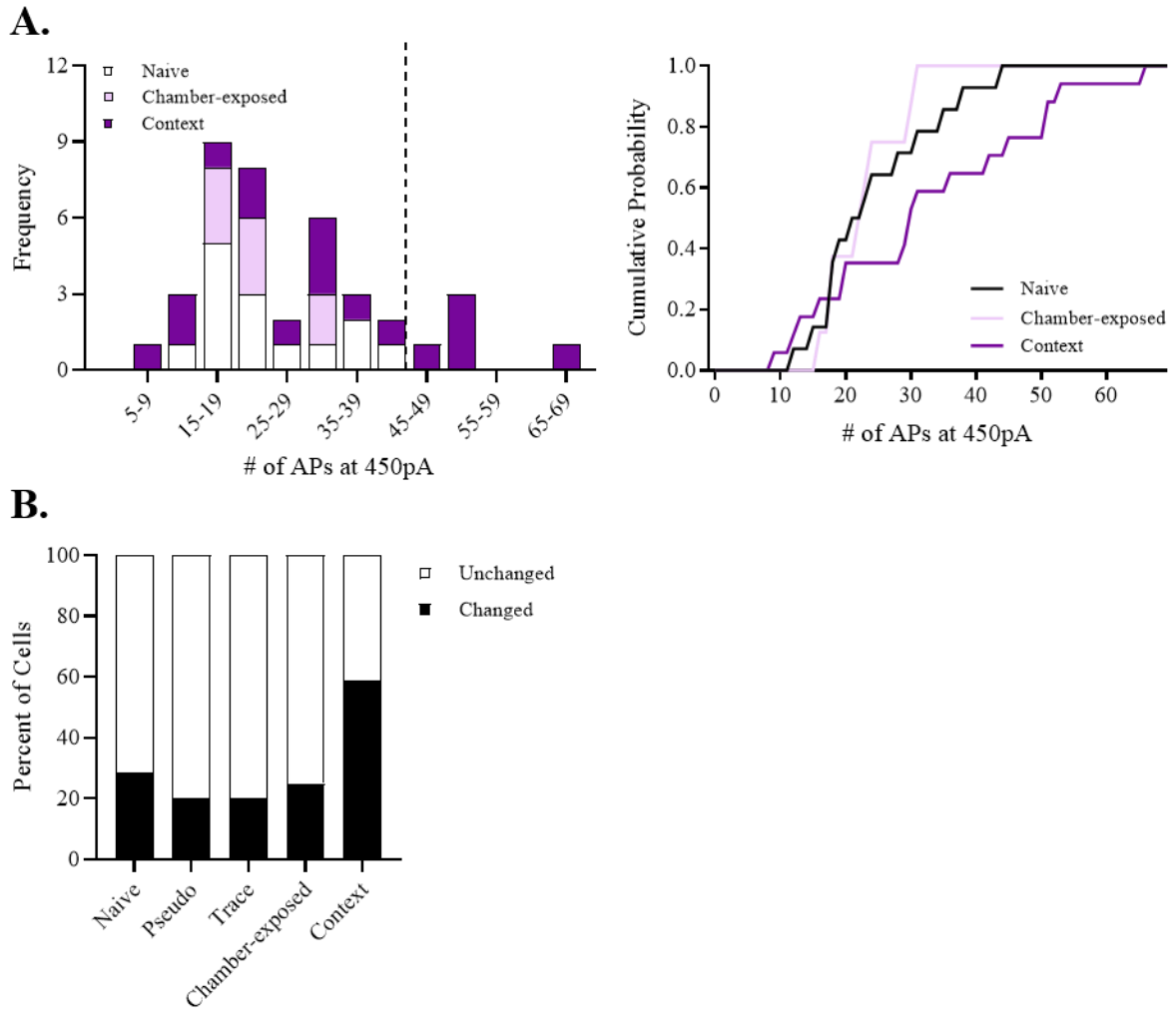

Figure 16. A subset of ventral hippocampal CA1 regular-spiking neurons display increased excitability following context fear conditioning. ***A,*** *Left,* Frequency histogram for number of action potentials elicited by a 450pA current injection. A subset of neurons from Context rats (29%) fired more action potentials than the most excitable Naïve neuron (indicated by dashed line). *Right,* Cumulative frequency distribution showing number of action potentials for ventral hippocampal CA1 neurons. The rightward shift for neurons from Context fear conditioned rats indicates increased intrinsic excitability in this group. ***B,*** Neurons whose action potential output in response to a 1 s, 450 pA current injection was greater than 2 SE above the mean of neurons from Naïve rats were classified as "Changed". Percent of "Changed" neurons from Context rats (59%) was greater than any other group.

Ventral hippocampal CA1 LS neurons from Trace-Good rats demonstrated reduced spike frequency adaptation (Figure 17A). A repeated-measures ANOVA revealed a significant effect of current intensity ($F_{(1.7, 63.5)} = 186.5$, $p < .001$), a non-significant effect of group ($F_{(3, 37)} = 2.2$, p

= .1), and a non-significant interaction effect ($F_{(5.2, 63.5)} = 1.5$, $p = .2$). However, repeated-measures ANOVA comparing LS Trace-Poor to Trace-Good neuronal excitability revealed a significant effect of current intensity ($F_{(8, 112)} = 90.8$, $p < .001$), a significant effect of group ($F_{(1, 14)} = 9.9$, $p < .01$), and a significant interaction effect ($F_{(8, 112)} = 5.3$, $p < .001$). Follow-up pairwise comparisons indicated neurons from Trace-Good rats fired significantly more APs than neurons from Trace-Poor rats at 250pA ($p < .01$). In contrast, spike frequency adaptation for LS neurons following context fear conditioning remained unchanged (Figure 17B). A repeated-measures ANOVA comparing Naïve, Chamber-exposed, and Context groups indicated a significant effect of current intensity ($F_{(1.4, 45.1)} = 105.8$, $p < .001$), but a non-significant effect of group ($F_{(2, 32)} = .6$, $p = .6$), and a non-significant interaction effect ($F_{(2.8, 45.1)} = 1.1$, $p = .4$).

Ventral Hippocampal CA1 Postburst AHP is Bidirectionally Altered in LS Neurons by Trace Fear Conditioning

Consistent with trace fear learning-related changes in spike frequency adaptation, the postburst AHP was reduced in LS neurons from Trace-Good rats (Figure 18A). Repeated-measures ANOVA comparing neurons from Naïve, Pseudo, Trace-Good, and Trace-Poor groups revealed a significant effect of time ($F_{(2.1, 73.1)} = 36.5$, $p < .001$), but no significant effect of group ($F_{(3, 35)} = .9$, $p = .5$), and no time by group interaction ($F_{(6.3, 73.1)} = .9$, $p = .5$). However, repeated-measures ANOVA comparing only neurons from Trace-Good and Trace-Poor groups indicated a significant effect of time ($F_{(2.1, 29.6)} = 37.3$, $p < .001$), a nonsignificant effect of group ($F_{(1, 14)} = 2.1$, $p = .2$), and a significant interaction between time and group ($F_{(2.1, 29.6)} = 3.4$, $p < .05$). Follow-up pairwise comparisons revealed significantly reduced postburst AHP amplitude for neurons from Trace-Good rats at 200 ms and 300 ms after current offset ($p \leq .05$). Analysis of peak AHP amplitude further suggested neurons from Trace-Good rats displayed reduced AHPs

(Figure 18C). One-way ANOVA comparing Naïve, Pseudo, Trace-Poor and Trace-Good groups revealed a trending effect of group ($F_{(3, 33)} = 2.3$, $p = .09$), with *post hoc* comparisons indicating neurons from Trace-Good rats displayed a significant reduction in peak AHP amplitude relative to neurons from Trace-Poor rats ($p < .05$). Furthermore, Student's *t*-test indicated the mAHP of neurons from Trace-Good rats was significantly reduced when compared to the mAHP of neurons from Trace-Poor rats ($t_{(14)} = -2.2$, $p < .05$; Table 4). There was no effect of behavior on time to reach peak AHP amplitude ($F_{(3, 32)} = 1.2$, $p = .3$; Figure 18C), or on the sAHP ($F_{(3, 35)} = 1.1$, $p = .4$; Table 4).

Figure 17

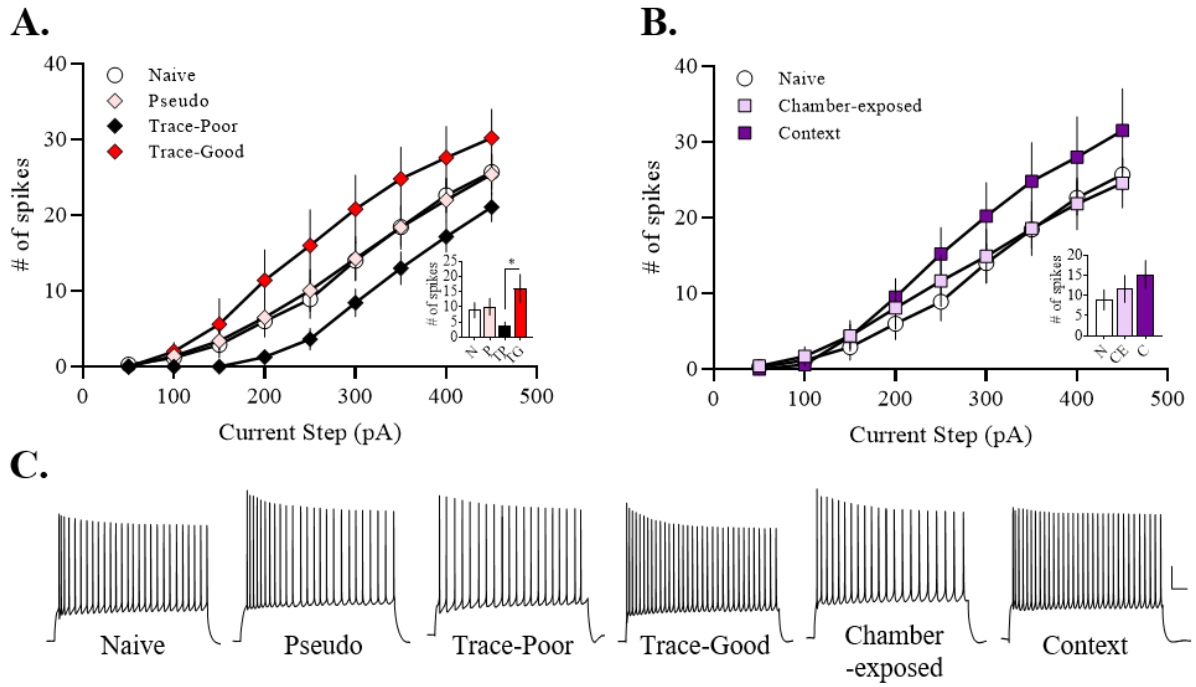

Figure 17. Trace fear conditioning bidirectionally modifies spike frequency adaptation in ventral hippocampal CA1 late-spiking neurons. ***A,*** LS neurons from Trace-Good rats display reduced spike frequency adaptation. *Inset,* LS neurons from Trace-Good rats display a significant increase in average number of spikes elicited by a 250pA current injection when compared to LS neurons from Trace-Poor rats. ***B,*** LS neurons from Context rats display no significant change in spike frequency adaptation relative to LS neurons from Chamber-exposed or Naïve rats. *Inset,* Average number of spikes elicited by a 250pA current injection remains unchanged following context fear conditioning. ***C,*** Representative traces showing number of spikes elicited by a 450pA current injection. *Calibration:* 20mV, 100ms; *Abbreviations:* late-spiking (LS); **p* < .05.

In contrast, analysis of the postburst AHP revealed no overall effect of context fear conditioning on this measure. Repeated-measures ANOVA comparing Naïve, Chamber-exposed, and Context groups indicated a significant effect of time ($F_{(2.0, 61.1)} = 51.8$, $p < .001$), but no effect of group ($F_{(2, 30)} = .3$, $p = .8$), and no time by group interaction ($F_{(4.1, 61.1)} = 1.3$, $p = .3$; Figure 18B). Additionally, there was no effect of group on the time to reach peak AHP ($F_{(2, 28)} = 1.5$, $p = .2$), peak AHP amplitude ($F_{(2, 30)} = 1.5$, $p = .3$; Figure 18D), mAHP ($F_{(2, 32)} = 1.3$, $p = .3$), or sAHP ($F_{(2, 32)} = .5$, $p = .6$; Table 4).

Together, these data suggest LS neurons likely contribute to successful trace fear learning, but not to context fear learning. The bidirectional excitability changes among LS neurons following trace fear conditioning suggests they play a specialized role in the learning process – maximizing firing activity to support good learning, but suppressing firing when learning is poor. This makes them uniquely suited to support associations across a prolonged time period.

Table 4. Effect of fear conditioning on mAHP and sAHP of ventral hippocampal CA1 late-spiking neurons

Group (# of cells)	mAHP (mV)	sAHP (mV)
Naïve (10)	-2.7 ± 0.4	-0.6 ± 0.1
Pseudo (13)	-2.7 ± 0.3	-0.5 ± 0.1
Trace		
Trace-Poor (11)	-3.5 ± 0.1	-0.4 ± 0.1
Trace-Good (5)	-2.3 ± 0.7*	-0.3 ± 0.1
Chamber-exposed (13)	-3.4 ± 0.3	-0.5 ± 0.1
Context (10)	-2.8 ± 0.4	-0.5 ± 0.1

Data are mean ± SE. The mAHP was measured as the peak of the AHP during the first 150 ms after current offset. The sAHP amplitude was measured 1 s after current offset. *$p < .05$ relative to Trace-Poor.

Figure 18

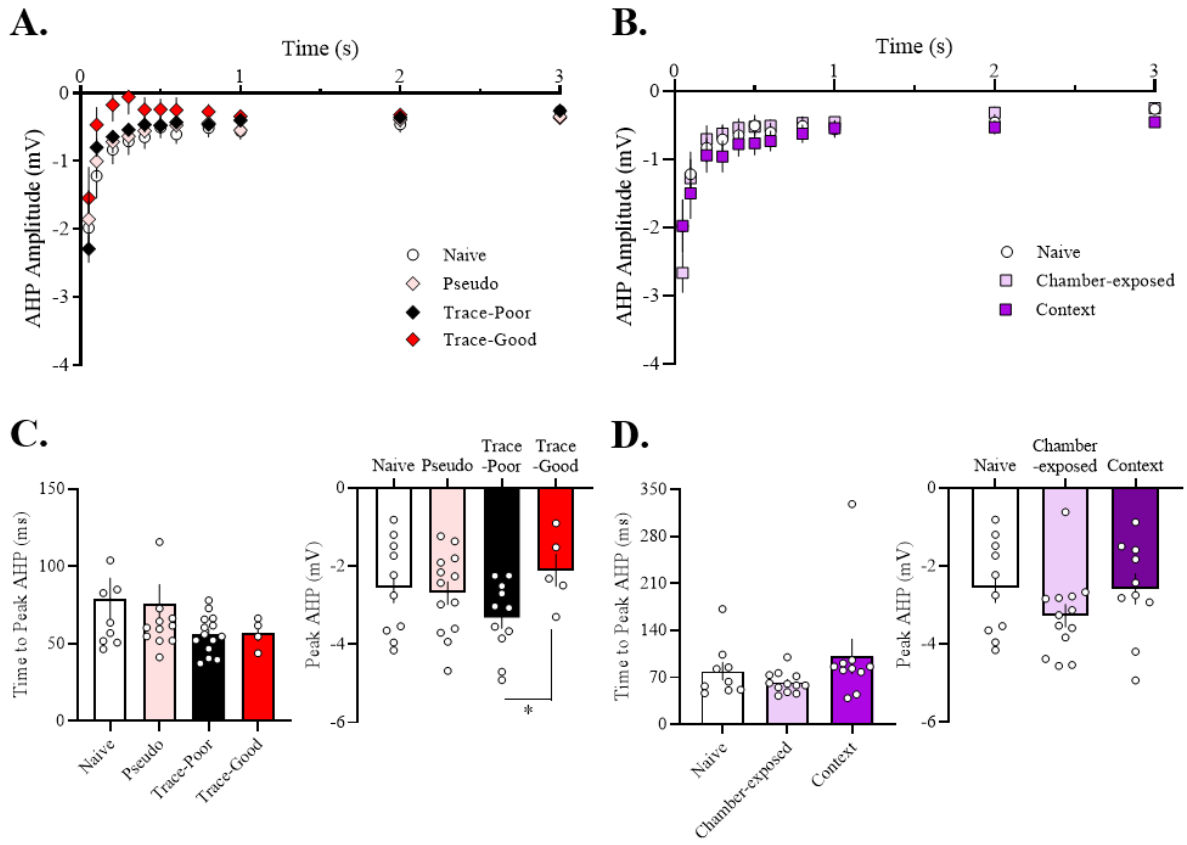

Figure 18. Trace fear conditioning bidirectionally alters peak postburst AHP amplitude in ventral hippocampal CA1 late-spiking neurons. ***A & B,*** Overall time course of the postburst AHP amplitude remains unchanged following trace fear conditioning (***A***), and following context fear conditioning (***B***). ***C,*** *Left,* Time to reach peak AHP is unchanged following trace fear conditioning. *Right*, Peak AHP amplitude is significantly reduced in neurons from Trace-Good rats when compared to neurons from Trace-Poor rats. ***D,*** There is no effect of context fear conditioning on time to peak AHP (*left*) or peak AHP amplitude (*right*). *$p < .05$.

Table 5. Effect of fear conditioning on membrane properties of ventral hippocampal CA1 late-spiking neurons

Group (# of cells)	RMP (mV)	R_N (MΩ)	Sag (mV)	$I_{threshold}$ (pA)	AP_{thresh} (mV)	AP_{amp} (mV)	AP_{width} (μs)	fAHP (mV)
Naïve (11)	-57.0 ± 1.1	94.8 ± 11.6	3.9 ± 0.5	201.4 ± 28.4	-40.4 ± 1.7	96.2 ± 3.4	735 ± 22	-7.1 ± 1.2
Pseudo (15)	-57.9 ± 1.3	83.5 ± 8.4	3.5 ± 0.3	205.0 ± 20.4	-41.5 ± 1.4	99.8 ± 1.7	740 ± 22	-6.8 ± 0.8
Trace								
Trace-Poor (11)	-57.7 ± 1.5	64.7 ± 2.6	3.1 ± 0.3	261.4 ± 16.7	-38.9 ± 2.1	94.0 ± 2.2	712 ± 20	-7.4 ± 1.6
Trace-Good (5)	-58.8 ± 1.6	95.9 ± 21.9	3.8 ± 0.6	183.0 ± 38.9	-40.3 ± 5.1	95.9 ± 4.7	731 ± 24	-9.9 ± 2.5
Chamber-exposed (14)	-56.2 ± 1.4	90.3 ± 10.3	4.1 ± 0.3	201.4 ± 24.8	-38.6 ± 2.2	96.3 ± 2.3	798 ± 39	-9.5 ± 1.4
Context (10)	-56.6 ± 1.6	105.1 ± 13.0	4.3 ± 0.5	174.5 ± 18.7	-42.6 ± 1.0	96.4 ± 1.8	790 ± 32	-7.3 ± 1.0

Data are mean ± SE. *Abbreviations:* resting membrane potential (RMP); neuronal input resistance (R_N); threshold current required to elicit an action potential ($I_{threshold}$); action potential threshold (AP_{thresh}); action potential amplitude (AP_{amp}); action potential half-width (AP_{width}); fast afterhyperpolarization (fAHP).

Discussion

The current experiments examine the effect of foreground context and trace fear learning, two forms of Pavlovian fear conditioning, on intrinsic excitability within ventral hippocampal CA1 neurons. Our data suggest that context fear conditioning increases excitability of ventral hippocampal CA1 RS neurons, while trace fear conditioning leads to no change in RS neuronal excitability. In contrast, trace fear conditioning bidirectionally alters intrinsic excitability of LS neurons – enhanced excitability is observed in neurons from good learners, while neurons from poor learners display suppressed excitability. Thus, learning-related changes of ventral hippocampal CA1 neuronal excitability are learning- and cell-type specific.

To our knowledge, this is the first study to examine the intrinsic plasticity of ventral hippocampal CA1 neurons following trace and context fear conditioning. Other evidence suggests trace fear learning alters dorsal hippocampal CA1 neuronal excitability (Kaczorowski & Disterhoft, 2009; McKay et al., 2009; Song et al., 2012), and that trace eyeblink conditioning differentially modifies excitability in dorsal and ventral hippocampal neurons (Weible et al., 2006). Consistent with the current experiments, these studies suggest that such changes in excitability are likely learning-specific, as they are not observed in neurons from control animals or poor learners (Kaczorowski & Disterhoft, 2009; McKay et al., 2009; Song et al., 2012; Weible et al., 2006).

The evidence from the present experiments suggests distinct firing types in the ventral hippocampal CA1 region selectively contribute to different fear associations. While RS neuronal excitability contributes to context fear learning, LS neuronal excitability changes support trace fear learning. Similar specificity of learning-related changes of excitability is observed in the subiculum following context fear conditioning, which selectively increases excitability of RS

neurons, but has no effect on BS neuronal excitability (Dunn et al., 2018). Our observation that trace fear, but not context fear, increases excitability in LS neurons from good learners is supported by the proposed function of the LS firing type. Due to the characteristic delay in spiking, and relatively suppressed adaptation, LS neurons are particularly well-suited to encode information over prolonged time periods (Beggs et al., 2000; Chu et al., 2003; Faulkner & Brown, 1999; Kurotani et al., 2013; Martina et al., 1999; McGann & Brown, 2000; Moyer et al., 2000; Storm, 1988; Tieu et al., 1999; Yousuf, Nye, et al., 2020). LS neuron activity may specifically support trace fear associations because they require that animals maintain a representation of the CS over time (i.e. over the TI) in order to associate it with the footshock US (Raybuck & Lattal, 2014). Future work is necessary to uncover exactly which aspects of trace fear acquisition and retrieval are supported by LS neuronal activity in ventral hippocampal CA1.

Given that learning-related increases of intrinsic excitability (i.e. reduced spike frequency adaptation) are typically accompanied by reduced AHP (e.g. Song et al., 2012), it was unusual to find that AHP measures displayed overall no change following context fear learning in RS neurons. However, we did observe a significant increase in time to reach peak AHP in RS neurons from trace fear conditioned rats. This suggests AP repolarization may vary in these neurons, and this change in repolarization phase could potentially confound any learning-related changes in the postburst AHP that might otherwise be observed. Additionally, recording methods can result in dramatically different observations in the AHP, with sharp microelectrode recordings having greater potential for uncovering more dramatic changes in physiological measures like AHP due to reduced dilution of intracellular signaling components relative to patch-clamp recordings. AHP measures can also depend on internal solution used in the patch pipette, with potassium gluconate-based internals like what was used in the present study

resulting in smaller AHPs but more stable recordings (Kaczorowski et al., 2007). Thus, our lack of observed changes in RS neuron postburst AHPs could be due to a possible floor effect. Regardless, we were able to observe AHP differences in LS neurons following trace fear conditioning, with neurons from good learners displaying a significant reduction in peak AHP voltage compared to neurons from poor learners.

Lastly, the current data indicate that context fear learning can induce excitability changes in a subset of ventral hippocampal CA1 RS neurons, suggesting these neurons may selectively contribute to context fear memory. Similar observations have been made in LA neurons following delay fear conditioning (Gouty-Colomer et al., 2016; Rumpel et al., 2005; Sehgal et al., 2014) and in IL-BLA projection neurons following trace fear conditioning (Song et al., 2015). Furthermore, a subset of LA neurons become activated during fear acquisition and retrieval, and selective deletion of these neurons disrupts fear expression (Han et al., 2007; Han et al., 2009). Thus, similar to other brain regions within the broader fear circuitry, the current data suggest that a specific subpopulation of ventral hippocampal CA1 RS neurons likely supports context fear learning. Future studies will be required to determine the specific mechanisms that contribute to such selective activation.

Chapter Four: General Discussion

The current experiments combine behavioral, molecular, and electrophysiological techniques to examine the contribution of the ventral hippocampus to context and trace fear memory. Our data suggest that the ventral hippocampus displays altered plasticity, as indicated by increased IEG expression and altered excitability, which is consistent with previous studies that demonstrate ventral hippocampal activity is required for trace and context fear associations (Beeman et al., 2013; Cox et al., 2013; Czerniawski et al., 2012; Czerniawski et al., 2009; Esclassan et al., 2009; Gilmartin et al., 2012; Kim & Cho, 2017; Maren & Holt, 2004; Pentkowski et al., 2006; Rogers et al., 2006; Rudy & Matus-Amat, 2005; Trivedi & Coover, 2004; Yoon & Otto, 2007; Zhang et al., 2001; Zhu et al., 2014). Further, the current experiments demonstrate ventral hippocampal CA1 exhibits heterogeneous firing types, and suggest that the physiological contribution of these neurons to different forms of associative fear memory likely vary as a function of intrinsic firing characteristics.

Role of Ventral Hippocampus in Context vs. Trace Fear Conditioning

Interestingly, while our western blots suggest that the ventral hippocampus displays increased Arc and pCREB protein expression following context but not trace fear retrieval, our patch-clamp recordings indicate altered excitability following both forms of fear learning. This raises an interesting question – if intrinsic excitability was altered in ventral hippocampal CA1 of trace fear conditioned animals, why did we not also observe altered IEG expression in the ventral hippocampus following trace fear retrieval? Previous work suggests that ventral hippocampal activity is specifically required for context fear, but not delay fear (Zhu et al., 2014), and that optogenetic silencing of ventral hippocampal terminals in PL during trace fear training disrupts context fear retrieval but not trace fear retrieval (Twining et al., 2020). These findings suggest

that ventral hippocampal neurons are preferentially tuned to support contextual over cued aspects of fear memory, and thus support our western blot findings that demonstrate context fear (not trace fear) retrieval increases ventral hippocampal Arc and pCREB expression.

Another possible scenario is that learning-related changes of plasticity occur in subpopulations of neurons within particular brain regions like the ventral hippocampus. This is supported by studies examining molecular (Han et al., 2007; Han et al., 2009; Reijmers et al., 2007; Zhou et al., 2009) and physiological (Dunn et al., 2018; Gouty-Colomer et al., 2016; Sehgal et al., 2014; Song et al., 2015; Weible et al., 2006) changes in various brain regions and in different learning tasks. Indeed, the current experiments demonstrate that trace fear conditioning increases excitability of ventral hippocampal CA1 neurons, but only within the LS firing type and only for LS neurons from rats that displayed relatively good trace fear memory. Thus, the lack of observed changes of either Arc or pCREB expression in the ventral hippocampus following trace fear retrieval could be due to divergent signaling patterns that are specific to subpopulations of ventral hippocampal neurons, which are obscured when looking at overall protein changes within entire brain regions. Thus, a more targeted approach that examines changes in IEG and CREB signaling within individual neuronal subpopulations (e.g. using immunohistochemistry) might uncover trace fear learning-related changes of plasticity.

Divergent Roles of Dorsal and Ventral Hippocampus in Trace Fear Conditioning

In contrast to previous research (Chia & Otto, 2013; Czerniawski et al., 2011; Hudgins & Otto, 2019), we did not observe increased Arc expression in the dorsal hippocampus of trace fear conditioned rats. As alluded to previously, this could be due to the fact that subsets of dorsal hippocampal neurons display increased Arc expression, while others do not, as demonstrated in Hudgins & Otto (2019). For example, if a single subregion of the dorsal hippocampus (e.g. CA3)

demonstrates increased Arc following trace fear retrieval, while the remaining subregions do not, this increase may not be robust enough to be detected by examining Arc expression in the entire dorsal hippocampus. Future work must therefore take into account the possibility that dorsal hippocampal subregions or subpopulations of dorsal hippocampal neurons are in fact supporting trace fear memory.

It is also important to note that some evidence suggests the ventral hippocampus plays a much more critical role in trace fear learning than the dorsal hippocampus (Cox et al., 2013; Czerniawski et al., 2012; Czerniawski et al., 2009; Rogers et al., 2006; Yoon & Otto, 2007). Trace fear retrieval is disrupted when APV infusions are targeted to the ventral hippocampus, but not the dorsal hippocampus (Czerniawski et al., 2012). Further, while muscimol inactivation of the dorsal hippocampus disrupts recent trace fear memory, muscimol inactivation of the ventral hippocampus disrupts both recent *and* remote trace fear retrieval (Cox et al., 2013). These studies together suggest that intact ventral hippocampal function is paramount for trace fear memory, and support the current data that demonstrate trace fear conditioning increases excitability among ventral hippocampal LS neurons from good learners. Future studies will be needed to investigate whether the ventral hippocampus could in fact be contributing to long-term fear memory by observing whether changes of intrinsic excitability persist over time.

Role of Ventral Hippocampal Circuits in Fear and Anxiety

Due to its extensive connectivity with other fear-related brain regions, including the amygdala and mPFC, the ventral hippocampus is especially well-suited to convey fear and anxiety associations within these networks. Disconnection of ventral hippocampus-PL communication using either contralateral or ipsilateral lesions impairs trace fear memory, suggesting this connectivity is necessary for trace fear memory retrieval (Gilmartin et al., 2012).

Further, ventral hippocampal inactivation may be driving this effect, as subsequent experiments indicated unilateral ventral hippocampal inactivation disrupted trace fear memory, regardless of whether PL was also inactivated (Gilmartin et al., 2012). Similar to trace fear memory, delay fear memory likely relies on network-level communication between the ventral hippocampus and mPFC/amygdala. Renewal of a delay fear memory increases c-fos expression in neurons projecting from the ventral hippocampus to the amygdala, and while ipsilateral disconnection of ventral hippocampus-to-PL or ventral hippocampus-to-amygdala communication failed to disrupt delay fear renewal, contralateral disconnection of these circuits abolished it (Orsini et al., 2011). These studies suggest that associative fear memories rely on communication between the ventral hippocampus and prefrontal cortex, as well as the amygdala.

Inhibitory avoidance learning also relies on ventral hippocampal-amygdala communication. Increased dorsal and ventral hippocampal intrinsic excitability following inhibitory avoidance learning are suppressed following inactivation of BLA, suggesting inhibitory avoidance is regulated by hippocampal-amygdala communication (Farmer & Thompson, 2012). Additionally, latency to cross to the shock-paired compartment increases when BLA-ventral hippocampal terminals are optogenetically activated immediately after footshock exposure, however, activation of these terminals immediately after initial context exposure (i.e. before exposure to the footshock) leads to no change in inhibitory avoidance learning (Huff et al., 2016). These results suggest that context-footshock associations are consolidated via BLA/ventral hippocampal connections, and that perhaps other pathways may be involved in consolidation of the non-aversive contextual aspects of learning.

Beyond associative fear learning, other evidence supports a role for ventral hippocampus-to-mPFC circuits in behavioral tasks that model anxiety. Optogenetic inhibition of ventral

hippocampal-mPFC terminals reduces anxiety-like behaviors in mice on the elevated plus maze (EPM) and open field test (Padilla-Coreano et al., 2016). Further, silencing of mediodorsal nucleus of the thalamus-mPFC terminals has no effect on open arm avoidance in the EPM, suggesting the observed reduction in anxiety behavior on this task following ventral hippocampal-mPFC terminal silencing is likely not due to a non-specific reduction of neuronal excitation within the mPFC (Padilla-Coreano et al., 2016). Overall, the evidence strongly supports a role for circuit-level communication between the ventral hippocampus and mPFC as well as amygdala in associative fear and anxiety-based learning tasks. Future work is necessary to determine whether context and/or trace fear learning-related changes of ventral hippocampal plasticity like the ones observed in the current experiments also lead to changes in ventral hippocampal circuit activation.

Heterogeneity of Physiological Firing Type Supports Learning

In the current experiments, ventral hippocampal CA1 intrinsic plasticity varied as a function of firing type and behavioral training, as well as memory strength. This is consistent with prior studies that have investigated fear learning-related changes in hippocampal (Dunn et al., 2018; Kaczorowski & Disterhoft, 2009; Song et al., 2012) and mPFC (Song et al., 2015) intrinsic plasticity. For example, RS neurons in PL display suppressed excitability following trace fear conditioning, while BS neurons in PL demonstrate enhanced excitability (Song et al., 2015). Additionally, context fear conditioning significantly increases the firing rate of RS subiculum neurons, but BS firing rates remain unchanged (Dunn et al., 2018). The relative proportion of specific firing types expressed within a single brain region can also be modified as a function of learning. The proportion of subiculum neurons displaying an RS phenotype

increases following context fear conditioning relative to naïve controls (Dunn et al., 2018), suggesting that perhaps firing patterns can be optimally tuned to support fear learning.

Although we did not observe any significant changes of firing type proportions as a function of behavior, we did observe that Context neurons tend to display RS properties rather than LS properties (49% vs. 29%), whereas Trace neurons tend display LS firing properties over RS properties (44% vs. 33%). This is particularly advantageous for the Trace group, as LS neurons are thought to support information encoding over prolonged time periods (Beggs et al., 2000; McGann et al., 2001; Storm, 1988; Tieu et al., 1999). Given that trace fear conditioning requires maintaining the CS representation across the TI to associate it with the US, our evidence showing increased LS excitability for neurons from Trace rats suggests they may be facilitating such an association (e.g. by delaying firing activity and/or adapting less to prolonged stimulation). Additionally, we found a considerable proportion of neurons from Trace and Context rats that displayed properties of both firing types, and as such were classified as RS/LS (19% and 17% respectively), while the relative percentage of Naïve neurons that were classified as RS/LS was much less (7%; Table 1). This further suggests that ventral hippocampal CA1 neurons may acutely tune their firing properties in such a way as to support different types of associative fear memory. This could perhaps be accomplished by altering the relative expression of specific ion channels (e.g. increased expression of voltage-gated K^+ channels to promote LS firing properties). Future work will be needed to address the physiological and molecular mechanisms that allow for such precise adjustments.

Final Conclusions and Future Directions

In summary, different forms of associative fear learning can lead to distinct changes in ventral hippocampal plasticity that likely support memory consolidation. Although several

studies have established that dorsal and ventral hippocampus are required for specific types of fear memory via inactivation or lesion techniques, relatively little work has examined how different forms of associative fear learning alter hippocampal plasticity, particularly in the ventral hippocampus. Because plastic changes such as IEG induction and altered intrinsic excitability are known to support various forms of memory, an examination of fear learning-induced ventral hippocampal plasticity will generate a broader understanding of its role in Pavlovian fear conditioning beyond merely observing what happens to learning in the absence of ventral hippocampal function. Here, we demonstrate for the first time that context fear memory retrieval increases expression of Arc as well as the phosphorylated active form of CREB in ventral hippocampal tissue, whereas trace fear retrieval leads to no change in IEG induction in this region. We also show for the first time that ventral hippocampal CA1 neurons consist of primarily RS and LS neuronal firing types, and these firing types demonstrate distinct changes of excitability following associative fear learning. While context fear conditioning increases RS neuronal excitability, trace fear conditioning also modifies LS neuronal excitability, but this change is closely linked with trace fear memory strength. Overall, our data provide ample evidence that suggests two distinct forms of associative fear learning, trace and foreground context fear, induce both molecular and cellular plasticity within the ventral hippocampus, indicating this region likely plays a definitive role in Pavlovian fear learning.

Future work is required to examine the region-specific and/or cell-type specific changes in IEG expression within the dorsal and ventral hippocampus following associative fear learning. Although our data suggest that context fear conditioning primarily drives IEG expression within the ventral hippocampus, we cannot conclude that trace fear conditioning does not also drive IEG induction in ventral neurons without determining whether there are changes in IEG

expression within neuronal subpopulations. Additionally, further exploration of the physiological mechanisms that underlie the observed changes in excitability within different firing types is warranted. Given the previously discussed possibility that neurons adjust their firing patterns based on environmental stimuli, and the present observation that hybrid firing patterns likely exist within the ventral hippocampal CA1 region, a thorough examination of ion channel expression and function from recorded neurons would provide additional insights into this phenomenon.

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2018	Neuroscience Research Symposium Abstract Winner University of Wisconsin – Milwaukee
2013-2016	Graduate Student Travel Award University of Wisconsin – Milwaukee
2014	Department of Psychology Summer Research Fellowship *The Effect of Apoaequorin on Trace Fear Conditioning in Aging Animals* University of Wisconsin – Milwaukee
2012-2014	Chancellor's Graduate Student Award University of Wisconsin - Milwaukee
2012	Sigma Xi Grant-in-Aid of Research *Effect of Oral Administration of Apoaequorin on Cell Death following Ischemia*

PUBLICATIONS

Ehlers, V. L., Yousuf, H., Smies, C. W., Moyer, J. R., Jr. (in preparation) Distinct patterns of Arc and pCREB protein expression in dorsal and ventral hippocampus following associative fear learning.

Ehlers, V. L., Yousuf, H., Smies, C. W., Natwora, B. R., Moyer, J. R., Jr. (in preparation) Fear conditioning alters intrinsic excitability of ventral hippocampal CA1 neurons in a learning- and cell-type-specific manner.

Ehlers, V. L., Smies, C. W., Moyer, J. R., Jr. (under review) Intrahippocampal infusion of the calcium-binding protein apoaequorin differentially modulates fear memory in adult and aged rats. *Brain and Behavior.*

Yousuf, H.*, **Ehlers, V. L.***, Sehgal, M., Song, C., Moyer, J. R., Jr. (2020). Modulation of intrinsic excitability as a function of learning within the fear conditioning circuit. *Neurobiology of Learning and Memory, 167*(107132). *co-primary authors

Song, C., **Ehlers, V. L.**, & Moyer, J. R., Jr. (2015). Trace fear conditioning differentially modulates intrinsic excitability of medial prefrontal cortex-basolateral complex of amygdala projection neurons in infralimbic and prelimbic cortices. *The Journal of Neuroscience, 35*(39), 13511-13524.

Sehgal, M., **Ehlers, V. L.**, & Moyer, J. R., Jr. (2014). Learning enhances intrinsic excitability in a subset of lateral amygdala neurons. *Learning & Memory, 21*(3), 161-170.

Sehgal, M., Song, C., **Ehlers, V. L.**, Moyer, J. R., Jr. (2013). Learning to learn – Intrinsic plasticity as a metaplasticity mechanism for memory formation. *Neurobiology of Learning and Memory, 105,* 186-199.

Holden J. M., Fitzgerald, M., Bussel, G., **Ehlers, V**. (2011). Triadimefon supports conditioned cue preference. *Behavioural Brain Research*, *221*(1), 307-310.

ABSTRACTS

Ehlers, V. L., Yousuf, H., Linske, M. D., Moyer, J. R., Jr. (2019). Developmental changes in the intrinsic excitability and morphology of ventral hippocampal CA1 neurons.
Poster presentation at the 2019 Society for Neuroscience meeting, Chicago, IL.

Ehlers, V.L., Yousuf, H., Smies, C. W., Moyer, J. R., Jr. (2018). Ventral hippocampal neuronal excitability and immediate early gene expression following trace fear learning.
Poster presentation at the 2018 Society for Neuroscience meeting, San Diego, CA.
Poster presentation at the 17th Molecular and Cellular Cognition Society meeting, San Diego, CA.

Ehlers, V.L., Tuma, J. A., Feldmann, K. L., Moyer, J. R., Jr. (2017). Apoaequorin differentially modulates fear conditioning in adult and aged rats.
Poster presentation at the UWM Neuroscience Symposium, University of Wisconsin – Milwaukee. March 2018.
Poster presentation at the 2017 Society for Neuroscience meeting, Washington, D.C.

Ehlers, V. L., Sehgal, M., Fulleylove-Krause, B. K., Hummer, B., Moyer, J. R., Jr. (2016). Quantifying changes in medial prefrontal cortex immediate early gene expression as a function of aging related cognitive decline.
Poster presentation at the UWM Neuroscience Symposium, University of Wisconsin – Milwaukee. March 2017.
Poster presentation at the 2016 Society for Neuroscience meeting, San Diego, CA.
Poster presentation at the 15th Molecular and Cellular Cognition Society meeting, San Diego, CA.

Ehlers, V. L., Feldmann, K. L., Moyer, J. R., Jr. (2015). Effects of the calcium-binding protein apoaequorin on acquisition of trace fear conditioning in adult and aging rats.
Poster presentation at the 2015 Society for Neuroscience meeting, Chicago, IL.

C. Song, **Ehlers, V. L.,** Aitken, J. C., Moyer, J. R., Jr. (2015). Delay fear conditioning enhances the intrinsic excitability of infralimbic neurons.
Poster presentation at 14th Molecular and Cellular Cognition Society meeting, Chicago, IL.
Poster presentation at the 2015 Society for Neuroscience meeting, Chicago, IL.
Adams, E. L., **Ehlers, V. L.,** Fettinger, N. B., Michels, S. C., Moyer, J. R., Jr. (2015). Oral administration of AQ is neuroprotective in an acute slice model of oxygen-glucose deprivation.
Poster presentation at the 2015 Society for Neuroscience meeting, Chicago, IL.

Ehlers, V. L., Adams, E. L., Fettinger, N. B., Michels, S. C., Moyer, J. R., Jr. (2014). The neurotherapeutic effects of the calcium binding protein apoaequorin. Poster presentation at the 2014 Society for Neuroscience meeting, Washington, D.C.

Song, C., **Ehlers, V. L.**, Aitken, J. C., Bula, T., Moyer, J. R., Jr. (2014). Trace fear conditioning and extinction differentially modulate intrinsic excitability of mPFC neurons that project to amygdala.
Poster presentation at the 2014 Society for Neuroscience meeting, Washington, D.C.

Moyer, J. R., Jr., **Ehlers, V. L.**, Song, C. (2014). Layer- and region-specific differences in the neurophysiological properties of medial prefrontal cortical neurons.
Poster presentation at the 2014 Society for Neuroscience meeting, Washington, D.C.

Adams, E. L., **Ehlers, V. L.**, Michels, S. C., Fettinger, N. F., Moyer, J. R., Jr. (2013). Orally administered apoaequorin protects neurons from oxygen-glucose deprivation.
Poster presentation at the 2013 Society for Neuroscience meeting, San Diego, CA.

Song, C., **Ehlers, V. L.**, Aitken, J. C., Bula, T., Moyer, J. R., Jr. (2013). Effect of trace fear conditioning and extinction on mPFC-amygdala projection neurons. Poster presentation at the 2013 Society for Neuroscience meeting, San Diego, CA.

Hochstetter, E. L., **Ehlers, V. L.**, Michels, S. C., Fettinger, N. B., Moyer, J. R., Jr. (2013). Oral administration of apoaequorin protects neurons from ischemic cell death. Poster presentation at the 2013 Meeting of The Milwaukee Chapter of the Society for Neuroscience. University of Wisconsin – Milwaukee, Milwaukee, WI.

Sehgal, M., **Ehlers, V. L.**, Moyer, J. R., Jr. (2013). Fear conditioning enhances intrinsic excitability in a subset of lateral amygdala neurons.
Poster presentation at the 2013 Meeting of The Milwaukee Chapter of the Society for Neuroscience. University of Wisconsin – Milwaukee, Milwaukee, WI.

Michels, S. C., Fettinger, N. B., Hochstetter, E. L., **Ehlers, V. L.**, Moyer, J. R., Jr. (2013). Methods for administration of apoaequorin in rats.
Poster presentation at the 2013 Meeting of The Milwaukee Chapter of the Society for Neuroscience, University of Wisconsin – Milwaukee, Milwaukee, WI.

Song, C., **Ehlers, V. L.**, Aitken, J. C., Bula, T., Moyer, J. R., Jr. (2013). Circuit-specific study of fear conditioning and extinction in mPFC neurons.
Poster presentation at the 2013 Meeting of The Milwaukee Chapter of the Society for Neuroscience, University of Wisconsin – Milwaukee, Milwaukee, WI.

Ehlers, V. (2010). Underdeveloped dendritic connections in the cerebellum of the BTBR mouse.
Poster presentation at the 20th Annual Tri-State Undergraduate Psychology Research Conference. Loras College, Dubuque, IA.

Ehlers, V. (2010). Triadimefon's effects on mouse behavior.
Poster presentation at the 4th Annual MidBrains Undergraduate Neuroscience Conference. St. Olaf College, Northfield, MN.

COLLOQUIA AND SYMPOSIA

Ventral hippocampal neuronal excitability and immediate early gene expression following fear learning. Presented at the 21st Annual Graduate Research Symposium, University of Wisconsin – Milwaukee. April 2019.

Apoaequorin differentially modulates fear conditioning in adult and aged rats. UWM Neuroscience Symposium, University of Wisconsin – Milwaukee. March 2018.

Aging-related changes of IEG expression following trace fear conditioning and extinction. Neuroscience Group Seminar, University of Wisconsin – Milwaukee. October, 2017.

Apoaequorin differentially modulates fear conditioning in adult and aged rats. Neuroscience Group Seminar, University of Wisconsin – Milwaukee. April 2017.

Effects of the calcium-binding protein apoaequorin on acquisition of trace fear conditioning in adult and aging rats. Presented at the 18th Annual Graduate Research Symposium, University of Wisconsin – Milwaukee. April 2016.

Effects of the calcium-binding protein apoaequorin on acquisition of trace fear conditioning in adult and aging rats. Presented at the UWM Neuroscience Mini-symposium, University of Wisconsin – Milwaukee. March 2016.

Aging-related cognitive decline and neuronal calcium dysregulation. Presented at the 17th Annual Graduate Research Symposium, University of Wisconsin – Milwaukee. April 2015.

Aging-related cognitive decline and neuronal calcium dysregulation. Neuroscience Group Seminar, University of Wisconsin – Milwaukee. Jan 2015.

Aging-related cognitive decline and neuronal calcium dysregulation. Neuroscience Group Seminar, University of Wisconsin – Milwaukee. May 2014.

TEACHING EXPERIENCE

University of Wisconsin – Milwaukee

Spring 2020	Teaching Assistant, Introduction to Statistics
Fall 2019	Teaching Assistant, Adv Physiological Psychology
Spring 2019	Teaching Assistant, Physiological Psychology
Fall 2018	Teaching Assistant, Adv Physiological Psychology
Spring 2018	Associate Lecturer, Adv Physiological Psychology
Fall 2012 – Spring 2018	Teaching Assistant, Introduction to Statistics
Spring 2015	Guest Lecturer, Introduction to Statistics, 1 class

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LABORATORY TECHNIQUES

Protein assay
Western blot
Stereotaxic surgery
Intracranial infusions
Fear conditioning
Immunohistochemistry
In vitro brain slice preparation
Patch clamp electrophysiology
Confocal microscopy