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Sex- and Age-associated Effects on the Retrosplenial Cortex Following Trace Fear Learning

Hanna Yousuf
University of Wisconsin-Milwaukee

SEX- AND AGE-ASSOCIATED EFFECTS ON THE RETROSPLENIAL CORTEX FOLLOWING TRACE FEAR LEARNING

by

Hanna Yousuf

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ABSTRACT

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Under the Supervision of Dr. James R. Moyer, Jr.

The rodent granular retrosplenial cortex (gRSC) forms reciprocal connections with the thalamus and hippocampus (Van Groen and Wyss, 1990, 1992; Sugar et al., 2011). The gRSC is well-positioned to coordinate information between higher-order brain structures to support complex forms of memory such as associative trace fear memories (Kwapis et al., 2014, 2015). Further, sex differences in fear learning and mechanisms underlying fear memories are observed in many of the brain regions implicated in trace fear learning such as the hippocampus, amygdala, and mPFC (Maren et al., 1994; Dalla et al., 2009; Gresack et al., 2009; Blume et al., 2017; Keiser et al., 2017), however, few studies have investigated sex differences in the RSC. The male gRSC has a diverse population of neuronal firing types, a likely requirement to encode complex associative memories. Cell types that are found in the rat gRSC include regular spiking (RS), regular spiking afterdepolarization (RS_{ADP}), burst firing, fast spiking, and late spiking neurons. The neuronal distribution drastically changes from the RS-dominant early adolescent to the RS_{ADP}-dominant mid-adolescent and adult gRSC. The afterdepolarization (ADP) component plays a key role in gRSC development but is also able to promote excitability and plasticity in fear-related regions (Azouz et al., 1996; Pike et al., 1999; Yousuf et al., 2020b). Thus, the first aim of this dissertation was to examine sex-dependent mechanisms underlying the ADP in RS_{ADP} neurons of the gRSC. Using whole-cell patch-clamp electrophysiology and morphological

analysis, we determined that N-methyl-D-aspartate (NMDA) receptor blockers reduce the ADP in female but not male RS_{ADP} neurons. Additionally, dendritic complexity and branching is reduced in male RS_{ADP} neurons compared to male RS neurons, whereas dendritic complexity is similar in female RS and RS_{ADP} neurons. These data suggest that while NMDA receptor activity mediates the ADP in females, dendritic morphology may regulate it in males.

The second aim of this dissertation used Pavlovian fear conditioning and patch-clamp electrophysiology to examine sex differences in the developmental trajectory of trace memories. Females show a positive linear trend in fear retrieval across early adolescence, mid-adolescence, and adulthood. Conversely, males exhibit a nonlinear trajectory where mid-adolescent rats have reduced fear retrieval compared to early adolescents and adults. Sex differences are noted only during early adolescence where males have stronger retrieval of trace fear compared to females. Similarly, sex differences in excitability of RS_{ADP} neurons in the gRSC are present during early adolescence, in which males fire more action potentials compared to females. Next, we investigated how fear retrieval alters distinct neuronal firing types in the male and female gRSC. In males, fear learning or exposure to novel stimuli reduces excitability of RS_{ADP} neurons in the early adolescent, mid-adolescent, and adult gRSC. However, in females, trace fear memory formation or exposure to novel stimuli suppresses excitability of both RS and RS_{ADP} neurons only in the adult gRSC. These data suggest that the male RSC is part of the trace fear circuitry early in development, whereas the female RSC may join the circuit later in development. Collectively, the results from this dissertation provide evidence that the male and female gRSC require distinct mechanisms to reach maturity, and are differentially affected by learning or novel experiences. Our work can provide insight regarding formation of maladaptive memories that underlie anxiety disorders that disproportionately affect women and adolescent girls.

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LIST OF ABBREVIATIONS

ADP: Afterdepolarization

AHP: Afterhyperpolarization

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

AP: Action potential

Arc: Activity-regulated cytoskeleton-associated protein

aRSC: anterior retrosplenial cortex

BLA: Basolateral amygdala

BNST: Bed nucleus of the stria terminalis

BS: Burst spiking

CaM: Calmodulin

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

CAMKKα: Calcium calmodulin kinase kinase α

CAN: Ca^{2+}-activated nonselective cation

CR: Conditioned response

CREB: cAMP Responsive Element Binding Protein

CS: Conditioned stimulus

E_2: 17β-estradiol

EPSC: Excitatory postsynaptic current

EPSP: Excitatory postsynaptic potential

ER: Estrogen receptors

ERK: Extracellular signal-regulated kinase

E-S: EPSP-spike

fAHP: Fast afterhyperpolarization

FS: Fast spiking

GABA: Gamma-aminobutyric acid

gRSC: Granular retrosplenial cortex

IEG: Immediate early gene

IPSP: Inhibitory postsynaptic potential

LS: Late spiking

LTD: Long-term depression

LTP: Long-term potentiation

mAHP: Medium afterhyperpolarization

mGluRs: Metabotropic glutamate receptors

mPFC: Medial prefrontal cortex

mRNA: messenger ribonucleic acid

NMDA: N-methyl-D-aspartate

pRSC: posterior retrosplenial cortex

PKA: cAMP-activated protein kinase

Postnatal day: PND

RS: Regular spiking

RS_{ADP}: Regular spiking afterdepolarization

RSC: Retrosplenial cortex

sAHP: Slow afterhyperpolarization

UR: Unconditioned response

US: Unconditioned stimulus

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CHAPTER ONE: Introduction and background information

Pavlovian fear conditioning

Pavlovian fear conditioning refers to learned associations between nonthreatening environmental stimuli and aversive events (Pavlov, 1927). Formation of fear memories is an evolutionary conserved process that allows organisms to survive against threat (Maren, 2001; Fanselow and Sterlace, 2014). When adequately expressed, fear increases the chances of survival, however, maladaptive fear memories can lead to anxiety disorders or posttraumatic stress disorder. Thus, Pavlovian fear conditioning not only serves as a model for anxiety disorders but it is also useful to investigate cellular mechanisms of learning and memory (Thompson and Krupa, 1994; Krasne and Glanzman, 1995; Holland and Gallagher, 1999). To date, there has been an exponential growth in different types of fear conditioning studies across many species (Fanselow and Sterlace, 2014). The earliest study of fear conditioning was conducted in infants, and is famously known as the "Little Albert" study (Watson and Rayner, 1920). In this study, a 9 month old infant (Albert) was conditioned to associate a white rat (conditioned stimulus; CS) with a loud noise (unconditioned stimulus; US). Following several pairings of the white rat and loud noise, Albert showed signs of distress (conditioned response; CR) when he was only presented with the rat. Similarly, in the laboratory, classical fear conditioning in rodents involves pairings of a neutral cue such as a light or tone (CS) with an aversive cue such as a mild footshock (US), which naturally elicits a stereospecific freezing or crouching response as the unconditioned response (UR). A learned association between CS and US occurs over multiple pairings of the two stimuli, which results in freezing behaviors (CR) to the CS alone. Thus, classical fear conditioning coupled with morphological, electrophysiological, and molecular techniques is an invaluable tool to elucidate mechanisms of learning and memory.

There are several types of fear conditioning paradigms, however, the more commonly used paradigms include context fear conditioning, delay fear conditioning, and trace fear conditioning. In contextual fear conditioning experiments, animals are placed in a context (CS) and receive a mild foot shock (US). When re-exposed to the training context, animals demonstrate a freezing response (CR) as they associate the environment with the aversive stimulus. Contextual regulation of associative fear learning and retrieval involves several brain structures including the medial prefrontal cortex (mPFC), hippocampus, and amygdala (Quirk et al., 2003; Herry et al., 2008; Orsini et al., 2011; Adhikari et al., 2011; Knapska et al., 2012; Sotres-Bayon et al., 2012). Another widely used type of fear conditioning is known as delay fear conditioning, in which a tone or a light (CS) is paired with a footshock (US). The CS offset overlaps with US onset, and elicits a freezing response (CR). The neural circuit for delay fear conditioning is simple and requires the brainstem and amygdala (Phillips and LeDoux, 1992; LeDoux, 2000; Kitamura et al., 2015). However, a subtle change in delay fear conditioning such as a brief temporal gap (30 s) or 'trace' interval between the CS and US (trace fear conditioning) can dramatically change the neural circuit. Explicit, declarative knowledge of the CS-US contingencies in trace fear learning (but not delay fear learning) requires the interaction of several subcortical and cortical brain regions, including the amygdala, hippocampus, mPFC, rhinal, and retrosplenial cortices (Knight et al., 2004; Esclassan et al., 2009a, 2009b; Gilmartin and Helmstetter, 2010; Kwapis et al., 2011; Gilmartin et al., 2012; Kwapis et al., 2015). Thus, trace fear conditioning is an excellent paradigm to investigate cellular and molecular mechanisms that underlie complex forms of learning that involve higher-order brain structures. Although many of the brain regions recruited in trace fear including the hippocampus, mPFC, and amygdala are extensively studied, the role of the

retrosplenial cortex (RSC) in trace fear learning remains poorly understood. Therefore, the overall goal of this dissertation is to understand the physiological properties of the RSC, and how they are altered as a consequence of trace fear learning.

Location, connectivity, and function of the retrosplenial cortex

The RSC is one of the largest cortical regions, extending over half the length of the cerebrum of the rat brain (Vann et al., 2009; Todd and Bucci, 2015). The anterior (aRSC) and posterior (pRSC) subregions (*Figure 1*) are known to be functionally distinct, in which the aRSC selectively processes the event-related (tone) component, and the pRSC processes the contextual component of the fear memory (Trask et al., 2021). Along the anterior-posterior axis, the RSC is further subdivided into the granular (Brodmann area 29; *Figure 1*) and dysgranular region (Brodmann area 30; *Figure 1*). Based on cytoarchitectural features, the granular RSC (gRSC) can be further subdivided into areas 29a, 29b, and 29c. Area 29a is the most ventral subdivision of the gRSC with a homogenous layer 2/3 (Vogt and Peters, 1981; Sugar et al., 2011; *Figure 1*). Dorsally adjacent to area 29a, area 29b consists of a densely packed layer 2/3. Compared to areas 29a and 29b, pyramidal cells bodies in area 29c are more randomly spaced (Van Groen and Wyss, 1990; Jones et al., 2005; Sugar et al., 2011; *Figure 1*). The dysgranular region (area 30) of the RSC does not have subregions but neuronal cell bodies in this region are larger and less densely packed compared to the gRSC (area 29; Krieg, 1946; Vogt and Peters, 1981; Van Groen and Wyss, 1990; Jones et al., 2005; *Figure 1*). The gRSC and dysgranular RSC are distinguishable by their reciprocal connections with different brain structures. For example, the gRSC forms connections with the anterior thalamic nuclei, whereas the dysgranular RSC is interconnected with the visual cortex (Vogt and Miller, 1983; Vann et al., 2009). The RSC (granular and dysgranular) forms reciprocal connections with the cingulate cortex, hippocampal formation (subiculum, presubiculum, and parasubiculum), entorhinal cortex, and parahippocampal region (Van Groen

and Wyss, 1990, 1992; Agster and Burwell, 2009; Sugar et al., 2011; Aggleton et al., 2012). The RSC acts as an intermediate node by forming reciprocal connections with the dorsolateral prefrontal cortex, providing an indirect route between the hippocampus and dorsolateral prefrontal cortex (Morris et al., 1999; Todd et al., 2019). Together, the anatomical connectivity of the RSC to the thalamus, hippocampus, and mPFC highlights its distinct role for fear memory formation.

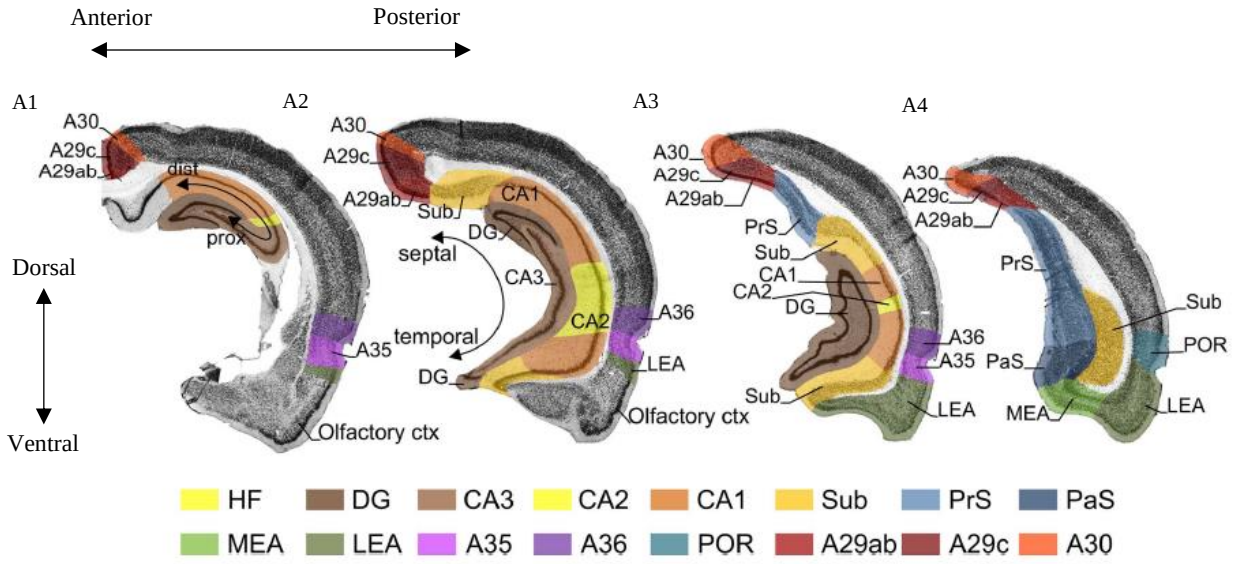


Figure 1. Representations of the retrosplenial cortex (RSC), hippocampal formation (HF), and the parahippocampal region in the rat brain. Four coronal sections of the rat brain. The RSC is subdivided into anterior (A1) and posterior (A2, A3, A4) regions. Along the anterior-posterior axis, the RSC is further subdivided into A29ab, A29c, and A30. The HF consists of the dentate gyrus (DG), CA3, CA2, CA1, and the subiculum (Sub). The PHR is subdivided into the presubiculum (PrS), parasubiculum (PaS), the entorhinal cortex, which has a lateral (LEA) and a medial (MEA) subdivision, the perirhinal cortex (PER; consisting of Brodmann areas A35 and A36) and the postrhinal cortex (POR). Adapted from Sugar et al., 2011.

Role of retrosplenial cortex in fear learning

The RSC is known to be involved in different types of fear conditioning including contextual fear conditioning. For example, one recent study used cFos genetic tagging to label mouse RSC cells that are active during contextual fear conditioning (Cowansage et al., 2014). Optogenetic reactivation of the tagged RSC cells in a novel (not training) context induces high freezing responses in mice (Cowansage et al., 2014). RSC lesioned rats are unable to acquire or retrieve contextual fear memories compared to controls (Keene and Bucci 2008a, 2008b; Robinson

et al., 2018). Further, infusions of a protein synthesis inhibitor, anisomycin (Kwapis et al., 2015) or N-methyl-D-aspartate (NMDA) receptor antagonists (Corcoran et al., 2011) in the rat RSC inhibits retrieval of context fear memories. Pharmacological blockade or lesions of the RSC attenuates retrieval of recent contextual fear conditioning (Keene and Bucci, 2008a, 2008b; Corcoran et al., 2011; Kwapis et al., 2015), however, RSC involvement is also evident in remotely acquired contextual fear memories where NMDA receptor blockade or lesions of the rodent RSC impairs retrieval of remote memories, approximately 8 weeks after contextual fear conditioning (Corcoran et al., 2011; Todd et al., 2016). Taken together, the RSC is necessary for retrieval of both recent and remote contextual fear memories.

Substantial evidence indicates that damage or inactivation of the RSC does not affect delay fear conditioning (CS and US overlap in time). Lesions or NMDA receptor inhibition to the rat RSC made prior to or after delay fear conditioning does not impair fear expression in rats (Keene and Bucci, 2008a, 2008b; Corcoran et al., 2011; Kwapis et al., 2014, 2015). The RSC has a limited role in a conditioned suppression task, which involves initial training to lever press for food reinforcement. In this task, a CS is paired with an aversive US, and typically rats suppress lever pressing (CR) in the presence of the CS alone. RSC and sham lesioned rats exhibit a similar number of lever presses in response to the CS (Todd et al., 2017). Similarly, the RSC has minimal involvement when the CS overlaps with a positive reinforcement (e.g. food; Keene and Bucci, 2008c; Robinson et al., 2011). RSC manipulations do not affect acquisition or retrieval of recent delay fear memories, however, one study shows that the rat RSC may selectively be involved in retrieval of remote delay fear memories in rats (Todd et al., 2016). Overall, except for remote delay fear memories, the RSC is not involved in delay fear learning across a range of stimuli, reinforcers, and conditioning paradigms.

The role of RSC is sensitive to the temporal relationship between the CS and US as it has shown to be necessary for trace fear conditioning (CS is separated from the US). Infusions of a protein synthesis inhibitor in the rat RSC prior to training impairs acquisition of trace fear conditioning (Kwapis et al., 2015). Likewise, infusions of NMDA receptor antagonists in the rat RSC following trace fear conditioning disrupts retrieval of trace fear memories (Kwapis et al., 2014, 2015). Using selective chemogenetic approaches, inactivation of the rat RSC disrupts retrieval of remote trace fear memories (Todd et al., 2016). Thus, the RSC's distinct involvement in trace fear learning shows that this region is critical for higher-order fear learning.

In summary, the rodent RSC is a key node in neural circuits mediating trace and context fear learning (Corcoran et al., 2011, 2013; Kwapis et al., 2014, 2015). These classical fear conditioning paradigms provide critical insight into how learning regulates intrinsic plasticity in many cortical and subcortical brain regions of the fear circuit including the amygdala (Rosenkranz and Grace, 2002; Sehgal et al., 2014), hippocampus (McKay et al., 2009; Song et al., 2012; Zhang et al., 2017), and mPFC (Santini et al., 2008; Sepulveda-Orengo et al., 2013; Song et al., 2015). To date, no studies have investigated how fear learning can alter neuronal plasticity in the RSC. The following section highlights mechanisms underlying intrinsic plasticity as well as our current understanding of the RSC physiology.

Plasticity of intrinsic membrane properties in the retrosplenial cortex

Associative fear memories or other types of memories lead to persistent, experience-dependent modifications in synaptic strength. Learning-related plastic changes can occur at the level of the synapse in the form of long-term potentiation (LTP) or long-term depression (LTD) or nonsynaptic changes that are intrinsic to the neuron (Daoudal and Debanne, 2003; Mozzachiodi and Byrne, 2010; Sehgal et al., 2013; Dunn and Kaczorowski, 2019; Yousuf et al., 2020a). Historically, synaptic plasticity has been extensively investigated as a cellular mechanism for

learning and memory. Much of the work on LTP has been focused on the perforant pathway of the hippocampus, which involves a high frequency stimulation to induce a robust and persistent enhancement of synaptic strength (Bliss and Lomo, 1973). Since the landmark findings of Bliss and Lomo, LTP has been observed in other fear-related brain structures including the amygdala and cerebellum (Racine et al., 1986; Chapman et al., 1990; Laroche et al., 1990). LTP occurs in a synapse-specific manner and multiple factors including stimulation patterns and neuromodulators can regulate LTP expression (Bliss and Lomo, 1973; Lynch et al., 1977; Lynch, 2004; Kumar, 2011; Lüscher and Malenka, 2012; Herring and Nicoll, 2016). However, learning-induced plasticity is not limited to modifications of the synapse as intrinsic membrane properties (resting and voltage-dependent channels) determine the likelihood of a neuron firing action potentials (APs) in response to synaptic inputs. Specifically, learning alters several intrinsic properties including AP amplitude, AP half-width, and afterdepolarization (ADP), which may influence neuronal excitability. Moreover, enhanced intrinsic excitability (reduced spike frequency adaptation) is correlated with enhanced learning (Disterhoft et al., 1986, 1988; *Figure 2*). Spike frequency adaptation is mediated by an afterhyperpolarization (AHP) current, and reduced AHP increases AP firing frequency (Disterhoft and Oh, 2006; Oh et al., 2010; *Figure 2*). The AHP acts as a negative feedback mechanism, and has three components: fast afterhyperpolarization (fAHP; within 2-5 ms of an AP), medium afterhyperpolarization (mAHP; 50-150 ms following a burst of APs), and slow afterhyperpolarization (sAHP; 1 s following a burst of APs; Storm, 1987, 1989; Sah and Bekkers, 1996; Kaczorowski et al., 2007; Song and Moyer, 2017). Fear learning increases intrinsic excitability, and reduces AHP in several brain structures including the amygdala (Sehgal et al., 2014), hippocampus (Kaczorowski and Disterhoft, 2009; McKay et al., 2009; Song et al., 2012; Dunn et al., 2018), and mPFC (Santini et al., 2008; Santini and Porter, 2010; Sepulveda-

Orengo et al., 2013; Soler-Cedeno et al., 2016). Taken together, it is evident that learning modulates both synaptic and nonsynaptic (intrinsic) ion channels, and receptors within the fear circuit (Mayford et al., 2012; Sehgal et al., 2013; Yousuf et al., 2020a).

It is clear that intrinsic and synaptic plasticity are mechanisms that are tightly coupled, such that enhanced intrinsic excitability promotes synaptic throughput in the brain (for extensive reviews see Sehgal et al., 2013; Yousuf et al., 2020a). However, how associative fear learning modulates intrinsic plasticity within the RSC has not yet been studied. So far, we have investigated that the RSC has a heterogeneous neuronal population, and the intrinsic and morphological properties of RSC neurons have unique characteristics, which may promote learning-related intrinsic excitability (Brennan et al., 2020; Yousuf et al., 2020b).

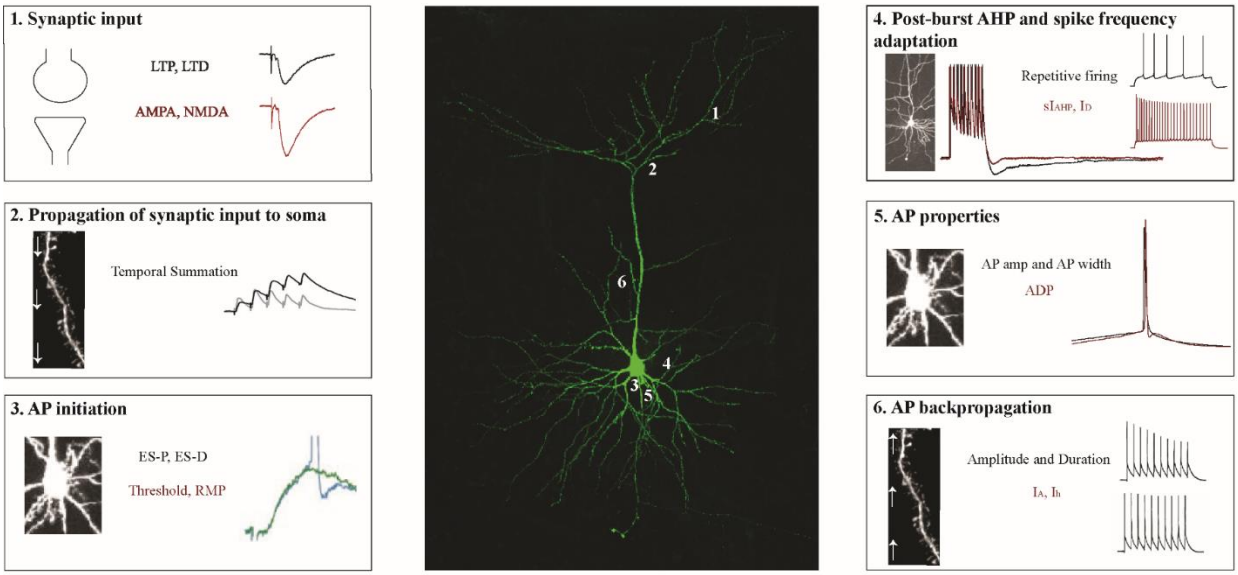


Figure 2. Synaptic and intrinsic properties modulate the flow of information within a neuron. Middle panel shows a confocal image of a retrosplenial cortical neuron that was filled with biocytin during whole-cell patch-clamp recording. Numbers 1, 2, 3, 4, 5 and 6 refer to the boxes in the left and right panels. (1) Most neuronal inputs originate via synapses on the dendrites and dendritic spines, which can undergo bidirectional plasticity in the form of LTP and LTD. Such plasticity is modulated by AMPA and NMDA receptor-mediated transmission as well as intrinsic membrane properties. (2) Following synaptic transmission, EPSPs are propagated from their site of generation towards the soma and AP zone. Propagation of EPSPs is influenced by dendritic cable as well as active membrane properties. (3) Once the signal reaches the soma, the increased likelihood for an EPSP to fire an AP is termed E-S potentiation. Factors including AP threshold and resting membrane potential determine AP initiation. (4) Increased neuronal excitability (e.g., reduction in the postburst AHP and/or spike frequency adaptation) promotes an output signal. (5) Other intrinsic factors including AP amplitude, AP duration, and the presence or absence of an ADP also influence neuronal excitability, which modulates neuronal processing and synaptic throughput. (6) Synaptic efficacy is also influenced by backpropagating APs, which are mediated by complex dendritic morphology, and dendritic ionic

conductances such as I_A currents. *Abbreviations*: long-term potentiation (LTP); long-term depression (LTD); excitatory postsynaptic potential (EPSP); EPSP-spike (E-S); action potential (AP); afterdepolarization (ADP). Electrophysiological traces in boxes 3 and 6 were adapted from Daoudal et al., 2002 (Copyright (2002) National Academy of Sciences, USA) and Tsubokawa et al., 2000 (Copyright (2000) Society for Neuroscience). Figure from Yousuf et al., 2020a.

As stated earlier, the RSC has two distinct subregions, gRSC and dysgranular that are morphologically and functionally distinct (Van Groen and Wyss, 1990, 1992; Van Groen et al., 2004; *Figure 1*). Because of its extensive connections with the thalamus and hippocampus, the majority of behavioral and electrophysiological studies have targeted the gRSC (Chen et al., 1994; Cho and Sharp, 2001; Van Groen et al., 2004; Garden et al., 2009; Pothuizen et al., 2009; Corcoran et al., 2011; Kwapis et al., 2015). We elucidated the existence of five distinct firing patterns in the adult gRSC, which include regular spiking (RS), regular spiking afterdepolarization (RS_{ADP}), late spiking (LS), burst spiking (BS), and fast spiking (FS) neurons (Yousuf et al., 2020b).

The most abundant firing type in the adult rat gRSC are RS_{ADP} neurons (63%, 71 of 113), which are identified as pyramidal (Yousuf et al., 2020b). RS_{ADP} neurons reside both in layers 2/3 (17%, 19 of 113) and layer 5 (46%, 52 of 113) of the adult gRSC. RS_{ADP} neurons in layer 2/3 and layer 5 are morphologically similar but differ in certain intrinsic membrane properties (Yousuf et al., 2020b). RS_{ADP} neurons in layer 2/3 have a significantly higher initial firing frequency compared to those in layer 5. In response to increasing current injections, RS_{ADP} neurons in layers 2/3 and 5 demonstrate robust spike frequency adaptation (Yousuf et al., 2020b). In addition to the gRSC, RS_{ADP} neurons are observed in other regions of the fear circuit including CA1 (Schwartzkroin, 1975; Storm, 1987; Azouz et al., 1996; Magee and Carruth, 1999; Yue and Yaari, 2004; Yue et al. 2005; Metz et al., 2005, 2007; Kaczorowski et al., 2007; Chen and Yaari, 2008) and CA3 (Brown and Randall, 2009) region of the hippocampus, mPFC (Haj-Dahmane and Andrade, 1997; Kalmbach et al., 2013), and lateral amygdala (Sugita et al., 1993). The ADP property can trigger burst firing in hippocampal neurons (Schwartzkroin, 1975; Azouz et al., 1996;

Jensen et al. 1996; Sanabria et al., 2001; Su et al., 2001), and bursting is a requirement for synaptic plasticity at the Schaffer collateral to CA1 synapse (Thomas et al., 1998; Pike et al., 1999). Thus, the ADP enhances excitability, which may play an important role in learning-related plasticity in the fear circuit (Disterhoft et al., 1986; Jensen et al., 1996; Moyer et al., 1996; Zhang and Linden, 2003; Santini et al., 2008).

Pharmacological manipulations or electrical stimulation can induce an ADP in CA1 (Azouz et al., 1996; Wu et al., 2004; Park et al., 2010) and CA3 (Young et al., 2004) regions of the rodent hippocampus. Ion channels and receptors that mediate the ADP are NMDA receptors (Wu et al., 2004; Grienberger et al., 2014), metabotropic glutamate receptors (mGluRs; Greene et al., 1994; Young et al., 2004; Park et al., 2010), and muscarinic receptors (Haj-Dahmane and Andrade, 1998; Yan et al., 2009; Lei et al., 2014). Activation of mGluRs and muscarinic receptors enhances activity of Cav2.3 R-type calcium channels in CA1 (Park et al. 2010), Ca^{2+}-activated nonselective cation (CAN) current in the neocortex (Greene et al. 1994), Na^{+} currents in the substantia nigra (Guatteo et al., 1999), and voltage-sensitive CAN current in mPFC and CA3 of the hippocampus (Caeser et al., 1993; Haj-Dahmane and Andrade, 1998) in rats. In the adult rat gRSC, we have shown that mGluRs partially mediate the ADP property as mGluR inhibitors (LY367385 – mGluR1 and MPEP – mGluR5) significantly reduce the ADP but do not completely abolish it (Yousuf et al., 2020b). This suggests that diverse receptors and channels contribute to the ADP, and their roles may differ across brain regions (Azouz et al., 1996; Haj-Dahmane and Andrade, 1997, 1998; Metz et al., 2005; Fowler et al., 2007; Chen and Yaari, 2008; Park et al. 2010; Yan et al., 2009).

RS_{ADP} neurons in the gRSC are the most prominent type of pyramidal neurons in the gRSC, however, we also observe BS pyramidal neurons, which generate a burst of 2 or more APs in

response to a suprathreshold depolarizing current injection. We only observe BS neurons in layer 5 (5%, 3 of 65) of the gRSC (Yousuf et al., 2020b), and identified a subset of neurons that oscillate between single RS_{ADP} to BS neurons. This oscillating pattern has previously been reported in neurons of the rat subthalamic nucleus, which is facilitated by the activation of L-type Ca^{2+} currents, Ca^{2+}-activated inward current, and Ca^{2+}-activated K^{+} currents (Beurrier et al., 1999; Su et al. 2001). Previous evidence suggests summation of ADPs can lead to burst or repetitive neuronal firing (Legendre and Poulain, 1992; Su et al. 2001; Kuehl-Kovarik et al., 2005). BS neurons exhibit similar physiological and morphological (Yousuf et al., 2020b) characteristics to RS_{ADP} neurons, and due to the large prevalence of ADPs in layer 5 of the gRSC, we speculate that BS neurons may be an extension of RS_{ADP} neurons.

It is important to understand the properties of different cell types as unique firing patterns may convey distinct types of information to promote flexible processes necessary for learning-related synaptic and/or intrinsic plasticity. Previous work has shown that trace fear learning reduces excitability in RS neurons of the rat mPFC, and increases excitability in BS neurons of the mPFC (Song et al., 2015). In contrast, contextual fear learning differentially enhances intrinsic excitability of RS neurons but not BS cells of the mouse hippocampus (Dunn et al., 2018). Further, learning results in a reduction in the proportion of BS cells, and overrepresentation of RS neurons in the hippocampus (Dunn et al., 2018). These findings indicate that RS and BS neurons convey specialized information, and may project to different brain regions. BS neurons that have distinct morphologies often project to thalamus, pons, and colliculus, whereas RS neurons are more likely to project to cortex or to the striatum (Gao and Zheng, 2004; Le Be et al., 2007). Overall, understanding cell type-specificity may provide critical insight into developing circuits, and precise mechanisms that mediate fear learning.

Development of electrophysiological properties within the fear circuit

The previous section discussed that the RSC of adult rats has a heterogeneous population of cell types, and each have a unique profile based on intrinsic and morphological properties. Observations in the mPFC (Song et al., 2015) and hippocampus (Dunn et al., 2018) indicate that specific cell types are differentially recruited during fear learning. However, we have also shown the firing types are altered as a consequence of age (adolescence to adulthood), and these changes may have implications regarding circuit- and learning-specific changes across development.

We have reported that RS neurons (without an ADP) are the most common firing type (~80%) in layer 5 gRSC of the juvenile rat between postnatal days (PND) 14-30, and are much less common in layer 5 of the adult (PND > 60) gRSC (~10%; *Figure 3; Table 1*). In contrast, RS_{ADP} neurons are rare (12%) in layer 5 of the juvenile gRSC but are frequently observed in layer 5 of the adult (~80%) gRSC (*Figure 3; Table 1*). During the lifespan, RS_{ADP} neurons in the gRSC begin to increase in number at approximately PND 35 (41%), whereas RS neurons decline (59%), and this pattern steadily continues into adulthood (PND > 60; *Figure 3; Table 1*). Adult RS_{ADP} neurons in layer 5 exhibit enhanced excitability compared to RS neurons of juvenile neurons (PND 14-30) in layer 5 of the gRSC (Yousuf et al., 2020b; *Figure 4*), suggesting that the ADP component in neurons may play a role in promoting excitability of gRSC neurons.

Similar to the gRSC, intrinsic properties of neurons in other fear-related brain regions such as the BLA undergo significant changes until PND 28 (Ehrlich et al., 2012). Specifically, between PND 7-28, drastic changes in intrinsic properties occur, such that the input resistance is reduced nearly 10-fold, and membrane time constant is reduced 4-fold in BLA neurons (Ehrlich et al., 2012). Likewise, during the first postnatal month, mPFC neurons of mice also have significantly decreased input resistance, resting membrane potential, AP half-width, and AP threshold in both

layers 3 and 5 (Kroon et al., 2019). In addition to alterations in intrinsic properties, morphological changes occur during development in the BLA and mPFC (Ryan et al., 2016; Kroon et al., 2019). Specifically, during adolescence, dendritic pruning occurs, in which synapses are eliminated and refined until they stabilize through adulthood (Huttenlocher, 1979; Petanjek et al., 2011). In neurons of the mouse mPFC, number of apical dendritic branches begin to decrease at PND 16 (Kroon et al., 2019), whereas dendritic length decreases in rat BLA neurons at PND 28 (Ryan et al., 2016). Dendritic structure of neurons can influence neuronal function, as dendritic surface area and conductances directly affect firing patterns (Mainen and Sejnowski, 1996). For example, reduced dendritic length is associated with increased ADP amplitude and burst firing (Bekkers and Häusser, 2007; Roberts et al., 2009; Šišková et al., 2014). Together, electrophysiological and morphological characteristics of the gRSC, mPFC, and BLA go through drastic changes from youth to adulthood. Understanding changes in intrinsic and morphological properties across the lifespan may give insight into development of circuits, and how external factors such as anxiety or stress could perturb developing fear networks. Additionally, electrophysiological recordings from juvenile rats and mice have highlighted the necessity to consider age as a variable especially in *in vitro* electrophysiological studies. It is important to note that many studies using *in vitro* recordings use younger rodents as historically, experiments are more difficult to conduct in older rodents (McCutcheon and Marinelli, 2009). Thus, it is critical to consider age as an important experimental variable as developmental changes in behavior and physiology have nonlinear trajectories as discussed in the section below (McCutcheon and Marinelli, 2009).

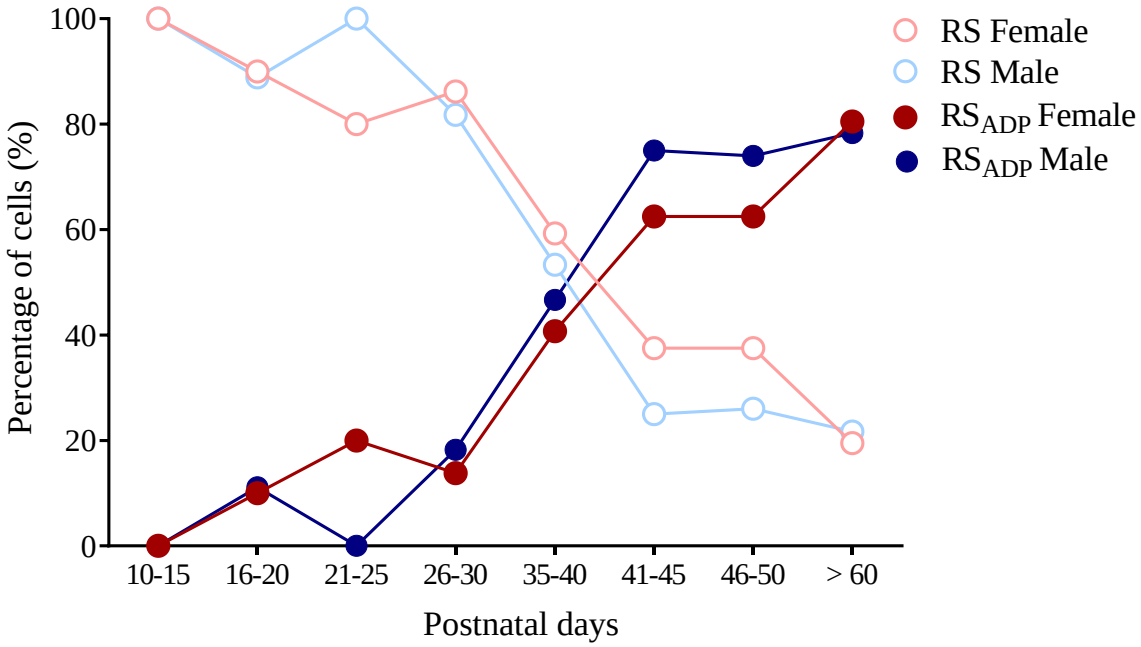


Figure 3. Developmental trajectories of RS and RS$_{ADP}$ neurons in the male and female gRSC. The open pink (female) and blue (male) circles represent RS neurons and the closed red (female), and blue (male) circles represent RS$_{ADP}$ neurons. During PND 10-15, RS neurons constitute 100% of layer 5 of the male and female gRSC and no RS$_{ADP}$ neurons are observed during this period. RS neurons decline at postnatal days 35-40 (~50%) and RS$_{ADP}$ neurons increase (45%). During adulthood (PND > 60), RS neurons are present in smaller percentages (20%) and RS$_{ADP}$ neurons are the common (80%) firing type of this region. *Abbreviations*: granular retrosplenial cortex (gRSC); regular spiking (RS); regular spiking afterdepolarization (RS$_{ADP}$); postnatal days (PND).

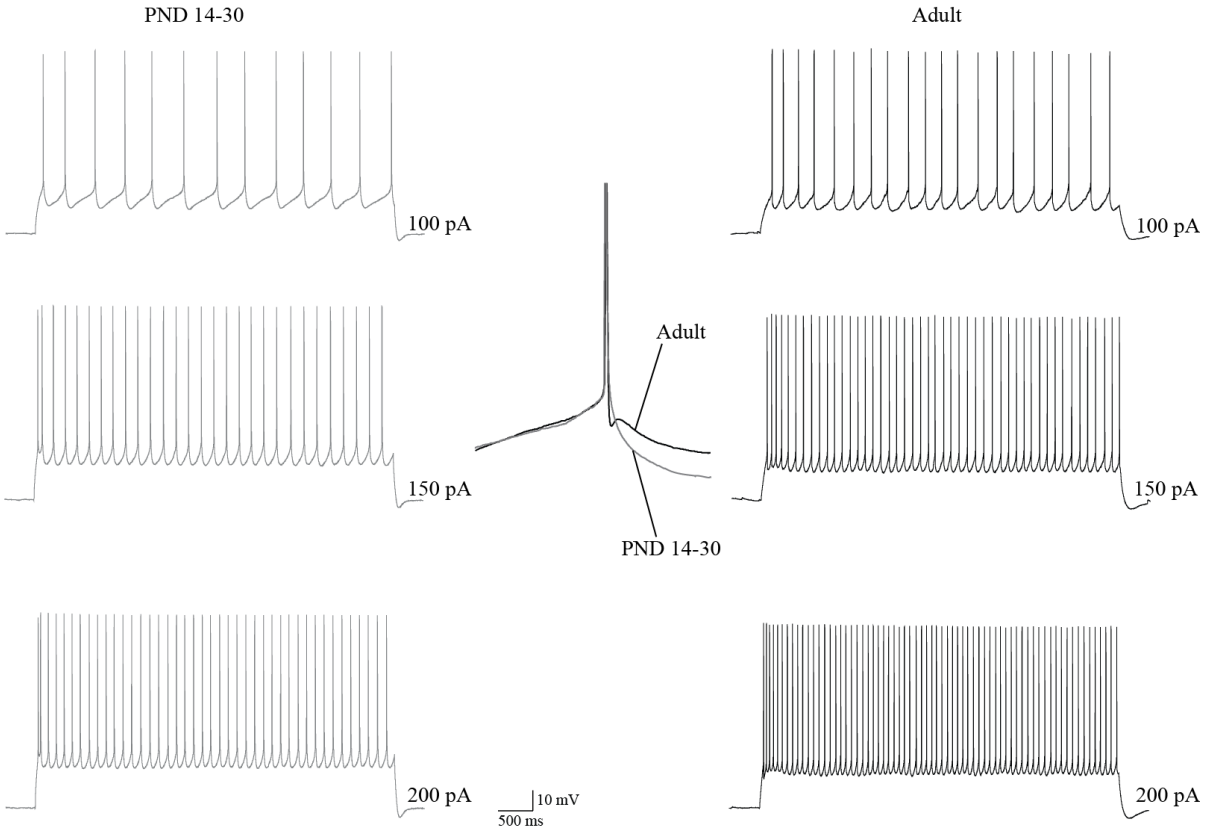


Figure 4. Intrinsic plasticity within granular retrosplenial cortex (gRSC) during development. The middle inset shows an overlay of voltage responses illustrating that layer 5 neurons from juvenile rats (PND 14-30) lack an ADP, whereas layer 5 neurons from adult rats have a prominent ADP. Left and right columns show a series of membrane voltage responses in response to a series of 5 s current injections. RS neurons from juvenile rats (left column) have reduced firing rats and a significantly larger slow afterhyperpolarization (sAHP; -2.03 $\pm$ 0.24 mV) compared to that of adult RS_{ADP} neurons (right column; sAHP -1.33 $\pm$ 0.18 mV; $t_{26} = 2.195$, $p < 0.05$, independent *t*-test). Figure from Yousuf et al., 2020b.

Development of fear behavior

Many drastic changes that occur in development such as emergence of ADP, suppressed AHP (Ehrlich et al., 2012; Yousuf et al., 2020b), and dendritic pruning (Ryan et al., 2016; Arruda-Carvalho et al., 2017; Zimmermann et al., 2019) occur around PND 30. This time window coincides with when rats and mice specifically learn trace fear memories (PND 28; Moye and Rudy, 1987; Barnet and Hunt, 2005) compared to context fear (PND 23; Rudy, 1993; Rudy and Morledge, 1994; Akers et al., 2012; Hunt and Barnet, 2016) or delay fear learning (PND 23; Rudy

and Morledge, 1994; Barnet and Hunt, 2005; Richardson and Hunt, 2010; Pattwell et al., 2011). In this section, we will review the ontogeny of fear behaviors in different fear paradigms.

Following standard or *foreground context* fear conditioning (no auditory stimulus is present), rats can express freezing behavior at PND 23 (Rudy, 1993; Rudy and Morledge, 1994) and mice freeze by PND 14 (Akers et al., 2012). Context freezing is at equivalent levels in 23, 27, 30, 35, 75, and 90 old day rats (Rudy, 1993; Hunt and Barnet, 2016; Santarelli et al., 2018). This linear developmental pattern in rodents is not observed in *background context* conditioning. It is important to note that when auditory cues (CS) are present, presentations of the aversive stimuli (US) can result in acquisition of fear to both the CS tone and the *background context* (Phillips and LeDoux, 1994; Fanselow, 2000). Background context fear emerges by PND 23 (Rudy, 1993; Raineki et al., 2010) and follows a nonlinear developmental trajectory (Pattwell et al., 2011). Mice between PND 23-27 exhibit substantial freezing to the *background context* and this freezing behavior is reduced between PND 29-39 (Pattwell et al., 2011). Freezing behaviors return following PND 39 and remain elevated until adulthood (PND 70; Pattwell et al., 2011). One possible reason for the suppressed freezing during adolescence (PND 29-39) may be that developmental processes such as synaptic pruning and reorganization of the fear-related circuitry may interfere with the mouse's ability to successfully retrieve context fear memories.

Several studies demonstrate that trace fear learning occurs in rats at PND 28 (Moye and Rudy, 1987; Barnet and Hunt, 2005). The delayed emergence of trace fear learning in rats compared to delay or context fear conditioning may be attributed to immaturity of the hippocampus (Moye and Rudy, 1987; Dumas and Rudy, 2010) and possibly the RSC. In contrast to the diminished contextual fear retrieval during adolescence (PND 29-39), multiple studies have shown that adolescent rats (PND 30-35) exhibit an enhanced propensity for trace fear conditioning

(Barnet and Hunt, 2005; Hunt et al., 2006; Schreiber and Hunt, 2013; Hunt and Barnet, 2016). One study directly compared delay and trace fear conditioning in three different ages of male rats: early adolescence (PND 23), mid-adolescence (PND 35), and adults (PND 90; Den and Richardson, 2013). Delay conditioned rats have similar freezing levels across all three ages, however, mid-adolescent rats (PND 35) exhibit the strongest trace fear conditioning compared to early adolescent and adult rats (Den and Richardson, 2013). Together, these findings suggest that compared to other age groups, mid-adolescent rats may be more predisposed to acquiring trace but not delay fear memories.

In summary, we have reviewed the nonlinear transitions in fear learning, neural plasticity, and connectivity that are uniquely associated with adolescence. These findings provide insight that the adolescent brain is distinctly different than that of an adult and dynamic changes in neural circuits during development may render the brain more sensitive to traumatic experiences (Pattwell et al., 2013; Hunt et al., 2016; Zimmermann et al., 2019). Although the above mentioned studies provide critical insight regarding the developmental patterns during adolescence, these studies have exclusively focused on male subjects, which leaves a substantial gap in the literature as anxiety disorders are highly prevalent in women and adolescent girls compared to men and adolescent boys (McLean et al., 2011; Kessler et al., 2012; Li and Graham, 2017).

Sex differences in learning and memory

Epidemiological studies have consistently shown that women are three times more likely to develop posttraumatic stress disorder compared to their male counterparts (Kessler et al., 1995; Kessler et al., 2012). Despite growing evidence in sex differences in fear memories in women and female rodents, approximately 32% of neuroscience-related research have exclusively used male subjects (Shansky and Woolley, 2016), and 2% of fear-related research have included female

subjects (Lebron-Milad and Milad, 2012). So far, there are inconsistencies related to sex differences in contextual fear learning in rodents. While some studies report higher context fear conditioning in male rodents compared to females (Maren et al., 1994; Kosten et al., 2006; Gresack et al., 2009; Mizuno et al., 2012; Sase et al., 2019), other studies show a reversed pattern where females have higher contextual freezing than males (Ris et al., 2005; Moore et al., 2010), and some studies demonstrate no sex differences (Wiltgen et al., 2001; Kosten et al., 2006; Dachtler et al., 2011). These contradictory findings may be due to several reasons such as differences in strain (Pryce et al., 1999), training protocols (Wiltgen et al., 2001), and shock intensities (Keiser et al., 2017; Sase et al., 2019). In one study, male mice have high freezing responses during a context text compared to females, however, this sex difference is alleviated when mice receive precontext exposure prior to contextual fear conditioning (Wiltgen et al., 2001). Further, more recent evidence indicates that female rats exhibit freezing behaviors differently than males (Gruene et al., 2015). In response to a CS, female rats exhibit fear behaviors in the form of short, rapid movements that are called 'darting' (Gruene et al., 2015). This active defensive response is seen only in 10% of male rats (Gruene et al., 2015). Taken together, females may use distinct behavioral strategies to acquire context associations as well as express fear responses differently than males.

In addition to context fear learning, sex differences are evident in rodent models of fear generalization. In this behavioral model, freezing or darting behaviors are measured in a context slightly different from the one in which rodents are originally fear-conditioned. Typically, more generalization of fear is observed if the contexts are most similar to the training contexts (Asok et al., 2018; Tronson and Keiser, 2019). Several studies have shown that female mice and rats show stronger context fear generalization compared to males (Lynch et al., 2013; Keiser et al., 2017; Asok et al., 2018). However, these sex differences are dependent on context preexposure (Keiser

et al., 2017; Asok et al., 2018), testing orders (Asok et al., 2018), and length of time since training (Lynch et al., 2013). Thus, these studies raise multiple questions about the specific strategies female rodents use to retrieve context-shock associations as well as sex differences in mechanisms of memory retrieval.

Sex differences in synaptic and intrinsic plasticity

A common assumption is that different neural mechanisms may necessarily result in different behavioral outcomes. However, distinct brain structures, molecular mechanisms, circuits, and neuronal cell types may be recruited to obtain the same performance in males and females. Here, we review sex differences in basal neuronal activity, synaptic and intrinsic plasticity in the fear conditioning circuit.

Sex differences in synaptic plasticity have been widely studied in the rodent hippocampus. The most prominent form of synaptic plasticity is NMDA receptor dependent and involves stimulation of glutamate release from the presynaptic terminals. Glutamate activates α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and depolarizes the postsynaptic neuron to relieve the Mg^{2+} block in NMDA receptors (Luscher et al., 2000; Liu and Cull-Candy, 2000). A high frequency presynaptic stimulation can open NMDA channels and allow entry of Ca^{2+} into the cell, which leads to LTP induction. Elevation of intracellular Ca^{2+} concentration triggers activation of multiple signaling cascades, which ultimately promote gene transcription and protein synthesis that are underlying machineries for memory (Hayashi et al., 2000; Lisman et al., 2012). Several studies report sex differences in glutamate signaling. For example, under basal conditions, hippocampal AMPA receptor synaptic responses are larger in female rats compared to males, and this may be due to increased phosphorylation of the GluR2 subunit of AMPA receptors (Monfort et al., 2015). The GluR2 subunit is involved in Ca^{2+} permeability and controlling the

assembly of AMPA receptors (Tanaka et al., 2000; Sans et al., 2003). In contrast, the magnitude of LTP is larger in male rats than in females in preforant (Maren et al., 1994; Maren, 1995), Schaffer collateral-CA1 (Yang et al., 2004; Monfort et al., 2015; Wang et al., 2018), and temporoammonic-CA1 (Qi et al., 2016) pathways. Compared to male rats, the reduced magnitude of LTP in the female rat hippocampus may be due to a decrease in tetanus-induced phosphorylation of GluR1 (subunit of AMPA receptors; Monfort et al., 2015; Qi et al., 2016). Since the magnitude of LTP depends on GluR1 insertion in the synaptic membrane, which is modulated by GluR1 phosphorylation (Shi et al., 1999; Hayashi et al., 2000; Esteban et al., 2003; Oh et al., 2006; Man et al., 2007), this could explain one potential mechanism for the diminished synaptic plasticity in the rat female hippocampus. A few studies have shown a lack of sex differences in the magnitude of hippocampal LTP in rats (Dursun et al., 2018; Jain et al., 2019). One reason for the inconsistencies in findings may be due to variability in stimulus protocols as theta-burst stimulations are more likely to elucidate a sex difference in the magnitude of LTP compared to high-frequency stimulations (Yang et al., 2004). Further, a low-frequency stimulation induces LTD in the female but not male hippocampus (Dursun et al., 2018). Thus, differences in the threshold for LTP may contribute to differences in encoding memories in males and females.

As stated above, some studies report a lack of sex differences in LTP (Dursun et al., 2018; Jain et al., 2019); however, they show that LTP induced distinct underlying mechanisms in male and female rats. For example, one study demonstrated that 60 min following LTP induction, the magnitude did not differ between the sexes but GluN1 (subunit of NMDA receptors) mRNA expression increases significantly in the male versus female hippocampus of rats (Dursun et al., 2018). Similarly, cAMP-activated protein kinase (PKA) inhibitor blocks LTP in the female hippocampus but had no effect on the male hippocampus of rats, suggesting that PKA activity is

necessary to promote synaptic strength in females (Jain et al., 2019). These studies support the argument that a similar outcome in each sex may still require different underlying neural mechanisms.

Although majority of studies focus on sex differences in synaptic plasticity of the hippocampus, one recent study demonstrated sex differences favoring females in other fear-related brain structures such as the rat BLA (Blume et al., 2017). *In vivo* and *in vitro* recordings show that in response to excitatory synaptic stimulation, BLA neurons from female rats have higher frequency and amplitude of excitatory postsynaptic currents (EPSCs) than males (Blume et al., 2017). These reports indicate that sex differences in synaptic plasticity may differ between brain regions within the fear circuit (Maren et al., 1994; Blume et al., 2017; Wang et al., 2018).

To date, very few studies have investigated sex differences in intrinsic excitability as a common misconception is that experience-dependent plasticity (such as associative learning) is synonymous with synaptic plasticity. However, synaptic plasticity is not an exclusive form of experience-dependent plasticity, but is tightly couple with other forms of plasticity such as intrinsic plasticity (Daoudal and Debanne, 2003; Mozzachiodi and Byrne, 2010; Sehgal et al., 2013; Dunn and Kaczorowski, 2019; Yousuf et al., 2020a). So far, one study demonstrates sex-specific differences in the intrinsic properties of the bed nucleus of the stria terminalis (BSNT), a key brain region in the fear circuitry (Goode and Maren, 2017; Miles and Maren, 2019). In response to increasing current injections, intrinsic excitability of mouse female BNST neurons is lower than males (Smithers et al., 2019). Further, based on intrinsic properties, cell types are classified as Type I and Type II neurons in the mouse BNST (Smithers et al., 2019). Sex differences are observed in intrinsic properties such as capacitance (Smithers et al., 2019), which influences synaptic efficacy and determines the speed of electrical signals that travel along the dendrites and

axons (Gentet et al., 2000). In the male BNST, capacitance is higher compared to females in Type I neurons, however, this sex difference is reversed in Type II neurons, in which females had a higher capacitance than males (Smithers et al., 2019). Together, a large amount of evidence suggests that sex differences are observed in rodent studies of LTP and intrinsic plasticity within the fear circuitry (Teyler et al., 1980; Maren et al., 1994; Oberlander and Woolley, 2016; Blume et al., 2017; Jain et al., 2019; Smithers et al., 2019).

Summary and significance

The RSC plays a substantial role in processing complex forms of associative memories such as trace fear memories. Although the RSC has recently been gaining more attention, the physiology of this region and how it undergoes learning-related plasticity remains to be elucidated. So far, we have reported that the gRSC has a heterogeneous population of neuronal firing types that changes dramatically over development (Yousuf et al., 2020b). RS_{ADP} neurons that are rarely found in the juvenile gRSC become the dominant firing type in adults (Yousuf et al., 2020b).

The ADP component is key to gRSC development, but may also be responsible for enhancing intrinsic excitability (Yousuf et al., 2020b), thereby inducing learning-related plastic changes in the gRSC. The ADP property in fear-related brain structures is composed of various ion channels and receptors including NMDA receptors (Wu et al., 2004; Grienberger et al., 2014). Additionally, the complex dendritic morphology plays a role in regulating and shaping the ADP component (Bekkers and Häusser, 2007; Roberts et al., 2009; Šišková et al., 2014). These reports raise important questions about the function and mechanisms underlying the ADP component in neurons of the gRSC. Further, the current body of literature related to the RSC has exclusively been focused on male subjects. As discussed in detail in chapter 1, sex differences are observed in cellular mechanisms underlying fear memories (Maren et al., 1994; Kosten et al., 2006; Gresack

et al., 2009; Dalla et al., 2009; Mizuno et al., 2012; Keiser et al., 2017; Sase et al., 2019), and in NMDA receptor activity in the gRSC (de Olmos et al., 2008). These data lead us to question if there are sex differences in the mechanisms mediating the ADP component in RS_{ADP} neurons of the gRSC. Thus, chapter 2 of this dissertation will investigate how learning, NMDA receptor activity, and dendritic morhpology regulate the prominent ADP in a sex-specific way. After establishing the mechanisms that drive the ADP property during development in chapter 2, our next question is to tackle exactly when during development (early adolescence, mid-adolescence or adulthood) do sex differences emerge in trace fear memory retrieval and intrinsic properties of gRSC neurons. Further, in chapter 3, we will also focus on how trace fear memory formation alters RS, RS_{ADP}, and BS neurons in layer 5 of the gRSC in a sex- and age-dependent way. The overall goal of these experiments is, 1) to elucidate sex differences in mechanisms underlying development of gRSC neurons, 2) to investigate when sex differences emerge in fear behavior as well as intrinsic plasticity of the gRSC, and 3) to understand age- and sex-dependent learning-related plasticity in the gRSC across the lifespan.

CHAPTER TWO: Sex-specific mechanisms regulating the afterdepolarization component in granular retrosplenial cortical neurons

Introduction

The RSC is a key node in the neural circuits that process context and trace fear memories (Corcoran et al., 2011; Tayler et al., 2013; Kwapis et al., 2015; Todd et al., 2016). The RSC is one of the largest cortical structures in the brain and is divided into the gRSC and dysgranular regions. Due to its dense reciprocal connections with the thalamus and hippocampus, the gRSC is often targeted as a critical structure in the fear circuit (Van Groen et al., 2004; Pothuizen et al., 2009; Corcoran et al., 2011; Kwapis et al., 2015). The RSC has a diverse population of neuronal firing types (Yousuf et al., 2020b), a likely requirement for encoding complex associative memories. In layer 5 of the juvenile (PND 14-30) rat gRSC, the neuronal distribution includes a large percentage of RS (81%) and a small percentage of RS_{ADP} (12%) neurons (Yousuf et al., 2020b). This distribution reverses over development as the adult gRSC constitutes a higher percentage of RS_{ADP} (72%) neurons and a lower percentage of RS (12%) neurons (Yousuf et al., 2020b), indicating that the appearance of the ADP is essential for gRSC development. Further, RS neurons in the juvenile gRSC have reduced intrinsic excitability compared to RS_{ADP} neurons in adults (Yousuf et al., 2020), which suggests that the prominent ADP property may drive excitability and learning-related plasticity in the gRSC. Studies in the rodent hippocampus show that the ADP promotes burst firing, and reduces AHP to facilitate intrinsic and synaptic plasticity (Schwartzkroin, 1975; Disterhoft et al., 1986; Azouz et al., 1996; Moyer et al., 1996; Thomas et al., 1998; Jensen et al. 1996; Pike et al., 1999; Sanabria et al., 2001; Su et al., 2001; Wu et al., 2004), necessary for the formation of associative fear memories. However, whether fear memory formation can induce an ADP in neurons of the gRSC has not yet been elucidated.

The ADP component is not unique to the gRSC but seen in other memory-related brain structures including the lateral amygdala (Sugita et al., 1993), hippocampus (Schwartzkroin, 1975; Storm 1987; Azouz et al., 1996; Magee and Carruth, 1999; Yue and Yaari, 2004; Yue et al., 2005; Metz et., al 2005, 2007; Kaczorowski et al., 2007; Chen and Yaari, 2008), and prefrontal cortex (Haj-Dahmane and Andrade, 1997, 1998; Kalmbach et al., 2013). Several factors are known to regulate the ADP including dendritic morphology and basic dendritic cable properties. For example, removal of dendrites from rat cortical neurons results in reduced ADP amplitude and burst firing (Bekkers and Häusser, 2007). However, this effect was region-specific as a reversed pattern was observed in rodent cerebellar Purkinje, hippocampal, and gonadotropin releasing-hormone neurons, where shorter dendritic length is associated with increased ADP amplitude and repetitive firing (Bekkers and Häusser, 2007; Roberts et al., 2009; Šišková et al., 2014). Whether dendritic morphology contributes to the ADP in gRSC neurons is unknown.

In addition to synaptic inputs and dendritic morphology, NMDA receptors mediate the ADP in fear-related brain regions (Wu et al., 2004). Bath-application of NMDA receptor antagonists reduces the ADP amplitude in rat hippocampal neurons (Wu et al., 2004). Although the mechanisms by which the RSC processes fear memories have been well-established (Corcoran et al., 2011; Cowansage et al., 2014; Kwapis et al., 2015; Jovasevic et al., 2015; Leaderbrand et al., 2016; Todd et al., 2016; Corcoran et al., 2018; de sousa et al., 2019; Opalka and Wang, 2020), these studies have exclusively focused on male subjects. So far, one study demonstrates sex differences in the rat gRSC where intraperitoneal injections of NMDA receptor blockers result in higher neurotoxicity in the female gRSC compared to males (de Olmos et al., 2008), which suggests that NMDA receptor activity is different in the male and female gRSC. These data raise

important questions regarding sex-dependent mechanisms underlying the ADP in neurons of the gRSC.

Here, we examined whether learning, NMDA receptor activity, and dendritic morphology regulate the ADP in a sex-specific manner in layer 5 of gRSC neurons. We first investigated if trace fear learning, an RSC-dependent task induces a greater proportion of RS_{ADP} neurons in the gRSC during three developmental periods. We found that learning does not change the proportion of RS or RS_{ADP} neurons in the gRSC at any age. Next, we examined if bath-application of NMDA receptor antagonist (D-AP5) abolishes the ADP in RS_{ADP} neurons and found that pharmacological blockade of NMDA receptors significantly reduces the ADP in female RS_{ADP} neurons but not in males. Finally, we investigated the role of ADP in dendritic branching and observed that male RS_{ADP} neurons have reduced dendritic complexity and branching compared to male RS neurons. However, in the female gRSC, both RS and RS_{ADP} neurons exhibit similar dendritic complexity, suggesting sex differences in dendritic regulation of the ADP. Understanding sex-dependent mechanisms underlying intrinsic properties in the gRSC will give us insight on the precise mechanisms through which males and females process fear-related memories.

Materials and methods

Subjects

Subjects were male and female early adolescent (PND 25-29), mid-adolescent (PND 42-49) and adult (PND > 60) F344 rats. For mid-adolescent rats, we examined preputial separation in males and vaginal opening in females to ensure that they had reached puberty. All rats were maintained in an AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care) accredited facility on a 14-hour light/10-hour dark cycle and housed individually with *ad*

libitum access to food and water. Procedures were conducted in accordance with the University of Wisconsin – Milwaukee Animal Care and Use Committee and NIH guidelines.

RSC slice preparation

Rats were deeply anaesthetized with isoflurane and brains were rapidly removed in ice-cold oxygenated sucrose-aCSF (composition in mM: 206 Sucrose, 26 $NaHCO_3$, 10 D-glucose, 2.80 KCl, 2 $MgSO_4 \cdot 7H_2O$, 1.25 $NaH_2PO_4 \cdot H_2O$, 1 $CaCl_2$, 1 $MgCl_2$, 0.40 Na-ascorbate). Brains were placed in ice-cold oxygenated sucrose-aCSF and blocked with two dissections, (anterior and posterior sections). Coronal brain slices (400 µm) containing the RSC (anteroposterior from, -1.80 to -3.80 mm) were cut in oxygenated sucrose-aCSF at ~ 0˚C using a vibrating tissue slicer (VT1200, Leica). Slices were then transferred to a holding chamber (Moyer and Brown, 1998) containing oxygenated aCSF (composition in mM: 124 NaCl, 26 $NaHCO_3$, 20 D-glucose, 2.80 KCl, 2 $MgSO_4 \cdot 7H_2O$, 1.25 $NaH_2PO_4 \cdot H_2O$, 2 $CaCl_2$) at 32-36℃ and allowed to recover for about 30 minutes. Brain slices were left in room temperature aCSF and allowed to recover for another 30 minutes before recordings.

Electrophysiological recordings

An Olympus BX51WI upright microscope outfitted with a submerged recording chamber and equipped with infrared DIC optics was used for visualizing the neuron and patch electrode. Slices were transferred to the chamber where they were perfused at a rate of 2.1 ml/min (maintained at 32-36℃ using an inline temperature controller). Whole-cell recordings were made from layer 5 of the anterior gRSC (AP ~ -1.80 to -3.80). For whole-cell recordings, electrodes (~ 4-5 MΩ) were prepared from thin-walled capillary glass and filled with the following 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, 0.2% Biocytin, pH to 7.3. All recordings were obtained in current clamp mode (holding

potential -65.0 ± 3 mV). Series resistance was fully compensated and consistently monitored to ensure the stability of recording conditions. Experiments were controlled by Multiclamp 700B software (Molecular Devices) and data were analyzed using clampfit (Molecular Devices).

Intrinsic properties of gRSC neurons were studied in current-clamp conditions using the following protocols (Song et al., 2015). (1) *V-I* relationships were obtained from a series of 0.5 s current injections (ranging from -300 to +50 pA) and the plateau voltage deflection was plotted against the current amplitude. Neuronal input resistance (R_N) was determined from the slope of the linear fit of the portion of the *V-I* plot where the voltage sweeps are symmetrical. AP properties were studied with an ascending series of 0.5 s or 1 s depolarizing pulses (5 pA increments). At rheobase, neurons were classified as RS_{ADP}, BS or RS (Yousuf et al., 2020b). Cells were classified by firing type based on how the cell responds to suprathreshold current injections. RS_{ADP} neurons were defined as neurons that fired a single AP at rheobase within ~ 350 ms of a 500 ms current injection. The ADP was identified as the depolarizing crest between the fAHP and the point at which membrane voltage returned to resting potential (Storm 1987; Yue and Yaari, 2004; Metz et al. 2005). This depolarization crest following a fAHP is completely absent in RS neurons of the gRSC. BS neurons were defined as neurons that fire two or more APs in short succession (~8 ms) at the beginning of the current injection either at rheobase or within 20 pA of rheobase. For all neurons, AP properties were studied from the first AP. Current threshold ($I_{threshold}$) was defined as the minimum amount of depolarizing current required to evoke an AP from a recorded neuron. $AP_{threshold}$ was defined as the membrane voltage at which dV/dt first exceeds 28 mV/ms (Kaczorowski et al., 2012). $AP_{amplitude}$ was measured relative to the $AP_{threshold}$, and fAHP was also measured relative to $AP_{threshold}$. $ADP_{amplitude}$ was measured relative to fAHP. For drug experiments, an average $ADP_{amplitude}$ was calculated pre- and post-drug application by measuring $ADP_{amplitude}$ at

rheobase. Lastly, $AP_{half\text{-}width}$ was measured as the width at half of the $AP_{amplitude}$. (3) Post-burst AHP was studied after a 50 Hz burst of 10 spikes, each of which was evoked by a 2 ms suprathreshold current injection (three times, at 20 s intervals). After the last AP, the post-burst AHP was measured at the peak amplitude. mAHP was measured at 150 ms and sAHP at 1 s following the offset of current injection. (4) Neuronal excitability was assessed by counting the number of spikes evoked in response to a series of 5 s depolarizing steps (range is from 0 to 450 pA in 25 pA increments). Only neurons with a resting membrane potential more negative than -50 mV and $R_N > 50$ MΩ were included.

Pharmacological agents

NMDA receptor antagonist (D-AP5, 100 μM; Wu et al., 2004) was purchased from Tocris (Ellisville, MO). Once a neuron was identified as RS_{ADP} in the layer 5 of the male or female gRSC, D-AP5 was bath-applied for 20 min.

Biocytin staining

All neurons were filled with biocytin to confirm the location and perform morphological analyses of cells in the gRSC. After completion of whole-cell recordings, slices were fixed in formalin and kept at 4℃ for at least 1 day and no longer than 10 days before further processing. To visualize neurons labeled by biocytin, the slices were washed with 0.1 M PBS for 5 min (three times). Slices were incubated in H_2O_2/methanol for 45 min, then another wash with 0.1 M PBS for 5 min (three times), followed by Triton X-100/BSA for 45 min. Slices were incubated with 1:500 Streptavidin-Alexa Fluor-488 (Invitrogen) for 120 min in the dark or overnight at 4℃ in the dark. Slices were washed with 0.1 M PBS for 5 min (three times), and subsequently mounted on slides, coverslipped with DAPI containing Ultra Cruz Mounting Medium (Santa Cruz Biotechonology, Houston TX), and sealed with nail polish.

Neuron imaging, reconstruction, and analysis

The neurons previously processed with Streptavidin-Alexa Fluor-488 were viewed under a fluorescence microscope at 2X and 100X and photographed using an Olympus BX51 upright microscope with attached CCD camera (Olympus microscopy). At 2X, an image of the hippocampus was captured to determine approximate anterior/posterior location of slice. The stained neurons were viewed under a confocal microscope (Olympus FV1000) using a 10X objective to capture a dual channel image (DAPI and Alexa Fluor-488) to determine laminar location of neurons. Confocal images were obtained using 20X and 100X objectives to capture additional Z-stacks and create three dimensional images of RSC neurons. The 20X image stacks were used to reconstruct stained neurons using Neurolucida 360 and subsequent Sholl analysis were performed with Neurolucida Explorer (MBF Bioscience, Williston, VT).

Fear conditioning

Trace fear conditioning was conducted in a Plexiglas and stainless-steel chamber (30.5 X 25.4 X 30.5 cm; Coulbourn Instruments, Whitehall, PA), which was located in a sound-attenuating box. The chamber had a standard grid floor consisting of 26 parallel steel rods (5 mm diameter and 6 mm spacing). The floor was connected to a precision adjustable shock generator (Coulbourn Instruments) to deliver a scrambled footshock (US). The sound-attenuating boxes include a ventilation fan, which produced a constant background noise of about 58 dB (measured by a sound level meter). The chamber was illuminated by a miniature incandescent lamp (28V) and was sprayed with 5% ammonium hydroxide solution prior to each training session. During training, the lights in the room were left on (illumination 20.9 lux) for the entire session.

The CS testing chambers included a Plexiglas and stainless-steel chamber, which served as a novel context for the auditory cue test. This chamber was located within a separate sound-attenuating box located in the same room as the training chambers. The testing chamber was

physically different from the training chamber as it had a curved wall, and the floor was black Plexiglass (instead of grid bars). The tray below the testing chamber contained clean bedding and was sprayed with 2% acetic acid before each test session to provide a distinct olfactory stimulus than the training chamber. During the testing session, lights in the room were turned off.

Rats received a one 6-trial session of auditory fear conditioning using a 15 s CS (80 dB white noise), followed by a 30 s stimulus-free trace interval, and then a 1 s footshock (US; 1 mA). A long (5.2 min $\pm$ 20%) intertrial interval was used to maximize CS and minimize context (i.e., training chamber) conditioning (Detert et al., 2008). Control rats were either CS only, which received only 6 CS presentations in the training chamber in the absence of the footshock (US), or experimentally naive rats (did not leave their home cage). To assess learning in conditioned rats, the amount of time spent freezing was measured using a PC running FreezeFrame 2.04 (Actimetrics Software, Coulbourn Instruments, Whitehall, PA), which controlled the delivery of all stimuli during training and testing.

Twenty-four hours after training, trace fear conditioned and CS only rats were brought back to the behavior room with the training and testing chambers. Both groups of rats were placed in the testing chamber and after a 2 min baseline, trace fear conditioned and CS only rats received 2 CS presentations (15 s, 80 dB; 2.9 min intertrial interval). Two minutes after the second CS, rats were removed from the testing chambers and anesthetized for preparation of RSC slices.

A remote CCTV video camera (model #WU-BP334; Panasonic Corp., Suzhou, China), mounted to the top of each behavioral chamber was used to record activity of each rat during training and testing. The video data was fed to a PC running FreezeFrame 2.04. Data was analyzed using FreezeView 2.04 (Actimetrics Software, Coulbourn Instruments) where a 1 s bout of

immobility was scored as freezing. Freezing was defined as the absence of all movement except that required for respiration (Blanchard and Blanchard, 1969).

Statistical analyses

All statistical analyses were conducted using IBM SPSS statistics (version 25). Data was analyzed using parametric statistics with two-tailed Student's *t* test, one-way ANOVA, two-way ANOVA, three-way ANOVA or repeated measures ANOVA as appropriate. For significant main effects ($\alpha = 0.05$), a Fisher's PLSD test was used for *post hoc* comparisons. All data were reported as the mean ± standard error of the mean.

Results

Trace fear learning does not alter proportions of RS and RS_{ADP} neurons in layer 5 of the early adolescent, mid-adolescent, and adult gRSC

Male (*n = 5*) and female (*n = 6*) early adolescent rats (PND 25-29) were trained to associate a tone and shock (6 pairings) that were separated by an empty period of time (30 s) and this group was referred to as the 'trace' group (*Figure 5A*). The CS only group were male (*n = 4*) and female (*n = 5*) early adolescent rats that were only exposed to 6 tone presentations and no footshocks during training (*Figure 5A*). Freezing levels in both trace and CS only groups were measured 30 s (trace interval) following the tone across the 6 trials. All 4 groups showed comparable freezing during training (*Figure 5A*). Two-way 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 ($F_{(6, 96)} = 15.72$, $p < 0.001$; *Figure 5A*). However, there was no significant trial by behavior by sex interaction ($F_{(6, 96)} = 0.61$, $p > 0.05$), and no significant effect of behavior ($F_{(1, 16)} = 0.38$, $p > 0.05$; *Figure 5A*). Twenty-four hours later, trace and CS only groups were brought back for testing in a novel environment (*Figure 5B*). To assess learning, both CS only and trace groups were presented

with 2 tone presentations in the testing chamber and freezing levels were measured 30 s following the tone presentations. Baseline freezing (the first 2 min in the testing chamber) was subtracted from the trace interval (*Figure 5B*). A two-way ANOVA during the tone test showed no significant effect of behavior ($F_{(1, 16)} = 1.67$, $p > 0.05$), sex ($F_{(1, 16)} = 0.01$, $p > 0.05$) or behavior by sex interaction ($F_{(1, 16)} = 0.47$, $p > 0.05$; *Figure 5B*). Immediately following retrieval of trace fear memories, recordings from the male and female gRSC revealed that RS neurons from trace (90%; *n = 5*), CS only (88%; *n = 4*) and naive homecage (82%; *n = 5*) were not different from each other (*Figure 5C*). Male RS_{ADP} neurons were also similar across trace (10%; *n = 5*), CS only (12%; *n = 4*), and naive (18%; *n = 5*) groups (*Figure 5C).* Similar trends were also observed in the female gRSC where the percentage of RS neurons did not change in naive (86%; *n = 6*), trace (75%, *n = 6*), and CS only (72%, *n = 5*) groups (*Figure 5D*). Female naive (14%; *n = 6*), trace (25%, *n = 6*), and CS only (28%, *n = 5*) RS_{ADP} neurons did not significantly alter as a function of trace fear learning (*Figure 5D*). A three-way ANOVA indicated that there was a significant effect of firing type ($F_{(1, 50)} = 183.43$, $p < 0.001$) but no significant firing type by behavior by sex interaction ($F_{(2, 50)} = 1.98$, $p > 0.05$) or firing type by behavior interaction ($F_{(2, 50)} = 0.00$, $p > 0.05$; *Figure 5C*; *Figure 5D*).

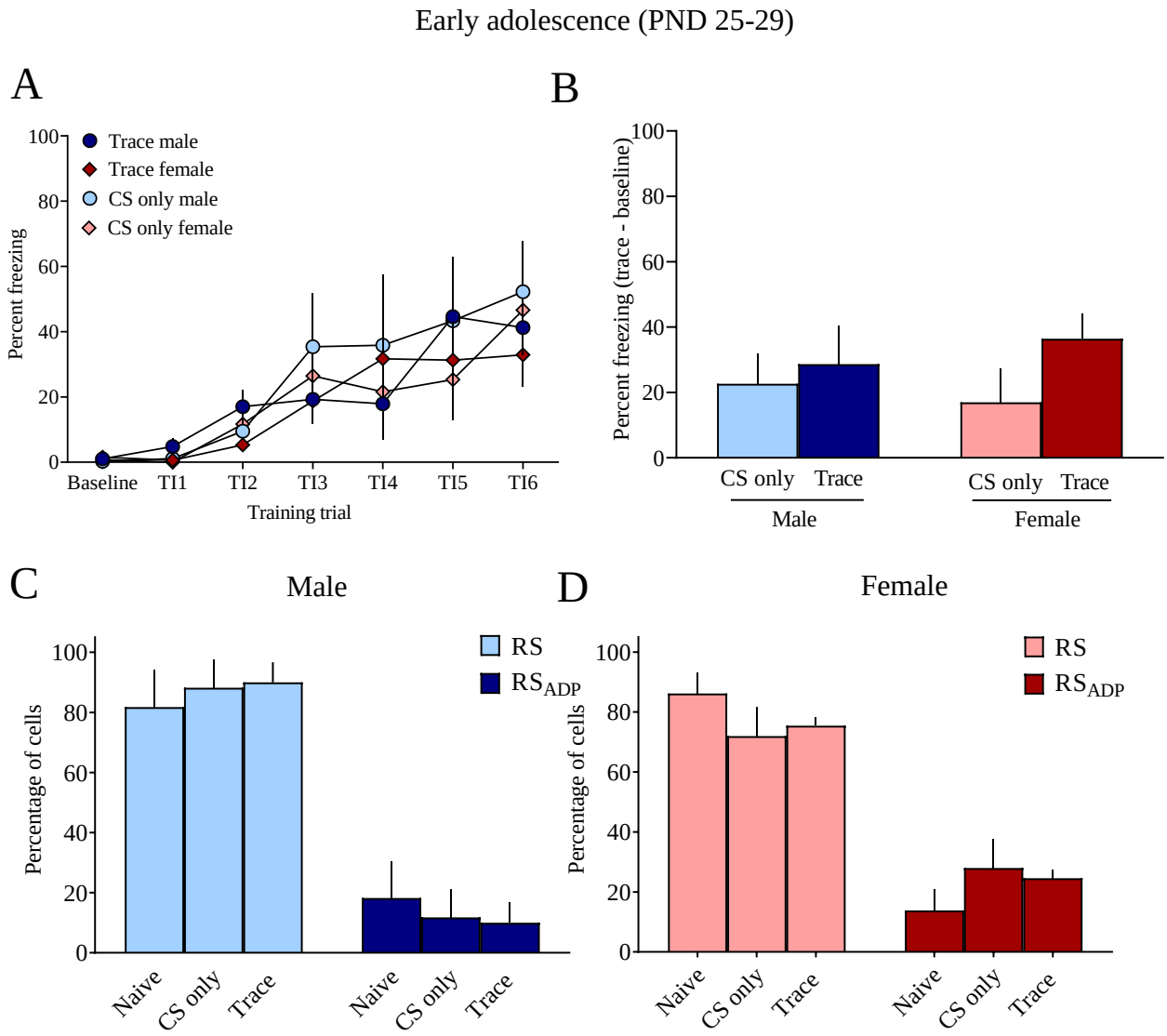


Figure 5. Behavioral training does not increase the proportion of RS$_{ADP}$ neurons in the early adolescent (PND 25-29) gRSC. (A) Male and female trace rats receive 6 tone-shock pairings that are separated by a 30 s interval. CS only rats receive 6 tone presentations and no footshocks. Rats show elevated freezing following trial 3. (B) Twenty-four hours later, trace and CS rats are brought to a novel environment and exposed to 2 tone presentations. Trace male and female groups show slightly higher freezing levels compared to the male and female CS only groups. (C, D) Behavioral training does not alter the percentage of RS or RS$_{ADP}$ neurons in the male or female gRSC. RS neurons are the majority firing type in naive, trace, and CS only groups. RS$_{ADP}$ neurons remain in small percentages in all three behavioral groups. *Abbreviations*: granular retrosplenial cortex (gRSC); postnatal day (PND); trace interval (TI); regular spiking (RS); regular spiking afterdepolarization (RS$_{ADP}$); conditioned stimulus (CS).

In mid-adolescent rats, two-way repeated measures ANOVA revealed a significant main effect of trial ($F_{(6, 114)} = 12.76$, $p < 0.001$), behavior ($F_{(1, 19)} = 10.84$, $p < 0.05$), and a trial by behavior interaction ($F_{(6, 114)} = 3.28$, $p < 0.05$, *Figure 6A*). However, there was no significant trial by sex interaction ($F_{(6, 114)} = 1.02$, $p > 0.05$) or trial by behavior by sex interaction ($F_{(6, 114)} = 1.31$,

$p > 0.05$; *Figure 6A*). *Post hoc* analysis showed that female trace rats ($n = 6$) had higher freezing levels compared to CS only females ($n = 4$) during trials 5 and 6, and male trace rats ($n = 6$) had higher freezing levels compared to CS only males ($n = 7$) during trials 2 and 3 ($p < 0.05$; *Figure 6A*). Following training, a two-way ANOVA during the tone test showed a significant effect of behavior ($F_{(1, 19)} = 13.52$, $p < 0.05$), but not a significant effect of sex ($F_{(1, 19)} = 2.29$, $p > 0.05$) or behavior by sex interaction ($F_{(1, 19)} = 0.56$, $p > 0.05$; *Figure 6B*). *Post hoc* analysis confirmed that trace male rats had significantly higher freezing compared to CS only males ($p < 0.05$; *Figure 6B*). Similarly, trace females had higher freezing than CS only females ($p < 0.05$), indicating that both male and female mid-adolescent rats had successful retrieval of trace fear memories (*Figure 6B*). Following retrieval of trace fear memories, recordings from the male gRSC revealed that RS neurons from trace (31%; $n = 6$), CS only (48%; $n = 7$) or naive homecage (25%; $n = 7$) were not different from each other (*Figure 6C*). Male RS_{ADP} neurons were also similar across trace (69%; $n = 6$), CS only (52%; $n = 7$), and naive (75%; $n = 7$) groups (*Figure 6C*). In the female gRSC, the percentage of RS neurons did not change in naive (37%; $n = 9$), trace (20%, $n = 6$), and CS only (28%, $n = 4$) groups (*Figure 6D*). Female RS_{ADP} neurons in naive (63%; $n = 9$), trace (80%, $n = 6$), and CS only (72%, $n = 4$) groups remained unchanged across all behavioral groups (*Figure 6D*). A three-way ANOVA indicated a significant effect of firing type ($F_{(1, 66)} = 31.07$, $p < 0.001$) but no significant firing type by behavior by sex interaction ($F_{(2, 66)} = 2.15$, $p > 0.05$) or firing type by behavior interaction ($F_{(2, 50)} = 1.11$, $p > 0.05$; *Figure 6C*; *Figure 6D*).

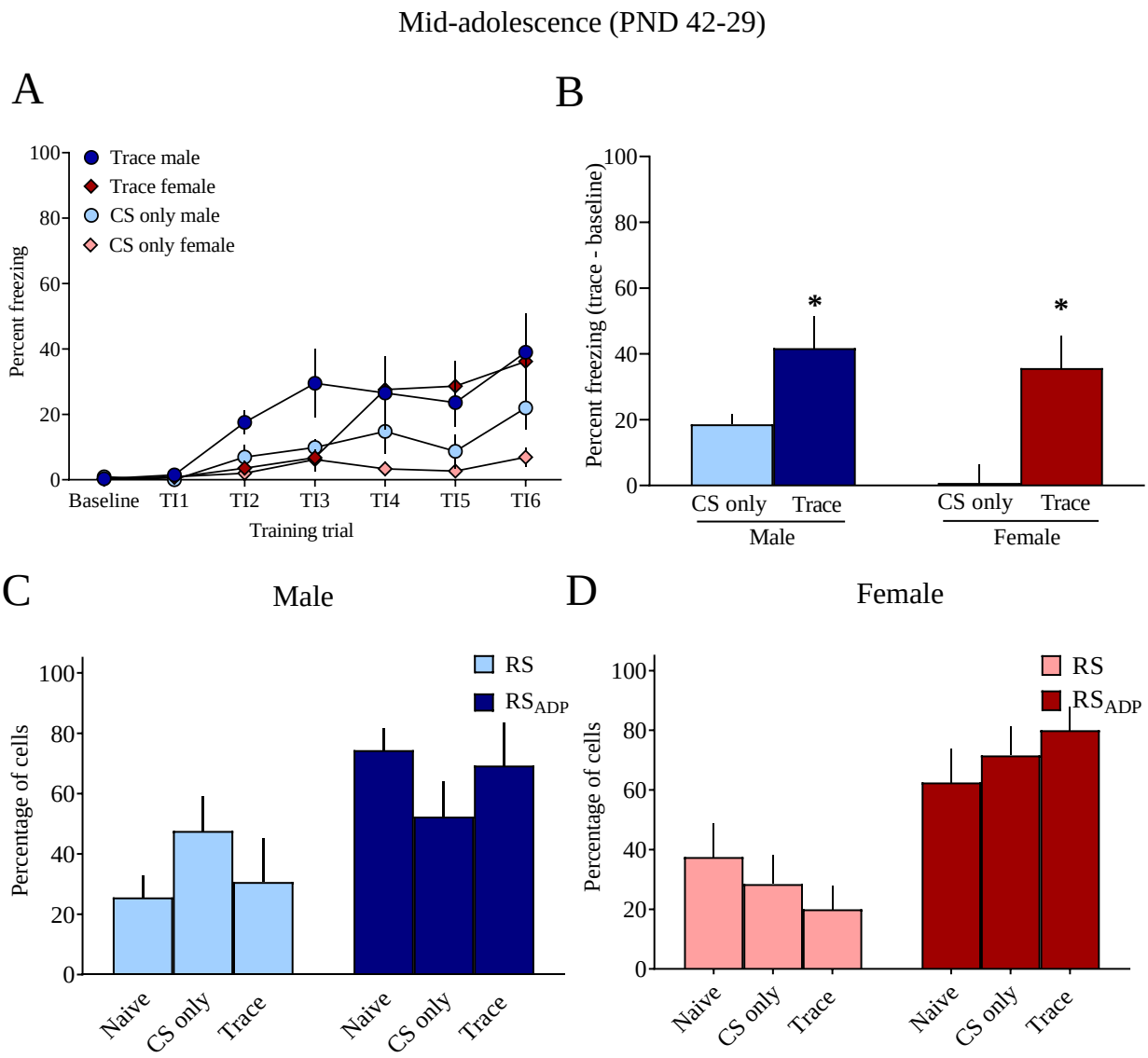


Figure 6. Retrieval of trace fear does not increase the proportion of RS$_{ADP}$ neurons in the mid-adolescent (PND 42-49) gRSC. (A) Male and female trace rats receive 6 tone-shock pairings that are separated by a 30 s interval. CS only rats receive 6 tone presentations and no footshocks. (B) Twenty-four hours later, trace and CS rats are brought to a novel environment and exposed to 2 tone presentations. Trace males have significantly higher freezing compared to CS only males and trace females have significantly higher freezing levels compared to CS only females. (C, D) Retrieval of trace fear memories does not alter the percentage of RS or RS$_{ADP}$ neurons in the male or female gRSC. *Abbreviations*: granular retrosplenial cortex (gRSC); postnatal day (PND); trace interval (TI); regular spiking (RS), regular spiking afterdepolarization (RS$_{ADP}$); conditioned stimulus (CS). (*$p < 0.05$).

In adults, two-way repeated measures ANOVA revealed a significant main effect of trial ($F_{(6, 156)} = 12.49$, $p < 0.001$), behavior ($F_{(1, 26)} = 15.81$, $p < 0.05$), trial by behavior interaction ($F_{(6, 156)} = 3.59$, $p < 0.05$), and trial by sex interaction ($F_{(6, 156)} = 2.16$, $p < 0.05$; *Figure 7A*). However, there was no significant trial by behavior by sex interaction ($F_{(6, 156)} = 0.03$, $p > 0.05$; *Figure 7A*).

Post hoc analysis confirmed that female trace rats (*n = 7*) froze significantly more than CS only females (*n = 5*) during trial 4, whereas male trace rats (*n = 10*) froze more than CS only males (*n = 8*) during trials 2, 3, 4, and 5 ($p < 0.05$; *Figure 7A*). During retrieval, a two-way ANOVA showed a significant effect of behavior ($F_{(1, 26)} = 19.69$, $p < 0.001$), but not an effect of sex ($F_{(1, 26)} = 1.29$, $p > 0.05$) or behavior by sex interaction ($F_{(1, 26)} = 0.09$, $p > 0.05$; *Figure 7B*). *Post hoc* analysis confirmed that during the retrieval test, trace males and females froze more than their CS only controls ($p < 0.05$; *Figure 7B*), suggesting successful retrieval in trace rats. After retrieval of trace fear memories, recordings from the male gRSC revealed that RS neurons from trace (23%; *n = 10*), CS only (43%; *n = 7*), and naive homecage (22%; *n = 25*) were not different from each other (*Figure 7C*). Male RS_{ADP} neurons were also similar across naive (78%; *n = 25*), trace (77%; *n = 10*), and CS only (57%; *n = 7*) groups (*Figure 7C).* In the female gRSC, the percentage of RS neurons did not significantly change in naive (20%; *n = 21*), trace (4%, *n = 7*), and CS only (39%, *n = 5*) groups (*Figure 7D*). Female naive (80%; *n = 21*), trace (96%, *n = 7*), and CS only (61%, *n = 5*) RS_{ADP} neurons did not significantly alter due to trace learning (*Figure 7D*). A three-way ANOVA indicated that there was a significant effect of firing type ($F_{(1, 138)} = 61.90$, $p < 0.001$) and firing type by behavior interaction ($F_{(2, 138)} = 5.06$, $p < 0.05$) but no significant firing type by behavior by sex interaction ($F_{(2, 138)} = 0.80$, $p > 0.05$; *Figure 7C*; *Figure 7D*). Together, these data suggest that retrieval of trace fear memories does not change the proportion of RS or RS_{ADP} neurons irrespective of developmental age.

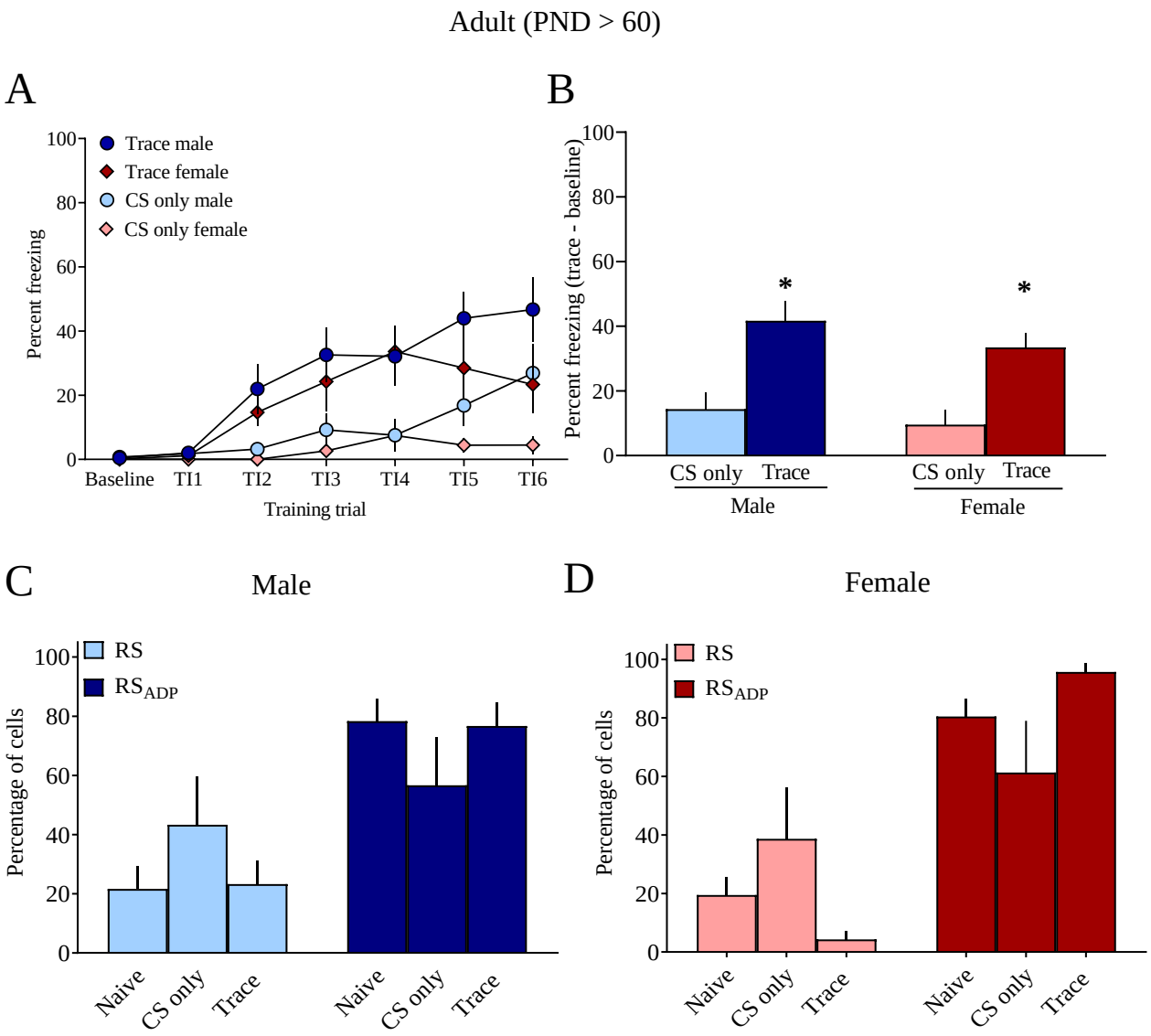


Figure 7. Retrieval of trace fear does not increase the proportion of RS$_{ADP}$ neurons in the adult (PND > 60) gRSC. (A) Male and female trace rats receive 6 tone-shock pairings that are separated by a 30 s interval. CS only rats receive 6 tone presentations and no footshocks. (B) Twenty-four hours later, trace and CS rats are brought to a novel environment and exposed to 2 tone presentations. Trace males have significantly higher freezing compared to CS only males and trace females have significantly higher freezing levels compared to CS only females. (C, D) Retrieval of trace fear memories does not alter the percentage of RS or RS$_{ADP}$ neurons in the male or female gRSC. *Abbreviations*: granular retrosplenial cortex (gRSC); postnatal day (PND); trace interval (TI); regular spiking (RS), regular spiking afterdepolarization (RS$_{ADP}$); conditioned stimulus (CS). (*$p < 0.05$).

Sex differences in the NMDA receptor-mediated ADP of gRSC neurons

The ADP property in RS_{ADP} neurons is mediated by several ion channels and receptors including Na^+ channels (Guatteo et al., 1999), mGluRs (Greene et al., 1994; Young et al., 2004; Park et al., 2010; Yousuf et al., 2020b), and NMDA receptors (Wu et al., 2004; Grienberger et al., 2014). Bath-application of NMDA receptor antagonist completely abolishes the ADP in male hippocampal neurons (Wu et al., 2004), and the female gRSC is more sensitive to NMDA receptor blockade than males (de Olmos et al., 2008), suggesting sex differences in the NMDA receptor-regulated ADP. We identified RS_{ADP} neurons in the female (*n = 10*) and male (*n = 10*) gRSC and bath-applied NMDA receptor antagonist, D-AP5 (100 µM) for approximately 20 min (*Figure 8*). A two-way repeated measures ANOVA showed a significant effect of drug ($F_{(1, 18)} = 5.25$, $p < 0.05$) but not a significant effect of sex ($F_{(1, 18)} = 0.14$, $p > 0.05$) or drug by sex interaction ($F_{(1, 18)} = 0.58$, $p > 0.05$; *Figure 8*). *Post hoc* analysis confirmed that D-AP5 significantly reduced the ADP amplitude in female ($p < 0.05$; *Figure 8A, 8B)* but not in male RS_{ADP} neurons ($p > 0.05$; *Figure 8A*; *Figure 8C*) of the gRSC. Thus, NMDA receptors regulate the prominent ADP component in the gRSC in a sex-dependent manner.

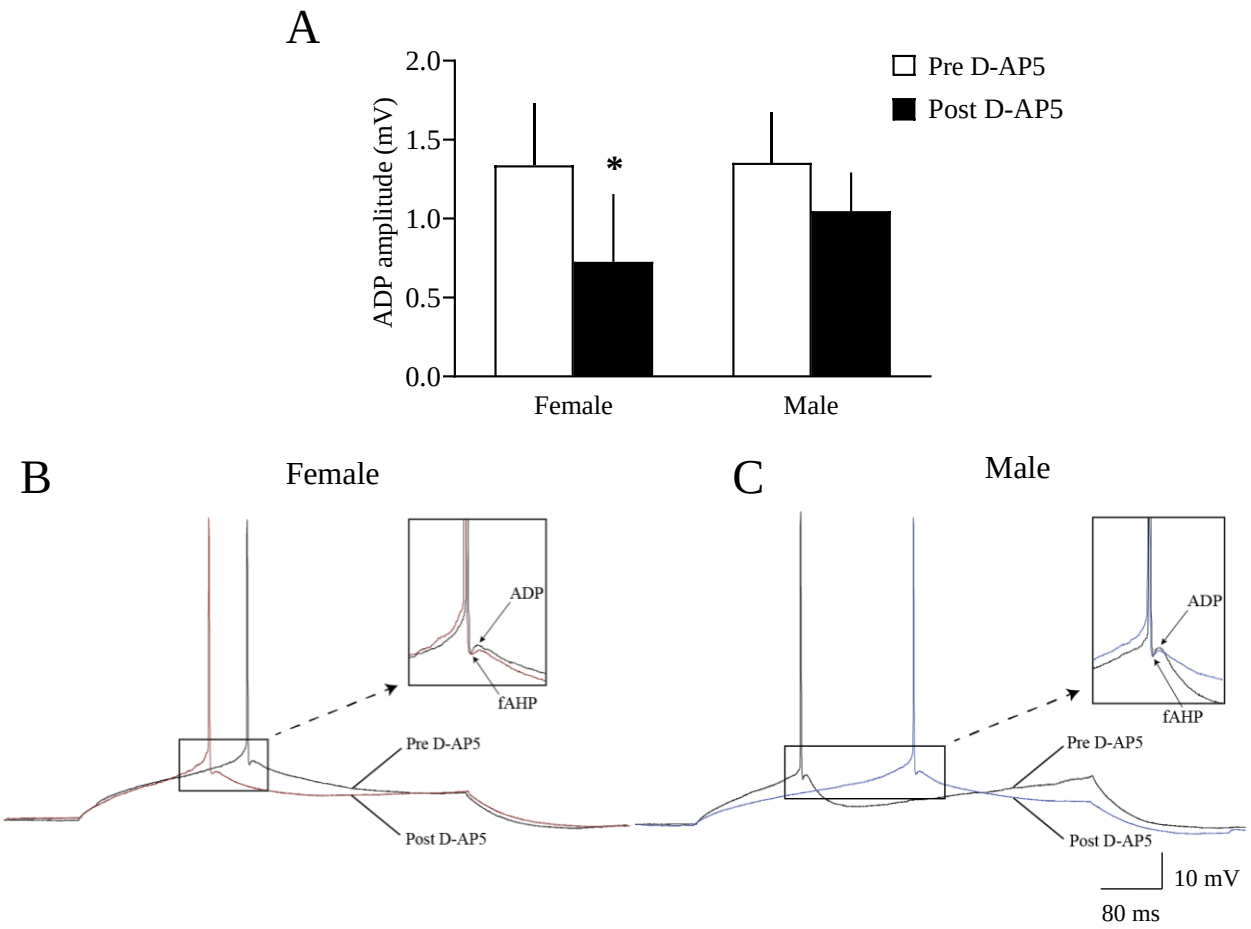


Figure 8. NMDA receptors mediate the ADP component in female RS_{ADP} neurons of the gRSC. (A) NMDA receptor blocker, D-AP5 significantly reduces the ADP ($p < 0.05$) in the female gRSC but does not completely reduces ADP amplitude in the male gRSC to a lesser extent (not significant). (B) Overlay of membrane voltages before (traces in black) and after (traces in red) bath-application of D-AP5. Note the substantial effect of D-AP5 in reducing the ADP amplitude in females. (C) Membrane voltage responses before (black) and after (blue) bath-application of D-AP5 in the male gRSC. D-AP5 does not significantly reduce the ADP in the male gRSC (*$p < 0.05$). *Abbreviations*: afterdepolarization (ADP); regular-spiking afterdepolarization (RS_{ADP}); granular retrosplenial cortex (gRSC); N-methyl-D-aspartate (NMDA).

Sex differences in ADP-associated dendritic morphology of gRSC neurons

Complex dendritic morphology regulates the ADP in rat cortical neurons and shorter dendritic length has been associated with increased ADP amplitude (Bekkers and Häusser, 2007; Roberts et al., 2009; Šišková et al., 2014). We sought to understand whether dendritic length and surface area contribute to ADP amplitude in male and female gRSC neurons. In males, a Sholl analysis indicated that dendritic complexity of basal dendrites in RS neurons (*n = 15*) was

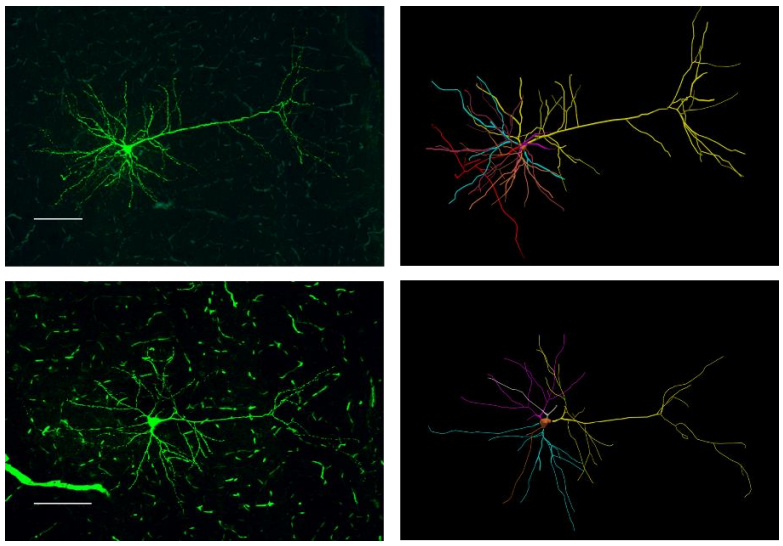
significantly enhanced compared to RS_{ADP} neurons (*n = 16; Figure 9A*; *Figure 9B*). Two-way repeated measures ANOVA revealed a significant effect of distance from soma ($F_{(29, 841)} = 146.92$, $p < 0.01$), firing type ($F_{(1,29)} = 12.22$, $p < 0.01$), and distance from soma by firing type interaction ($F_{(29, 841)} = 5.38$, $p < 0.01$; *Figure 9B*). *Post hoc* analysis showed that basal dendrites of RS neurons were significantly more complex between 70-220 µm and 260-290 µm distance from soma ($p < 0.05$; *Figure 9B*). No significant differences were observed in apical dendritic complexity of RS (*n = 15*) and RS_{ADP} (*n = 16*) neurons of the male gRSC (*Figure 9A*; *Figure 9C*). Two-way repeated measures ANOVA revealed a significant effect of distance from soma ($F_{(29, 841)} = 28.32$, $p < 0.01$), but not a significant effect of firing type ($F_{(1, 29)} = 1.54$, $p > 0.05$), or a distance from soma by firing type interaction ($F_{(29, 841)} = 1.99$, $p > 0.05$; *Figure 9C*). To visualize overall dendritic complexity, we combined basal and apical dendritic intersections of RS and RS_{ADP} neurons (*Figure 9A*; *Figure 9D*). Two-way repeated measures ANOVA demonstrated a significant effect of distance from soma ($F_{(29, 841)} = 149.62$, $p < 0.01$), firing type ($F_{(1,29)} = 9.36$, $p < 0.01$), and distance from soma by firing type interaction ($F_{(29, 841)} = 6.17$, $p < 0.01$; *Figure 9D*). *Post hoc* analysis showed that dendrites of RS neurons were significantly more complex between 60-190 µm, 210-220 µm, 250 µm, and 270-290 µm distance from soma ($p < 0.05$; *Figure 9D*). Thus, dendritic complexity of male RS_{ADP} neurons is substantially reduced compared to RS neurons.

Male

A

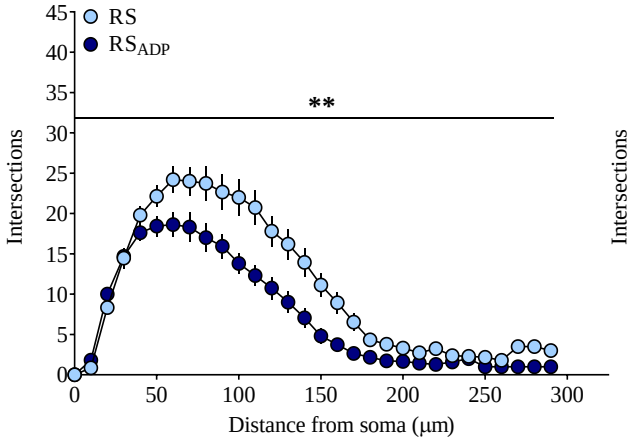
RS neuron

RS_{ADP} neuron



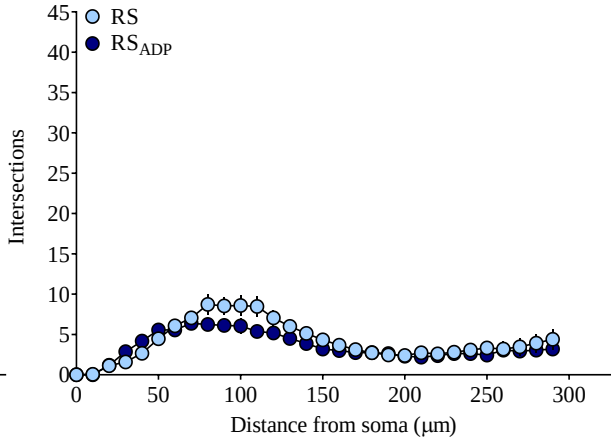
B

Basal dendrites



C

Apical dendrites



D

Basal + apical dendrites

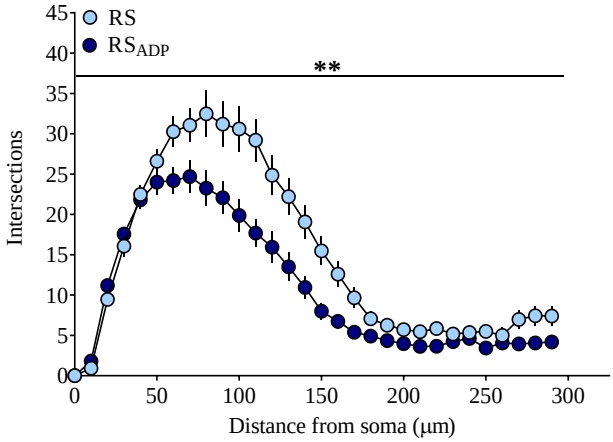


Figure 9. Dendritic morphology of RS and RS_{ADP} neurons within the male gRSC neurons. (A) Morphology of male RS and RS_{ADP} neurons in layer 5 gRSC. Shown are representative confocal Z-stack projections (*left*) and corresponding 3-dimensional reconstructions (*right*). (B) Sholl analysis comparing basal dendritic complexity of RS (light blue) and RS_{ADP} (dark blue) neurons. Basal dendrites of RS neurons have significantly more intersections and branching compared to those of RS_{ADP} neurons. (C) Apical dendrites of RS and RS_{ADP} neurons have similar branching. (D) Combined basal and apical dendrites of RS_{ADP} neurons have significantly reduced dendritic branching compared to those of RS neurons (**$p < 0.01$). Scale bar represents 100 µm. *Abbreviations*: regular spiking (RS); regular-spiking afterdepolarization (RS_{ADP}); granular retrosplenial cortex (gRSC).

Next, we examined if ADP amplitude correlated with dendritic length and surface area in male gRSC neurons (*n = 15*; *Figure 10*). We found that ADP amplitude did not correlate with basal ($r = -0.17$, $p > 0.05$; *Figure 10A*) or apical ($r = 0.07$, $p > 0.05$; *Figure 10B*) dendritic lengths. ADP amplitude did not correlate with basal ($r = -0.03$, $p > 0.05$; *Figure 10C*) or apical ($r = 0.44$, $p > 0.05$; *Figure 10D*) surface areas either. Together, RS_{ADP} neurons have reduced dendritic branching compared to RS neurons, and this may be due to the presence of the ADP. However, the ADP amplitude does not contribute to dendritic complexity.

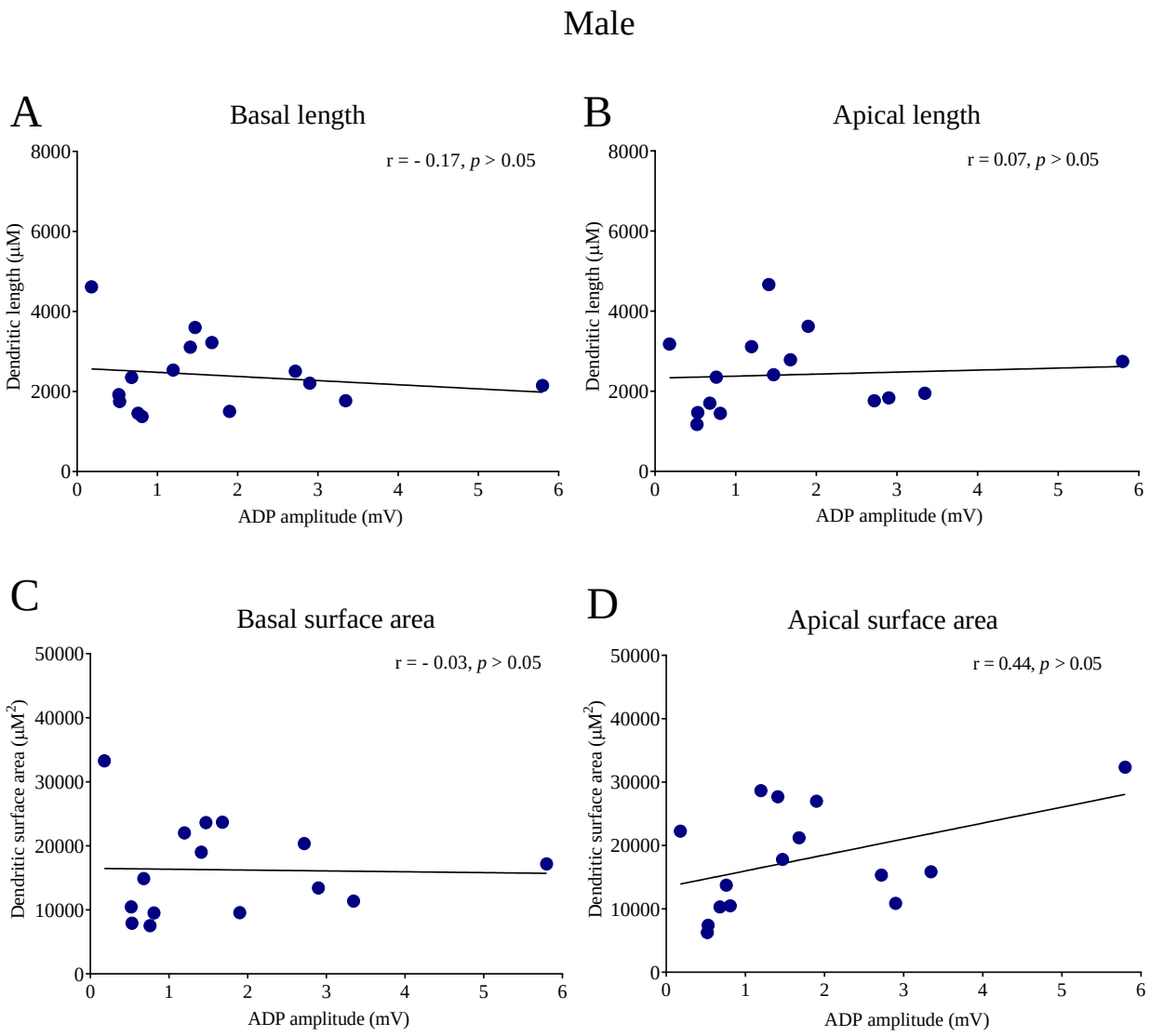


Figure 10. ADP amplitude does not correlate with dendritic length or surface area in the male gRSC. (A, B) ADP amplitude in male RS_{ADP} neurons does not correlate with basal or apical dendritic lengths ($p > 0.05$). (C, D) ADP amplitude in RS_{ADP} neurons does not correlate with basal or apical dendritic surface areas ($p > 0.05$). *Abbreviations*: afterdepolarization (ADP); granular retrosplenial cortex (gRSC).

Unlike layer 5 of the male gRSC, a Sholl analysis in females indicated that dendritic complexity of basal dendrites in RS neurons (*n = 14*) was not different than RS_{ADP} neurons (*n = 19*; *Figure 11A*; *Figure 11B*). Two-way repeated measures ANOVA revealed a significant effect of distance from soma ($F_{(29, 899)} = 128.84$, $p < 0.01$), but not a significant effect of firing type ($F_{(1,31)} = 1.68$, $p > 0.05$) or distance from soma by firing type interaction ($F_{(29, 899)} = 0.53$, $p > 0.05$; *Figure 11B*). No significant differences were observed in apical dendritic complexity of RS (*n = 14*) and

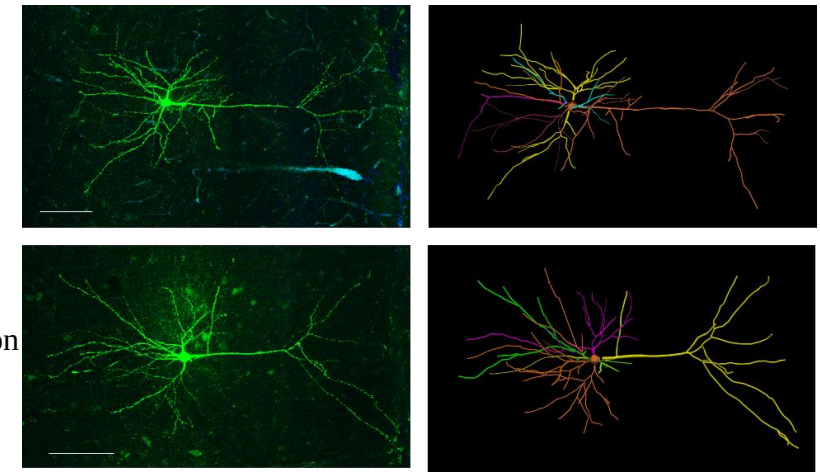
RS_{ADP} (*n = 19*) neurons of the male gRSC (*Figure 11A*; *Figure 11C*). Two-way repeated measures ANOVA revealed a significant effect of distance from soma ($F_{(29, 899)} = 28.32$, $p < 0.01$), but not a significant effect of firing type ($F_{(1, 31)} = 0.00$, $p > 0.05$), or a distance from soma by firing type interaction ($F_{(29, 899)} = 0.80$, $p > 0.05$; *Figure 11C*). To visualize overall dendritic complexity, we combined basal and apical dendritic intersections of RS and RS_{ADP} neurons (*Figure 11A, 11D*). Two-way repeated measures ANOVA demonstrated a significant effect of distance from soma ($F_{(29, 899)} = 122.26$, $p < 0.01$), but not a significant effect of firing type ($F_{(1,31)} = 1.20$, $p > 0.05$), and distance from soma by firing type interaction ($F_{(29, 899)} = 0.40$, $p > 0.05$; *Figure 11D*). Thus, dendritic complexity is similar in RS and RS_{ADP} neurons of the female gRSC.

Female

A

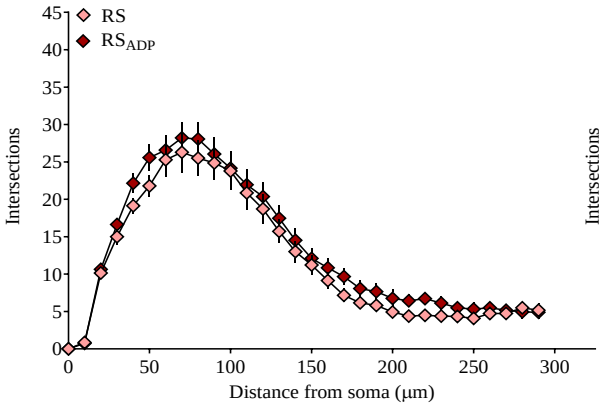
RS neuron

RS_{ADP} neuron



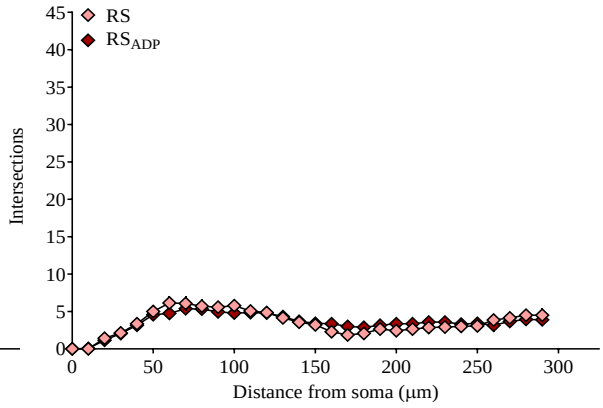
B

Basal dendrites



C

Apical dendrites



D

Basal + apical dendrites

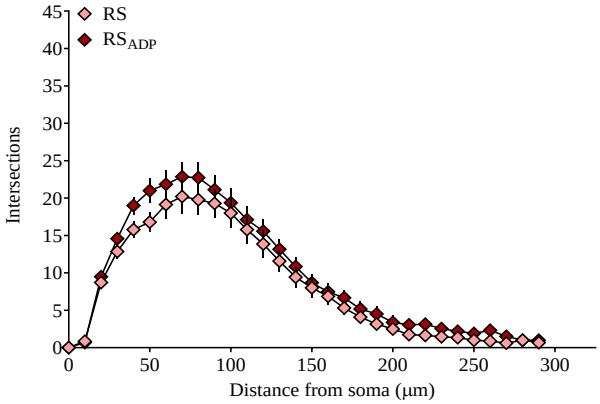


Figure 11. Dendritic morphology of RS and RS_{ADP} neurons within the female gRSC neurons. (A)Morphology of female RS and RS_{ADP} neurons in layer 5 gRSC. Shown are representative confocal Z-stack projections (*left*) and corresponding 3-dimensional reconstructions (*right*). (B) In females, Sholl analysis comparing basal dendritic complexity of RS (pink) and RS_{ADP} (red) neurons. Basal dendrites of RS neurons have similar number of intersections and branching compared to RS_{ADP} neurons. (C) Apical dendrites of RS and RS_{ADP} neurons have similar branching. (D) Combined basal and apical dendrites of RS_{ADP} neurons have similar dendritic branching to RS neurons. *Abbreviations*: regular spiking (RS); regular-spiking afterdepolarization (RS_{ADP}); granular retrosplenial cortex (gRSC). Scale bar represents 100 µm.

ADP amplitude did not correlate with dendritic length or surface area in female layer 5 gRSC neurons (*n = 18*; *Figure 12*). We found that ADP amplitude did not correlate with basal ($r = 0.11$, $p > 0.05$; *Figure 12A*) or apical ($r = -0.34$, $p > 0.05$; *Figure 12B*) dendritic lengths. Similar to males, ADP amplitude did not correlate with female basal ($r = 0.27$, $p > 0.05$; *Figure 12C*) or apical surface areas in females ($r = -0.30$, $p > 0.05$; *Figure 12D*). Thus, the presence of the ADP or the ADP amplitude does not influence dendritic morphology in the female gRSC.

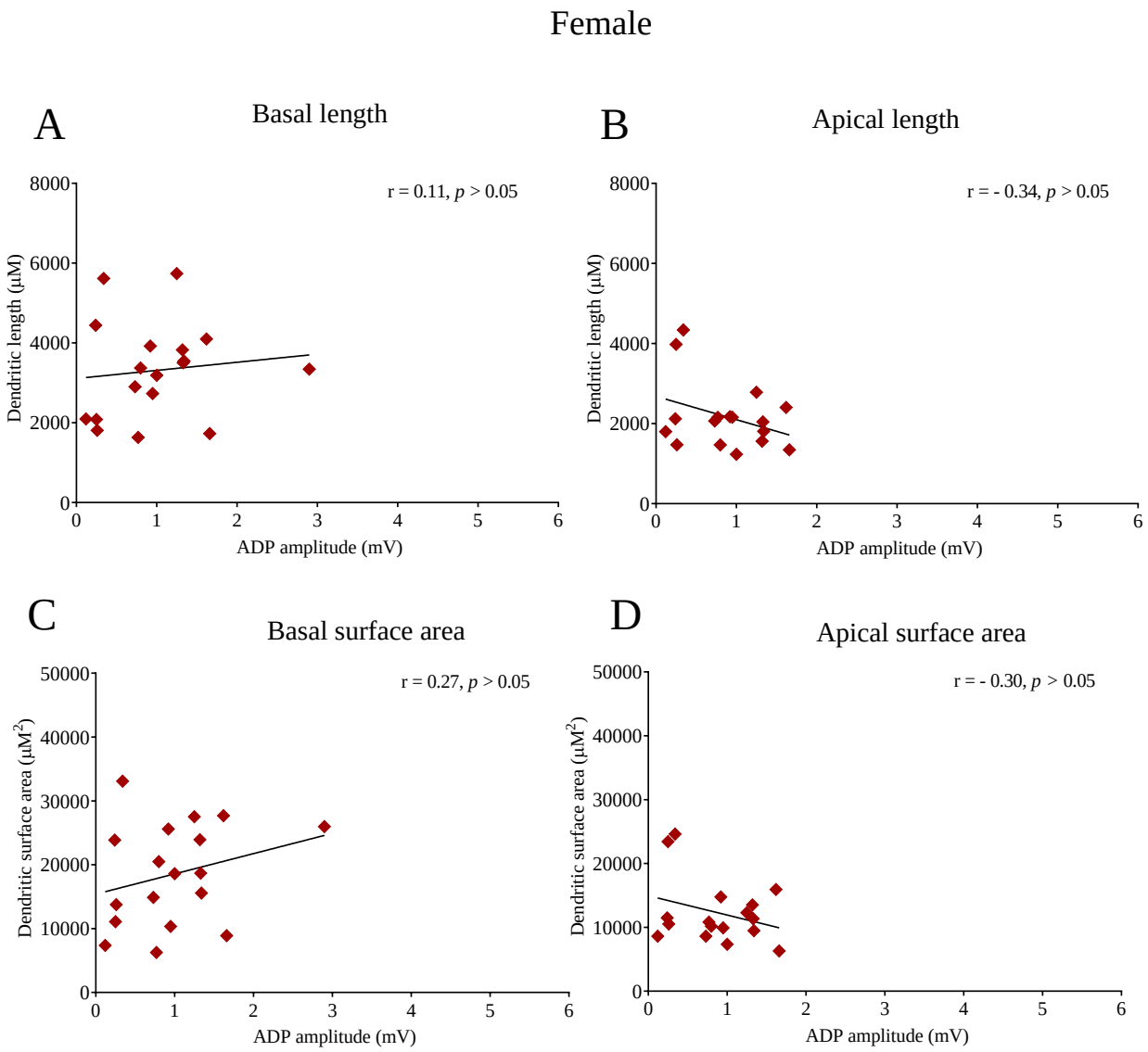


Figure 12. ADP amplitude does not correlate with dendritic length or surface area in the female gRSC. (A, B) ADP amplitude in female RS_{ADP} neurons does not correlate with basal or apical dendritic lengths ($p > 0.05$). (C, D) ADP amplitude in female RS_{ADP} neurons does not correlate with basal or apical dendritic surface areas ($p > 0.05$). *Abbreviations*: afterdepolarization (ADP); granular retrosplenial cortex (gRSC).

Discussion

Here, we examined whether learning, dendritic morphology, and NMDA receptor activity regulate the prominent ADP component, which emerges as a function of age in the gRSC (Yousuf et al., 2020b). Our results demonstrate that trace fear memory formation does not change the percentage of RS or RS_{ADP} neurons in the gRSC of early adolescent, mid-adolescent or adult rats. Further, we found that pharmacological blockade of NMDA receptors significantly reduces the ADP amplitude in female RS_{ADP} neurons but not in males. Our data also reveal that dendritic complexity in male RS_{ADP} neurons was significantly decreased compared to RS neurons but RS and RS_{ADP} neurons have similar dendritic branching in females. Overall, our results provide insight related to sex differences in mechanisms modulating the ADP, an intrinsic property that drives excitability in fear-related brain regions (Wu et al., 2004; Thomas et al., 1998; Pike et al., 1999).

Role of trace fear learning in induction of the ADP

Nearly 85% of neurons in the naive early adolescent gRSC are RS neurons and this number steadily declines to ~20% over development. During early adolescence, RS_{ADP} neurons are 15% of the gRSC and these percentages substantially increase during adulthood (80%). We found that retrieval of trace fear memories does not alter the percentage of RS neurons or RS_{ADP} neurons at any age. One possibility that we did not see an increased number of RS_{ADP} neurons in early adolescent rats is because they may not have successfully retrieved trace fear memories. During the tone test, CS only rats had reduced (but not significant) freezing compared to trace rats. These behavioral results could be because early adolescent rats were not trained at the same time, which may have added variability to the experiment. However, mid-adolescent and adult rats exhibit successful trace retrieval where CS only rats have significantly reduced freezing compared to trace rats. Despite the evidence of successful retrieval in mid-adolescent and adult male and female rats,

there was a lack of learning-related changes in proportions of RS and RS_{ADP} neurons in the gRSC in the two age groups. Thus, successful retrieval of trace fear memories does not enhance the percentage of RS_{ADP} neurons in the rat gRSC.

Previous reports indicate that learning alters the proportion of different firing types (Dunn et al., 2018). The subiculum of naive male rats has 56% of RS and 44% of BS cells, and retrieval of contextual fear significantly increases RS cells to 81% and reduces BS cells to 19% (Dunn et al., 2018). Additionally, proportion of RS and BS cells is altered in the subiculum of rats that are exposed to the training chamber without a shock (Dunn et al., 2018). These data suggest that fear learning or novel contexts alone is sufficient to remodel the intrinsic properties of the subiculum. We were expecting to see a substantial increase in RS_{ADP} neurons following learning as the ADP has been shown to influence both synaptic and intrinsic plasticity. For example, synaptic inputs promote an ADP in hippocampal neurons (Wu et al., 2004; Brown and Randall., 2009). Further, the ADP regulates critical learning-related intrinsic mechanisms such as the AHP (Wu et al., 2004). An enhancement of AHP in hippocampal neurons is associated with learning deficits in aged rabbits (Moyer et al., 1992), and a reduced AHP increases intrinsic plasticity in several limbic brain structures (Disterhoft et al., 1986; Moyer et al., 1996; Santini et al., 2008; Kaczorowski and Disterhoft, 2009; McKay et al., 2009; Song et al., 2012; Sehgal et al., 2014; Dunn et al., 2018). Pharmacological manipulations that suppress the AHP can facilitate learning in aged rabbits (Kowalska and Disterhoft, 1994; Moyer et al., 1992; Oh et al., 1999; Power et al., 2001), and abolishing the AHP leads to the induction of an ADP in hippocampal neurons (Wu et al., 2004). Thus, the ADP plays a large role in modulating other intrinsic membrane properties such as the AHP to influence intrinsic plasticity and synaptic efficacy.

Role of NMDA receptors in mediating the ADP

We provide the first evidence that pharmacological blockade of NMDA receptors reduces the ADP amplitude in gRSC neurons. In the male rat hippocampus, the ADP is mediated by NMDA receptors and pharmacologically abolishing the sAHP enhances the ADP amplitude (Wu et al., 2004). One mechanism that explains the associations between NMDA receptors, ADP, and sAHP is that the hyperpolarized sAHP facilitates the Mg^{2+} block of activated NMDA receptors (Wu et al., 2004). A suppressed sAHP may promote temporal summation of high-frequency inputs, which leads to the depolarization of neurons and removal of the Mg^{2+} block (Wu et al., 2004). The lack of Mg^{2+} block leads to synaptic inputs and triggers an enhanced NMDA receptor-mediated response in the form of an ADP (Wu et al., 2004). Together, a suppressed sAHP may enhance NMDA receptor activity to promote an ADP in gRSC neurons.

Bath-application of NMDA receptor blockers significantly reduces the ADP amplitude in the female gRSC (46%) and to a lesser extent in males (23%). These data are not surprising as previous studies have reported that the female gRSC is more sensitive to the neurotoxic effects of NMDA receptor antagonists (de Olmos et al., 2008). In the gRSC, NMDA receptor blockade only partially reduces the ADP but completely abolishes the ADP in hippocampal neurons (Wu et al., 2004). Similarly, mGluR antagonists reduce the ADP by only 30% in the gRSC (Yousuf et al., 2020b). The ADP in other fear-related brain regions is mediated by diverse ion channels and receptors including Na^{+}, Ca^{2+}, and muscarinic receptors (Caeser et al., 1993; Azouz et al., 1996; Greene et al., 1996; Guatteo et al., 1999; Haj-Dahmane and Andrade, 1998; Yan et al., 2009; Park et al., 2010; Lei et al., 2014), and how they regulate the ADP may vary across different brain regions. Thus, unlike the hippocampus, NMDA receptors play a partial role in shaping the ADP in gRSC neurons.

Role of dendritic morphology in regulating the ADP

Dendritic morphology and basic dendritic cable properties can modulate intrinsic plasticity as well as synaptic efficacy (for reviews see Spruston, 2008; Spruston et al., 2016). A non-active mechanism may occur, which involves an AP in the soma depolarizing the dendritic membrane (which has a larger surface area and capacitance). After the somatic AP has repolarized, the charge from the dendritic membrane travels back to the soma and produces an ADP that is enhanced by active dendritic conductances regulated by Na^+ and Ca^{2+} channels. These ion channels are usually present in the dendrites and allow large active depolarizing effects that are slower and occur later relative to APs in the soma (Andreasen and Lambert, 1995; Magee and Carruth, 1999; Williams and Stuarty, 1999; Bean, 2007). Based on this mechanism, we speculate that dendrites act as a capacitive load, and shorter dendritic length or surface area would allow dendritic positive charges to travel to the soma more efficiently, resulting in larger ADP amplitudes of neurons.

We demonstrated that in the male gRSC, the dendrites in RS neurons are significantly more complex than RS_{ADP} neurons. Interestingly, ADP amplitude does not correlate with basal and apical dendritic length or surface area. These data provide indirect evidence that the presence of the ADP may contribute to the decline in dendritic branching in male RS_{ADP} neurons but the amplitude itself does not have an effect on dendritic complexity. In females, RS and RS_{ADP} neurons have similar dendritic branching and ADP amplitude does not correlate with basal and apical dendritic length or surface area. These data suggest that the role of dendritic branching and length in mediating the ADP may be sex-specific. Future work using novel techniques such as dendrotomy can unveil the precise role of dendrites in promoting the ADP in neurons of the male and female gRSC.

In conclusion, these data are the first to investigate different mechanisms that regulate the prominent ADP component that appears as a consequence of age in the rat gRSC. We found that

NMDA receptor activity and dendritic morphology mediate the ADP differently in the female and male gRSC. Future work examining these sex-dependent mechanisms will give crucial insight on how the gRSC undergoes intrinsic plasticity in males and females. Understanding sex differences in learning-related plasticity can allow the development of tailored sex-specific treatments for learning and memory disorders.

CHAPTER THREE: Sex- and age-dependent effects on experience-dependent intrinsic plasticity in the granular retrosplenial cortex

Introduction

Pavlovian fear conditioning is a widely used model to investigate mechanisms underlying maladaptive fear memories. There are several types of fear conditioning paradigms including delay fear conditioning, in which rodents are trained to associate a CS (e.g. tone) with an aversive stimuli (US; e.g. footshock). The CS coterminates with the US and elicits a freezing response. The neural circuit for delay fear conditioning is simple, and requires the brainstem and the amygdala (Phillips and LeDoux, 1992; LeDoux, 2000; Kitamura et al., 2015). However, a subtle change in delay fear conditioning such as an empty period of time between the CS and US (trace fear conditioning) can dramatically change the neural circuit. Explicit, declarative knowledge of the CS-US contingencies in trace fear learning (but not delay fear learning) requires the RSC (Kwapis et al., 2015). Specifically, the gRSC subregion of the RSC forms reciprocal connections with the hippocampus, and has a heterogeneous population of neuronal firing types (RS, RS_{ADP}, BS; Yousuf et al., 2020b), making it an ideal candidate to support complex associative learning (Van Groen and Wyss, 1990; Sugar et al., 2011; Kwapis et al., 2015). Although many of the brain regions in the trace fear circuitry including the mPFC, hippocampus, and amygdala have been well-studied (Knight et al., 2004; Esclassan et al., 2009a, 2009b; Gilmartin and Helmstetter, 2010; Kwapis et al., 2011; Gilmartin et al., 2012), little is known about learning-related plasticity within the RSC.

Developmental age-related changes occur in fear behavior, and the physiology underlying several fear-related brain regions (Vasilyev and Barish, 2002; Pattwell et al., 2011; Ehrlich et al., 2012; Ryan et al., 2016; Arruda-Carvalho et al., 2017; Zimmermann et al., 2019; Yousuf et al., 2020b). Specifically, in the male rat gRSC, drastic changes occur around PND 30 where there is

an increase of RS_{ADP}, and a decrease of RS neurons (Yousuf et al., 2020b). The emergence of the ADP at ~PND 30 overlaps with when rodents are able to acquire trace fear memories (PND 28; Moye and Rudy, 1987; Barnet and Hunt, 2005), a behavioral task that relies on the RSC (Kwapis et al., 2015; Todd et al., 2016). These data raise the question about how certain intrinsic properties such as the ADP may contribute to the acquisition and retrieval of trace fear learning during development.

Majority of the studies investigating developmental patterns in learning are focused on males. Epidemiological evidence indicates that females are twice as likely to develop anxiety disorders than males (Kessler et al., 1995, 2012). Sex differences in fear learning can emerge prior to or after puberty due to the organizational and activational effects of sex steroid hormones (Toledo-Rodriguez and Sandi, 2007; Dalla and Shors, 2009; Vigil et al., 2016; Juraska and Willing, 2017). Previous studies have reported sex differences in RSC-dependent tasks such as trace eyeblink conditioning (Weible et al., 2000; Dalla et al., 2009) and contextual fear learning in adult rodents (Maren et al., 1994; Ris et al., 2005; Kosten et al., 2006; Gresack et al., 2009; Moore et al., 2010; Mizuno et al., 2012; Sase et al., 2019). Further, sex differences in synaptic plasticity have been noted in the adult rodent hippocampus (Maren et al., 1994; Yang et al., 2004; Monfort et al., 2015; Qi et al., 2016; Wang et al., 2018). Exactly when sex differences emerge in trace fear learning, and how learning induces plasticity in the gRSC is unknown.

We investigated how retrieval of trace fear memories is different between sexes, and three different age groups: early adolescence (PND 25-29), mid-adolescent (PND 42-49), and adults (PND > 60). We found a nonlinear trajectory in trace fear retrieval in males (but not females) where male mid-adolescent rats exhibit the lowest freezing levels compared to other age groups. Sex differences in retrieval of trace memories were also observed only during early adolescence,

and not any other ages. Second, we determined the developmental age when sex differences emerge in neurons of the gRSC, and how sex and age play a role in experience-dependent changes in these neurons. We found male RS_{ADP} neurons have enhanced intrinsic excitability compared to females, and this sex difference is present from early adolescence until adulthood. Further, sex differences in RS neurons emerge during adulthood. Learning and novel context influence gRSC neurons in a cell-type specific manner where intrinsic excitability of male RS_{ADP} neurons (but not male RS) is altered during all ages, whereas only adult female RS and RS_{ADP} neurons are affected by learning and novel experiences. Our results give insight regarding the sex- and age-specific recruitment of the RSC in processing complex fear memories or novel stimuli.

Materials and methods

Same as chapter 2.

Results

Sex and age differences in retrieval of trace fear memories

Male ($n = 4$ for each age group) and female ($n = 4$ for each age group) early adolescent (PND 25-29), mid-adolescent (PND 42-49), and adult (PND > 60) rats were conditioned to associate a tone with a footshock (6 pairings) that was separated by a 30 s interval. Freezing levels in all 6 groups were measured 30 s (trace interval) following the tone across the 6 trials. Two-way repeated measures ANOVA with trial as a within-subjects factor, and behavior group as a between-subjects factor revealed a significant main effect of trial ($F_{(6, 108)} = 47.87$, $p < 0.001$; *Figure 13A*). There was a significant trial by sex interaction ($F_{(6, 108)} = 2.24$, $p < 0.05$), and a sex by age interaction ($F_{(2, 18)} = 3.51$, $p = 0.051$; *Figure 13A*). However, there was no significant trial by sex by age interaction ($F_{(12, 108)} = 1.21$, $p > 0.05$), and no significant effect of age ($F_{(2, 18)} = 0.88$, $p > 0.05$) or sex ($F_{(1, 18)} = 0.63$, $p > 0.05$; *Figure 13A*). *Post hoc* analysis confirmed that following trial 3, all rats showed significantly higher freezing levels ($p < 0.05$) compared to baseline (first 2 min in the training chamber), suggesting that all groups had successful acquisition of trace fear learning (*Figure 13A*). Twenty-four hours later, trace and CS only groups were brought back for testing in a novel environment (*Figure 13B*; *Figure 13C*). To assess learning, all rats were presented with 2 tone presentations in the testing chamber and freezing levels were measured 30 s following the tone presentations. Baseline freezing (the first 2 min in the testing chamber) was subtracted from the trace interval (*Figure 13B*; *Figure 13C*). Two-way ANOVA demonstrated a significant effect of sex ($F_{(1, 18)} = 4.84$, $p < 0.05$) but not a significant effect of age $F_{(2, 18)} = 1.78$, $p > 0.05$) or a significant age by sex interaction ($F_{(2, 18)} = 1.64$, $p > 0.05$; *Figure 13B*; *Figure 13C*). *Post hoc* analysis concluded that early adolescent males froze significantly more than early adolescent

females ($p < 0.05$; *Figure 13C*). Further, male mid-adolescent males had reduced freezing compared to early adolescent and adult males ($p = 0.08$; *Figure 13B*). Thus, sex differences in retrieval of trace fear memories were observed only during early adolescence.

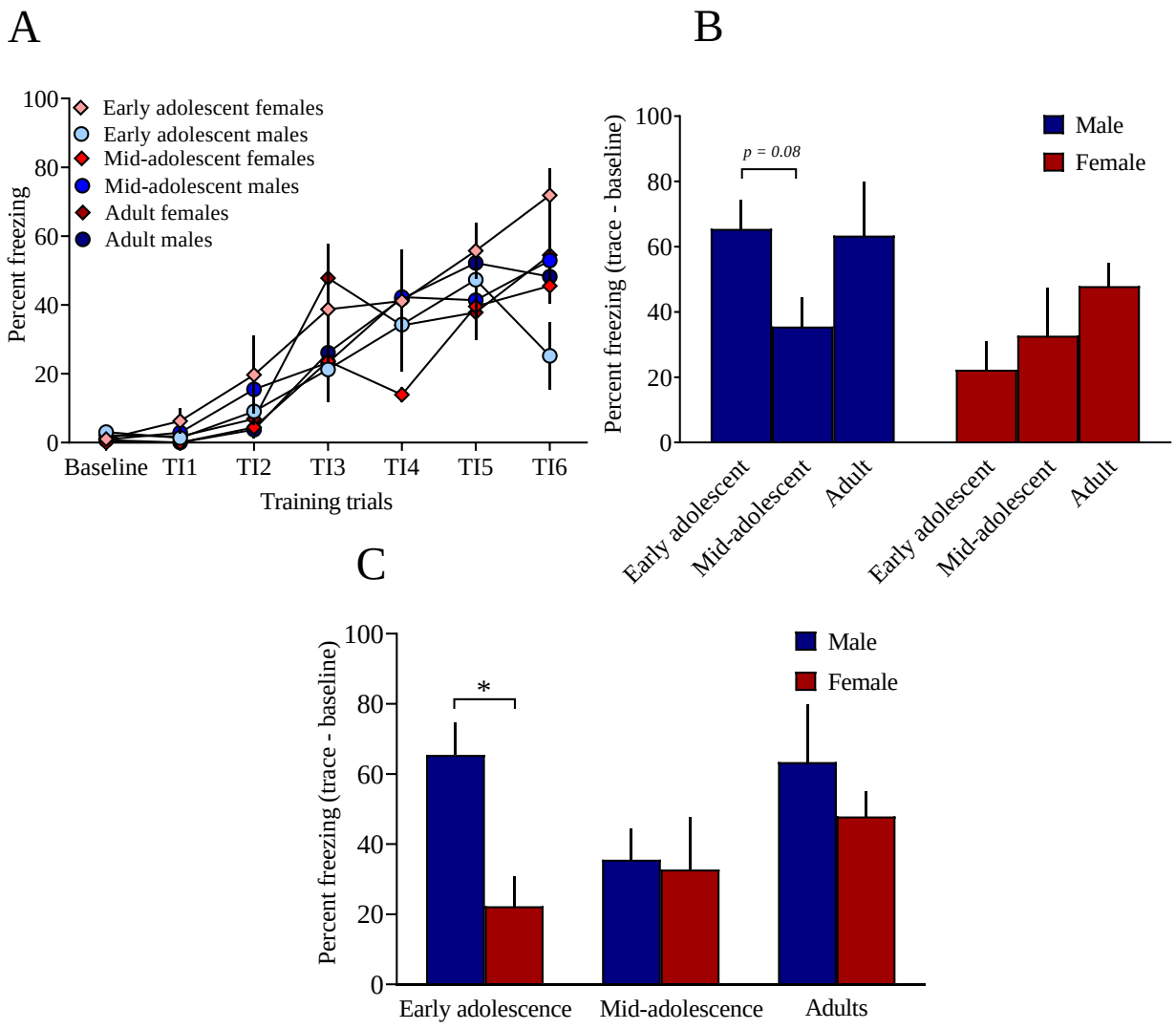


Figure 13. Age and sex differences in retrieval of trace fear memories. (A) Early adolescent (PND 25-29), mid-adolescent (PND 42-49), and adult (PND > 60) male and female rats receive 6 tone-shock pairings that are separated by a 30 s interval. (B) Twenty-four hours later, all rats are brought to a novel environment and exposed to 2 tone presentations. Blue bar graphs represent retrieval of trace fear memories in males during three different developmental periods. Mid-adolescent male rats freeze less than early adolescent and adult males. Red bar graphs represent retrieval of trace fear in females in three age groups and no differences are observed across development. (C) Bar graphs representing sex differences within each age group. Early adolescent male rats freeze significantly more than females. *Abbreviations*: postnatal day (PND); trace interval (TI). (*$p < 0.05$).

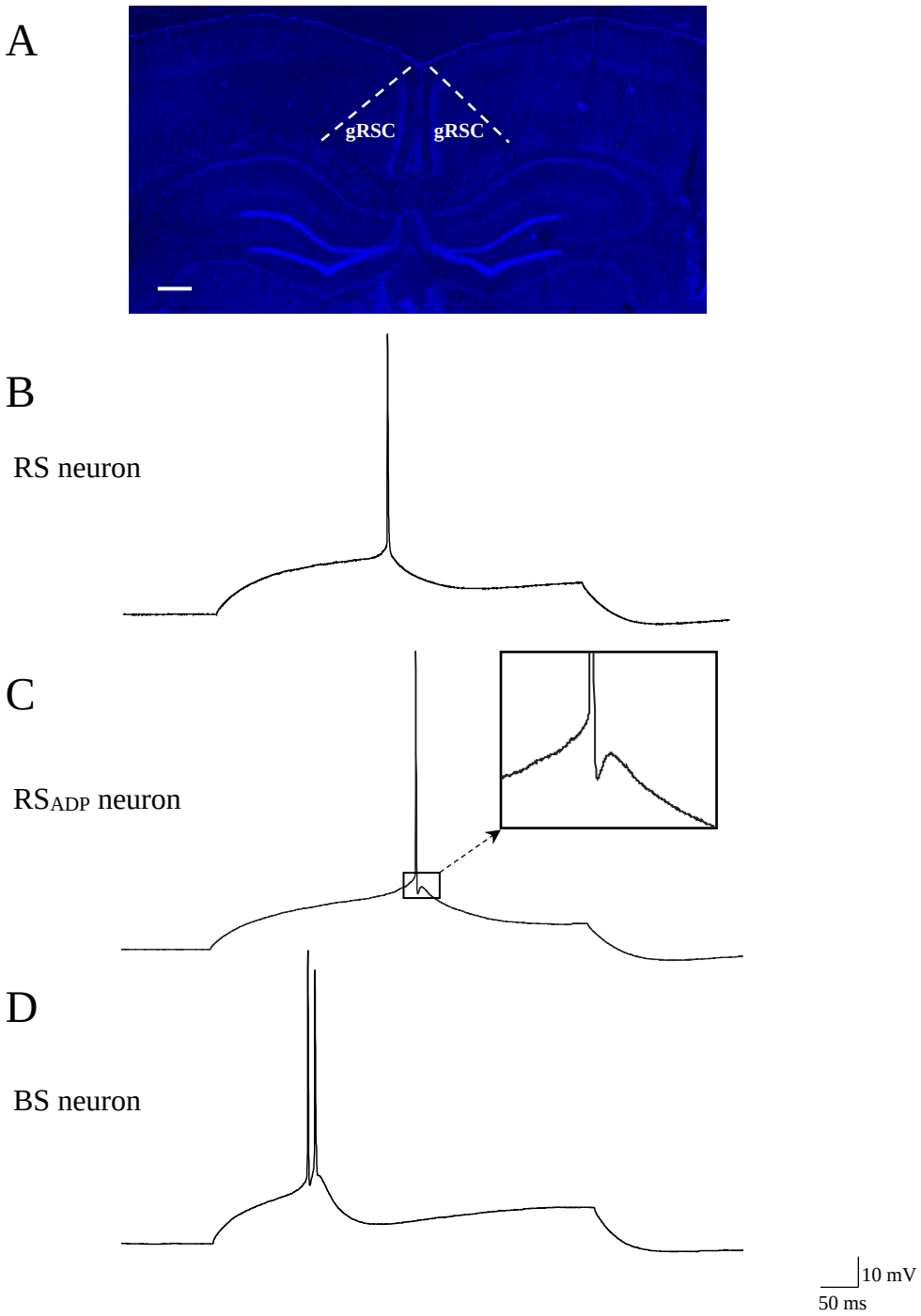


Figure 14. Neuronal firing types in layer 5 of the gRSC. (A) Fluorescence image of a coronal section of the rat gRSC. A low power (X4) DAPI image illustrating the borders of the gRSC. Traces representing a (B) RS neuron (C) RS_{ADP} neuron and (D) BS neuron. *Abbreviations*: granular retrosplenial cortex (gRSC); regular spiking (RS); regular spiking afterdepolarization (RS_{ADP}); burst spiking (BS). Scale bar, 50 µm.

	RS%	RS_{ADP}%	BS%	Other%
PND 10-15				
Male	83%	0%	0%	17%
Female	100%	0%	0%	0%
PND 16-20				
Male	85%	11%	0%	4%
Female	58%	8%	17%	17%
PND 21-25				
Male	100%	0%	0%	0%
Female	80%	20%	0%	0%
PND 26-30				
Male	82%	18%	0%	0%
Female	85%	13%	2%	0%
PND 35-40				
Male	41%	38%	0%	21%
Female	42%	38%	17%	3%
PND 41-45				
Male	23%	70%	7%	0%
Female	36%	52%	12%	0%
PND 46-50				
Male	24%	70%	3%	3%
Female	35%	54%	11%	0%
PND > 60				
Male	19%	64%	9%	8%
Female	15%	70%	12%	3%

Table 1. Developmental changes in neuronal firing type distribution in layer 5 gRSC. Data indicate the percentage of each firing type observed at different developmental ages. Other includes FS and LS neurons. *Abbreviations*: postnatal day (PND); regular spiking (RS); regular spiking afterdepolarization (RS_{ADP}); burst spiking (BS); fast spiking (FS); late spiking (LS); granular retrosplenial cortex (gRSC).

Sex differences in experience-dependent intrinsic plasticity in the early adolescent (PND 25-29) gRSC

During early adolescence (PND 25-29), RS neurons are the predominant firing type in the male and female layer 5 gRSC (~80%; *Figure 14*; *Table 1*). Intrinsic excitability of RS neurons was similar in the naive early adolescent gRSC of males (*n = 20*) and females (*n =26*; *Figure 15*; *Table 2*). Two-way repeated measures ANOVA demonstrated a significant effect of current ($F_{(18, 792)} = 147.831$, $p < 0.001$), indicating that the number of APs increased in response to ascending

current injections to the soma (*Figure 15A*). There was no effect of sex ($F_{(1, 44)} = 0.00$, $p > 0.05$) or current by sex interaction ($F_{(18, 792)} = 2.45$, $p > 0.05$; *Figure 15A*).

RS_{ADP} neurons constitute approximately 20% of the early adolescent gRSC (*Figure 14; Table 1*). In response to increasing current injections, male RS_{ADP} neurons (*n = 7*) had increased number of APs compared to females (*n = 7*; *Figure 15B*; *Table 2*). Two-way repeated measures ANOVA indicated a significant effect of current ($F_{(18, 198)} = 77.10$, $p < 0.001$), and current by sex interaction ($F_{(18, 198)} = 3.19$, $p < 0.05$) but not a significant effect of sex ($F_{(1, 11)} = 4.43$, $p = 0.06$; *Figure 15B*). *Post hoc* analysis confirmed that at current injections higher than 300 pA, male RS_{ADP} neurons fire more APs compared to females ($p < 0.05$; *Figure 15B*). Thus, sex differences are prevalent in intrinsic excitability of RS_{ADP} neurons but not RS neurons of the early adolescent gRSC.

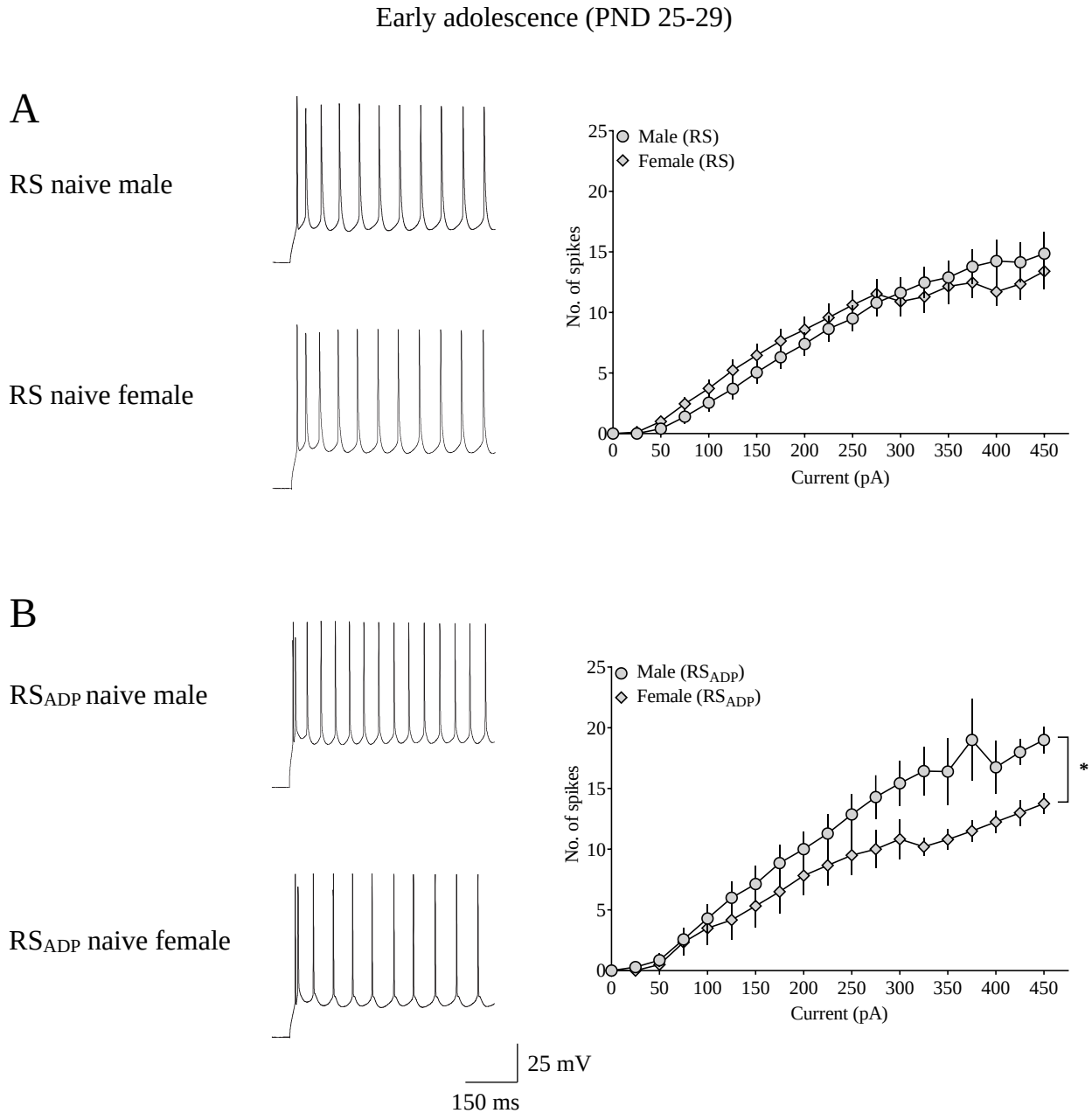


Figure 15. Sex differences in intrinsic excitability of RS_{ADP} neurons in the early adolescent (PND 25-29) gRSC. Representative traces at 300 pA injections. (A) RS neurons in the male (grey circle) and female (grey diamond) gRSC fire similar numbers of APs in response to increasing somatic current injections. (B) At current injections higher than 300 pA, male RS_{ADP} neurons fire more APs compared to females. *Abbreviations*: granular retrosplenial cortex (gRSC); postnatal day (PND); regular spiking (RS), regular spiking afterdepolarization (RS_{ADP}); action potential (AP). (*$p < 0.05$).

	RS		RS_{ADP}	
	Male	**Female**	**Male**	**Female**
	n = 20	*n = 26*	*n = 7*	*n = 7*
Membrane Properties				
RMP (mV)	-67.1 ± 0.4	-66.3 ± 0.5	-65.3 ± 0.3	-63.0 ± 0.6*
R_N (MΩ)	105.3 ± 11.2	144.5 ± 14.8*	92.1 ± 8.0	146.4 ± 27.0
$AP_{threshold}$ (mV)	-36.5 ± 1.0	-37.3 ± 0.8	-40.3 ± 1.0	-37.7 ± 0.7
$AP_{amplitude}$ (mV)	70.4 ± 2.3	74.2 ± 1.1	78.3 ± 2.9	76.8 ± 3.4
$AP_{half\text{-}width}$ (ms)	1.6 ± 0.1	1.5 ± 0.0	1.3 ± 0.1#	1.4 ± 0.1
$ADP_{amplitude}$ (mV)	n/a	0.7 ± 0.2	n/a	0.9 ± 0.3
fAHP (mV)	-3.7 ± 0.8	-5.6 ± 0.6	-6.0 ± 0.7	-6.3 ± 1.3
mAHP (mV)	-3.9 ± 0.5	-3.5 ± 0.4	-3.4 ± 0.7	-4.2 ± 0.8
sAHP (mV)	-1.7 ± 0.1	-1.7 ± 0.1	-1.5 ± 0.2	-1.9 ± 0.2

Table 2. Sex differences in membrane properties of RS and RS_{ADP} neurons in the early adolescent (PND 25-29) gRSC. Significantly different from RS or RS_{ADP} male (*, $p < 0.05$). Significantly different from male RS (#, $p < 0.05$). *Abbreviations*: regular spiking (RS); regular spiking afterdepolarization (RS_{ADP}); postnatal day (PND); action potential (AP); afterdepolarization (ADP); fast afterhyperpolarization (fAHP); medium afterhyperpolarization (mAHP); slow afterhyperpolarization (sAHP); resting membrane potential (RMP); input resistance (R_N); granular retrosplenial cortex (gRSC).

Sex differences favoring males were observed in RS_{ADP} but not RS neurons of the early adolescent rat (*Figure 15*). We next examined whether retrieval of trace fear memories would alter intrinsic excitability of gRSC neurons (RS or RS_{ADP}) in a sex-dependent manner. Male (*n = 5*) and female (*n = 6*) early adolescent rats (PND 25-29) were trained to associate a tone and shock (6 pairings) that were separated by an empty period of time and this group was referred to as the 'trace' group (*Figure 16A*). The CS only group were male (*n = 4*) and female (*n = 5*) early adolescent rats that were only exposed to 6 tone presentations and no footshocks during training (*Figure 16A*). All 4 groups showed comparable freezing during training (*Figure 16A*). Two-way repeated measures ANOVA revealed a significant main effect of trial ($F_{(6, 96)} = 15.72$, $p < 0.001$; *Figure 16A*). However, there was no significant trial by behavior by sex interaction ($F_{(6, 96)} = 0.61$,

$p > 0.05$), and no significant effect of behavior ($F_{(1, 16)} = 0.38$, $p > 0.05$; *Figure 16A*). Twenty-four hours later, trace and CS only groups were brought back for testing in a novel environment (*Figure 16B*). A two-way ANOVA during the tone test showed no significant effect of behavior ($F_{(1, 16)} = 1.67$, $p > 0.05$), sex ($F_{(1, 16)} = 0.01$, $p > 0.05$) or behavior by sex interaction ($F_{(1, 16)} = 0.47$, $p > 0.05$; *Figure 16B*).

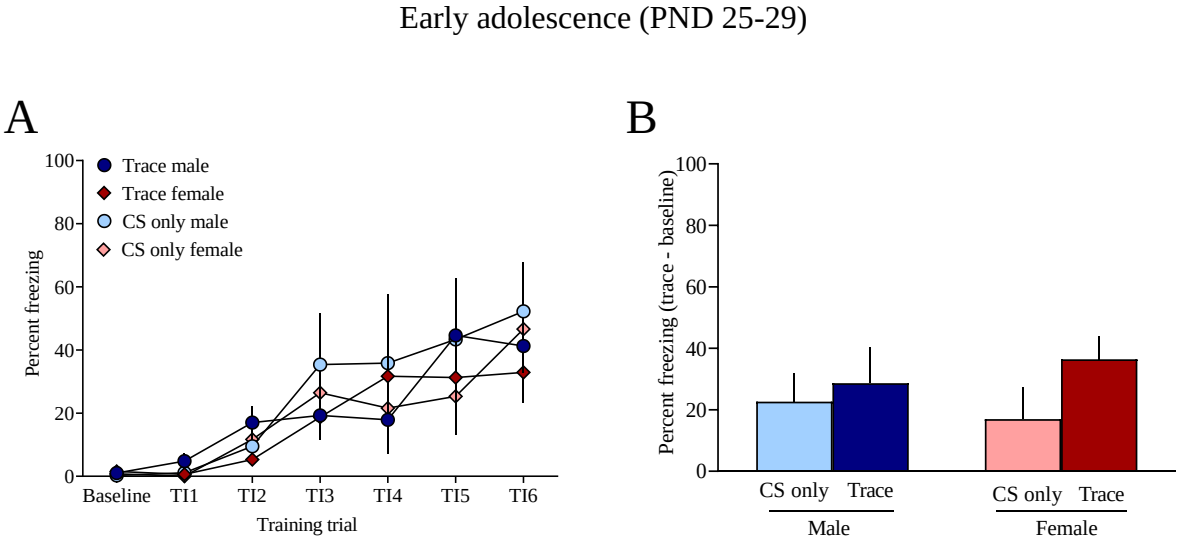


Figure 16. **Acquisition and retrieval of trace fear in male and female early adolescent (PND 25-29) rats.** (A) Male and female trace rats receive 6 tone-shock pairings that are separated by a 30 s interval. CS only rats receive 6 tone presentations and no footshocks. Rats show elevated freezing following trial 3. (B) Twenty-four hours later, trace and CS rats are brought to a novel environment and exposed to 2 tone presentations. Trace male and female groups show slightly higher freezing levels compared to the male and female CS only groups. *Abbreviations*: granular retrosplenial cortex (gRSC); postnatal day (PND); trace interval (TI); conditioned stimulus (CS).

Following acquisition and retrieval of trace fear memories (*Figure 16A*; *Figure 16B*), RSC slices were prepared for whole-cell patch-clamp recordings. There were no differences in intrinsic excitability of RS neurons in naive (*n = 20*), trace (*n = 24*), and CS only (*n = 24*) early adolescent males (*Figure 17A; Table 3*). Similarly, in females, intrinsic excitability was similar in naive (*n = 26*), trace (*n = 29*), and CS only (*n = 22*) groups (*Figure 17B; Table 3*). Three-way repeated measures ANOVA showed a significant effect of current ($F_{(18, 2484)} = 413.11$, $p < 0.001$), suggesting that RS neurons fire more APs in response to increasing current injections to the soma (*Figure 17A*; *Figure 17B*). There was no significant current by sex interaction ($F_{(18, 2484)} = 1.99$, p

> 0.05), current by behavior interaction ($F_{(36, 2484)} = 0.50$, $p > 0.05$) or current by sex by behavior interaction ($F_{(36, 2484)} = 1.12$, $p > 0.05$; *Figure 17A*; *Figure 17B*). Therefore, trace fear does not alter excitability of RS neurons in layer 5 of the male or female early adolescent gRSC.

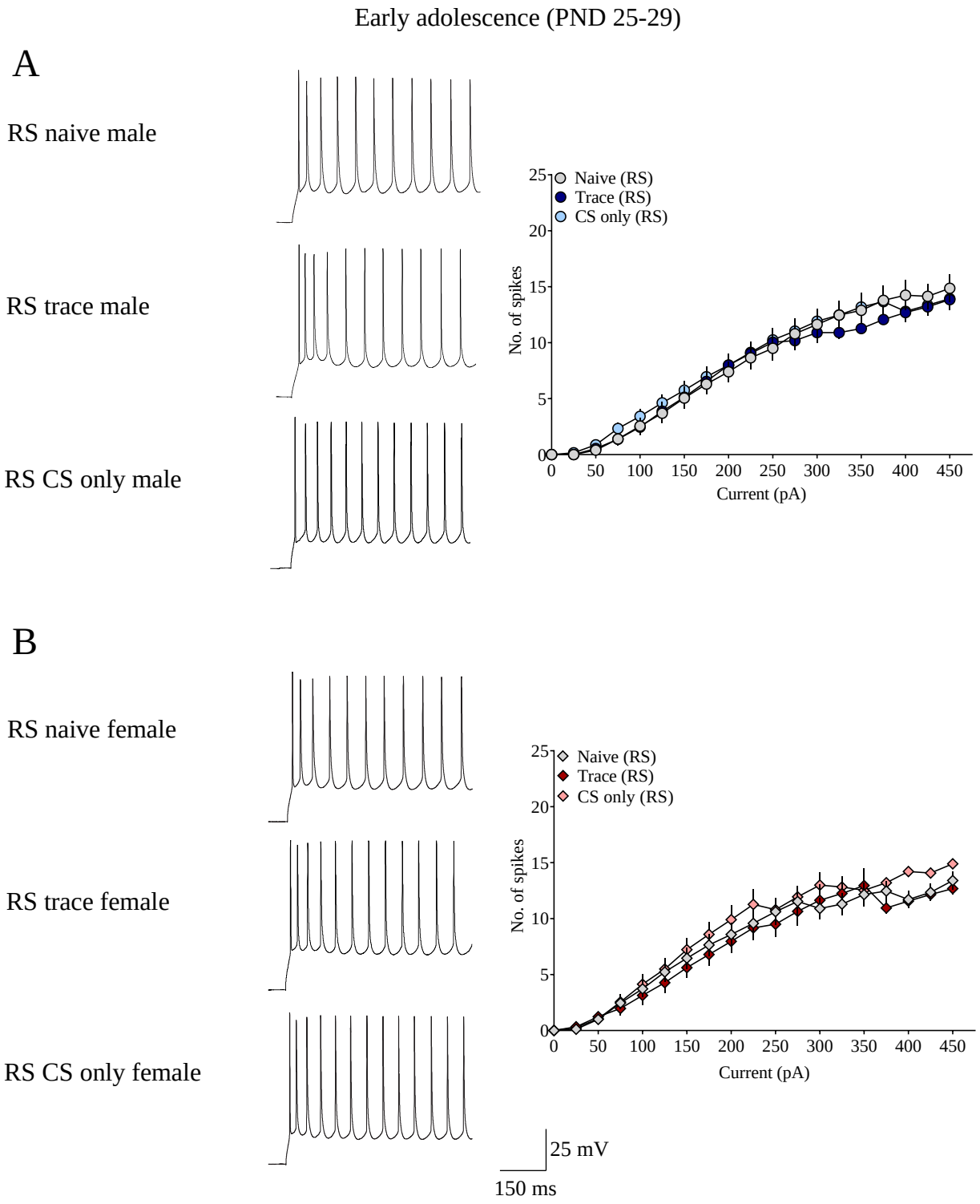


Figure 17. Behavioral training does not alter excitability of RS neurons in the early adolescent (PND 25-29) gRSC. Representative traces at 300 pA injections. (A) In males, intrinsic excitability of RS neurons in trace rats (dark blue circle) does not differ from naive (grey circle) or CS only (light blue circle) rats. (B) In females, excitability of RS neurons in trace (red diamond), naive (grey diamond), and CS only (pink diamond) are similar in all three groups. *Abbreviations*: granular retrosplenial cortex (gRSC); postnatal day (PND); trace interval (TI); regular spiking (RS); conditioned stimulus (CS).

	Male			Female		
	Naive	**Trace**	**CS only**	**Naive**	**Trace**	**CS only**
	n = 20	*n = 24*	*n = 24*	*n = 26*	*n = 29*	*n = 22*
Membrane Properties						
RMP (mV)	-67.1 ± 0.4	-66.4 ± 0.4	-66.0 ± 0.6	-66.3 ± 0.5	-66.3 ± 0.3	-66.1 ± 0.5
R_N (MΩ)	105.3 ± 11.2	110.3 ± 9.2	15.9 ± 9.2	144.5 ± 14.8	143.4 ± 22.1	155.2 ± 19.7
$AP_{threshold}$ (mV)	-36.5 ± 1.0	35.6 ± 0.7	-36.7 ± 1.0	-37.3 ± 0.8	-37.0 ± 0.8	-37.5 ± 0.7
$AP_{amplitude}$ (mV)	70.4 ± 2.3	73.9 ± 1.6	71.9 ± 2.0	74.2 ± 1.1	73.1 ± 1.3	69.4 ± 2.0
$AP_{half\text{-}width}$ (ms)	1.6 ± 0.1	1.5 ± 0.1	1.5 ± 0.0	1.5 ± 0.0	1.4 ± 0.0	1.6 ± 0.1
fAHP (mV)	-3.7 ± 0.8	-5.9 ± 0.5*	-6.1 ± 0.7*	-5.6 ± 0.6*	-7.0 ± 0.7	-4.2 ± 0.5#
mAHP (mV)	-3.9 ± 0.5	-3.6 ± 0.4	-3.5 ± 0.3	-3.5 ± 0.4	-4.0 ± 0.3	-3.4 ± 0.4
sAHP (mV)	-1.7 ± 0.1	-1.5 ± 0.1	-1.7 ± 0.1	-1.7 ± 0.1	-1.7 ± 0.2	-1.6 ± 0.1

Table 3. Effect of trace fear retrieval and CS exposure in membrane properties of RS neurons in the early adolescent (PND 25-29) gRSC. Significantly different from naive male (*, $p < 0.05$). Significantly different from trace female (#, $p < 0.05$). *Abbreviations*: regular spiking (RS); postnatal day (PND); action potential (AP); afterdepolarization (ADP); fast afterhyperpolarization (fAHP); medium afterhyperpolarization (mAHP); slow afterhyperpolarization (sAHP); resting membrane potential (RMP); input resistance (R_N); granular retrosplenial cortex (gRSC); conditioned stimulus (CS).

Early adolescent rats went through acquisition (*Figure 16A*) and retrieval (*Figure 16B*) of trace fear learning. Recordings from layer 5 of the male gRSC demonstrated that retrieval of trace fear reduced excitability of RS_{ADP} neurons (not significant) in layer 5 of the early adolescent gRSC (*Figure 18A*; *Table 4*). RS_{ADP} neurons from trace rats (*n = 2*) fired fewer APs compared to naive (*n = 7*) and CS only (*n = 3*) male rats (*Figure 18A*; *Table 4*). However, in females, RS_{ADP} neurons in naive (*n = 7*), trace (*n = 10*), and CS only (*n = 9*) rats fired a similar number of APs across all groups (*Figure 18B*; *Table 4*). Three-way repeated measures ANOVA revealed a significant effect of current ($F_{(12, 372)} = 175.91$, $p < 0.001$), but no signficant current by sex interaction ($F_{(12, 372)} = 1.11$, $p > 0.05$), current by behavior ($F_{(24, 372)} = 1.98$, $p > 0.05$), or current by sex by behavior interaction ($F_{(24, 372)} = 1.00$, $p > 0.05$; *Figure 18A*; *Figure 18B*). Taken together, retrieval of trace fear memories did not influence RS neurons in the male or female early adolescent gRSC.

However, trace fear reduced excitability of male RS_{ADP} neurons but had no effect on female RS_{ADP} neurons of the early adolescent gRSC.

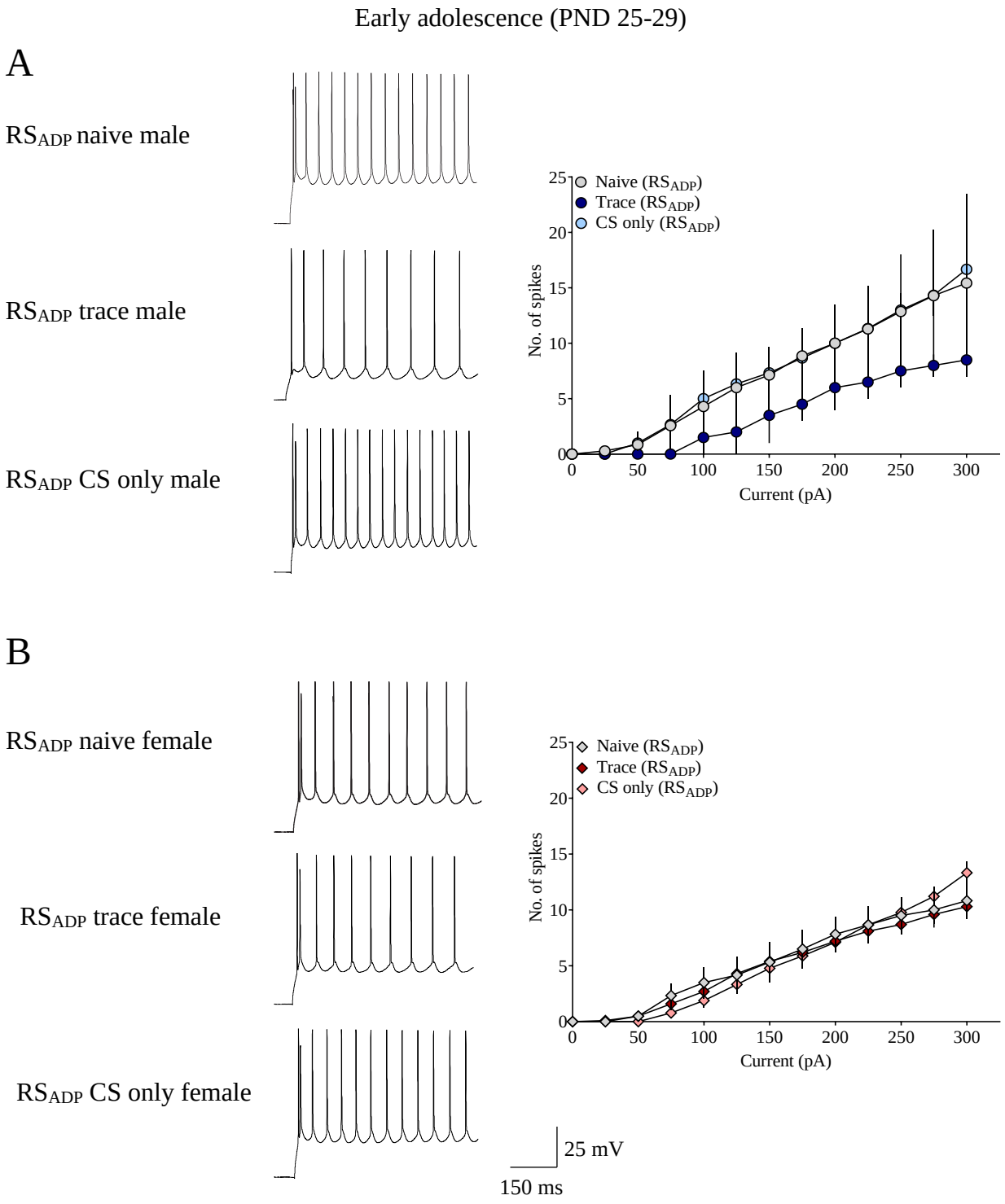


Figure 18. Behavioral training reduces excitability of male RS_{ADP} neurons in the early adolescent (PND 25-29) gRSC. Representative traces at 300 pA injections. (A) Intrinsic excitability of male RS_{ADP} neurons in trace rats (dark blue circle) is reduced compared to naive (grey circle) or CS only (light blue circle) rats. (B) In females, excitability of RS_{ADP} neurons in trace (red diamond), naive (grey diamond), and CS only (pink diamond) are similar in all three groups. *Abbreviations*: granular retrosplenial cortex (gRSC); postnatal day (PND); regular spiking afterdepolarization (RS_{ADP}); conditioned stimulus (CS).

	Male			Female		
	Naive	**Trace**	**CS only**	**Naive**	**Trace**	**CS only**
	n = 7	*n = 2*	*n = 3*	*n = 7*	*n = 10*	*n = 9*
Membrane Properties						
RMP (mV)	-65.3 ± 0.3	-65.6 ± 2.9	-65.3 ± 1.1	-63.0 ± 0.7	-65.5 ± 0.5	-65.5 ± 0.5
R_N (MΩ)	92.4 ± 8.0	89.0 ± 38.0	115.7 ± 41.7	146.4 ± 27.0	102.7 ± 11.6	107.3 ± 13.3
$AP_{threshold}$ (mV)	-40.3 ± 1.1	-35.6 ± 0.9	-40.6 ± 2.4	-37.7 ± 0.7	-38.2 ± 1.0	-39.3 ± 0.4
$AP_{amplitude}$ (mV)	78.3 ± 2.9	68.7 ± 11.1	85.1 ± 2.4	76.8 ± 3.7	76.7 ± 2.9	73.3 ± 3.2
$AP_{half\text{-}width}$ (ms)	1.3 ± 0.1	1.6 ± 0.6	1.2 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	1.3 ± 0.1
$ADP_{amplitude}$ (mV)	0.7 ± 0.2	1.1 ± 0.1	0.4 ± 0.1	0.9 ± 0.3	0.7 ± 0.2	0.8 ± 0.2
fAHP (mV)	-6.0 ± 0.8	-2.4 ± 4.5	-8.9 ± 2.2	-6.3 ± 1.3	-4.7 ± 1.0	-4.5 ± 0.6
mAHP (mV)	-3.4 ± 0.7	-2.1 ± 2.7	-3.9 ± 1.3	-4.2 ± 0.8	-3.3 ± 0.7	-5.1 ± 0.5
sAHP (mV)	-1.5 ± 0.2	-1.1 ± 0.2	-1.7 ± 0.2	-1.9 ± 0.2	-1.4 ± 0.3	-1.9 ± 0.1

Table 4. Effect of trace fear retrieval and CS exposure in membrane properties of RS_{ADP} neurons in the early adolescent (PND 25-29) gRSC. *Abbreviations*: regular spiking afterdepolarization (RS_{ADP}); postnatal day (PND); action potential (AP); afterdepolarization (ADP); fast afterhyperpolarization (fAHP); medium afterhyperpolarization (mAHP); slow afterhyperpolarization (sAHP); resting membrane potential (RMP); input resistance (R_N); granular retrosplenial cortex (gRSC); conditioned stimulus (CS).

Sex differences in experience-dependent intrinsic plasticity in the mid-adolescent (PND 42-49) gRSC

During mid-adolescence (PND 42-49), RS neurons are in smaller percentages in the male and female layer 5 gRSC (~30%; *Figure 14*; *Table 1*). Intrinsic excitability of RS neurons did not differ between males (*n = 10*) and females (*n =18*; *Figure 19A*; *Table 5*). Two-way repeated measures ANOVA demonstrated a significant effect of current ($F_{(18, 468)} = 89.37$, $p < 0.001$), indicating that the number of APs increased in response to increasing current injections (*Figure 19A*). There was no effect of sex ($F_{(1, 26)} = 0.40$, $p > 0.05$) or current by sex interaction ($F_{(18, 468)} = 1.09$, $p > 0.05$; *Figure 19A*).

RS_{ADP} neurons are the predominant firing type in the mid-adolescent gRSC (~70%; *Figure 14; Table 1*). Naive male RS_{ADP} neurons (*n = 22*) had increased excitability compared to those in

the female gRSC (*n = 20*; *Figure 19B*; *Table 5*). Two-way repeated measures ANOVA indicated a significant effect of current ($F_{(18, 720)} = 147.06$, $p < 0.001$), current by sex interaction ($F_{(18, 720)} = 4.04$, $p < 0.05$), and a significant effect of sex ($F_{(1, 40)} = 5.1$, $p < 0.05$; *Figure 19B*). *Post hoc* analysis confirmed that at current injections higher than 250 pA, male RS_{ADP} neurons fire more APs compared to females ($p < 0.05$; *Figure 19B*). Thus, similar to the early adolescent gRSC, sex differences are noted in intrinsic excitability of RS_{ADP} neurons in the mid-adolescent gRSC.

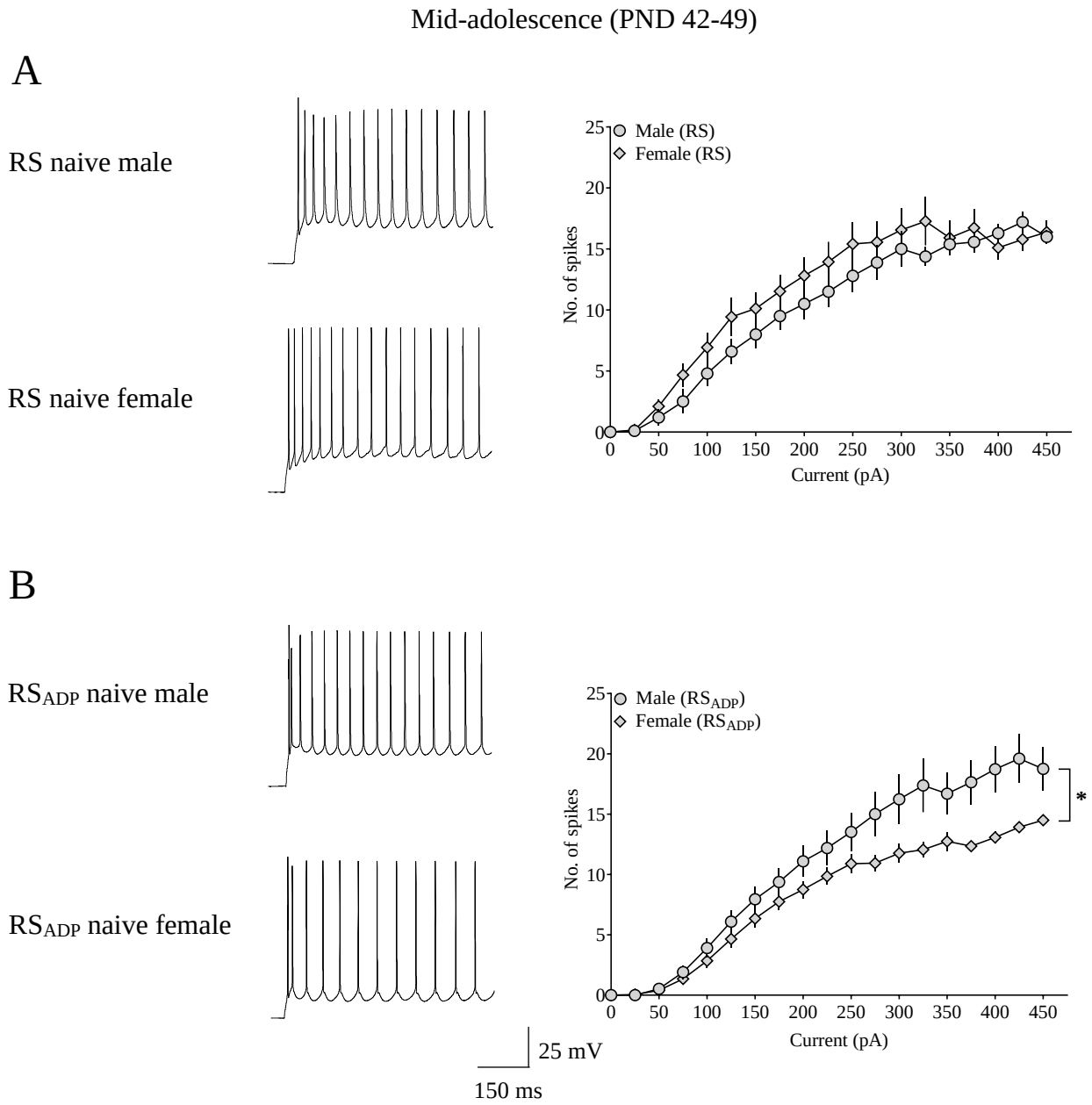


Figure 19. Sex differences in intrinsic excitability of RS$_{ADP}$ neurons in the mid-adolescent (PND 42-49) gRSC. Representative traces at 300 pA injections. (A) RS neurons in the male (grey circle) and female (grey diamond) gRSC fire a similar number of APs in response to increasing somatic current injections. (B) At current injections higher than 250 pA, male RS$_{ADP}$ neurons have significantly higher number of APs compared to females. *Abbreviations*: granular retrosplenial cortex (gRSC); postnatal day (PND); regular spiking afterdepolarization (RS$_{ADP}$); action potential (AP). (*$p < 0.05$).

	RS		RS_{ADP}	
	Male	**Female**	**Male**	**Female**
	n = 11	*n = 18*	*n = 27*	*n = 25*
Membrane Properties				
RMP (mV)	-67.1 ± 0.3	-66.5 ± 0.5	-66.1 ± 0.5	-66.3 ± 0.4
R_N (MΩ)	194.4 ± 37.5*	193.2 ± 28.1*	126.6 ± 10.6	116.0 ± 9.4
$AP_{threshold}$(mV)	-35.9 ± 1.3	-37.6 ± 1.0	-38.1 ± 0.7	-37.4 ± 0.6
$AP_{amplitude}$(mV)	71.3 ± 2.3	71.1 ± 2.4	75.0 ± 1.6	72.4 ± 1.6
$AP_{half\text{-}width}$ (ms)	1.3 ± 0.0	1.3 ± 0.1	1.2 ± 0.0	1.2 ± 0.0
$ADP_{amplitude}$ (mV)	n/a	n/a	1.2 ± 0.2	1.5 ± 0.3
fAHP (mV)	-11.5 ± 1.1	-9.1 ± 0.7	-9.2 ± 0.8	-8.8 ± 0.6
mAHP (mV)	-3.5 ± 0.4	-3.2 ± 0.4	-3.8 ± 0.4	-3.6 ± 0.4
sAHP (mV)	-1.8 ± 0.2	-1.6 ± 0.2	-1.6 ± 0.1	-1.6 ± 0.1

Table 5. Sex differences in membrane properties of RS and RS_{ADP} neurons in the mid-adolescent (PND 42-49) gRSC. Significantly different from RS_{ADP} in male or female (*, $p < 0.05$). *Abbreviations*: regular spiking (RS); regular spiking afterdepolarization (RS_{ADP}); postnatal day (PND); action potential (AP); afterdepolarization (ADP); fast afterhyperpolarization (fAHP); medium afterhyperpolarization (mAHP); slow afterhyperpolarization (sAHP); resting membrane potential (RMP); input resistance (R_N).

Male and female mid-adolescent rats were conditioned to associate a tone and a footshock (separated by a 30 s trace interval; *Figure 20A*). Two-way repeated measures ANOVA showed a significant main effect of trial ($F_{(6, 114)} = 12.76$, $p < 0.001$), behavior ($F_{(1, 19)} = 10.84$, $p < 0.05$), and a trial by behavior interaction ($F_{(6, 114)} = 3.28$, $p < 0.05$; *Figure 20A*). However, there was no significant trial by sex interaction ($F_{(6, 114)} = 1.02$, $p > 0.05$) or trial by behavior by sex interaction ($F_{(6, 114)} = 1.31$, $p > 0.05$; *Figure 20A*). *Post hoc* analysis showed that female trace rats ($n = 6$) had higher freezing levels compared to CS only females ($n = 4$) during trials 5 and 6 and male trace rats ($n = 6$) had higher freezing levels compared to CS only males ($n = 7$) during trials 2 and 3 ($p < 0.05$; *Figure 20A*). Following trace fear conditioning, a two-way ANOVA during the tone test showed a significant effect of behavior ($F_{(1, 19)} = 13.52$, $p < 0.05$), but not a significant effect of

sex ($F_{(1, 19)}$ = 2.29, p > 0.05) or behavior by sex interaction ($F_{(1, 19)}$ = 0.56, p > 0.05; *Figure 20B*). *Post hoc* analysis confirmed that trace male rats had significantly higher freezing compared to CS only males (p < 0.05; *Figure 20B*). Similarly, trace females had higher freezing than CS only females (p < 0.05), suggesting that both male and female mid-adolescent rats had successful retrieval of trace fear memories (*Figure 20B*).

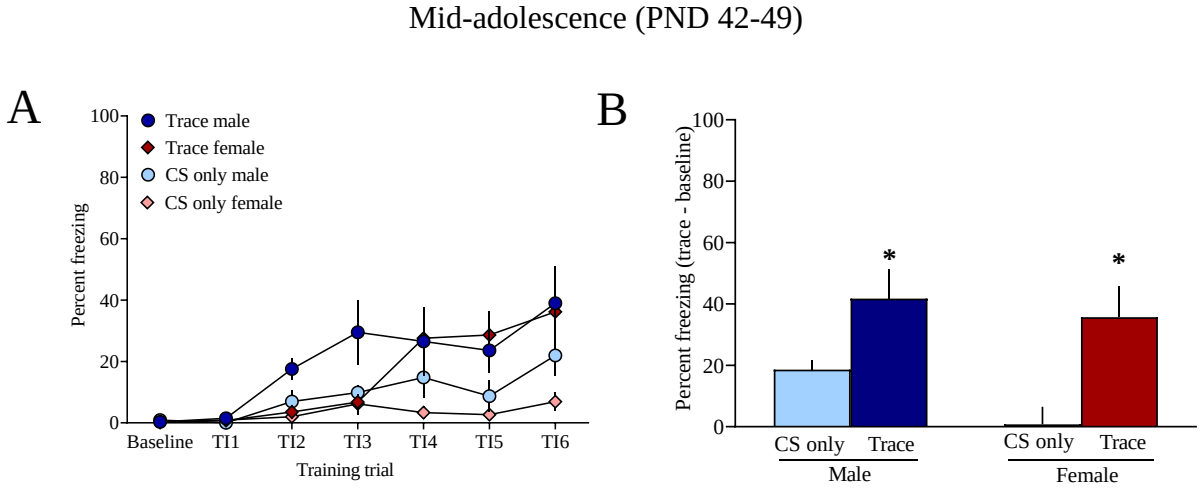


Figure 20. Acquisition and retrieval of trace fear in male and female mid-adolescent (PND 42-49) rats. (A) Male and female trace rats receive 6 tone-shock pairings that are separated by a 30 s interval. CS only rats receive 6 tone presentations and no footshocks. (B) Twenty-four hours later, trace and CS rats are brought to a novel environment and exposed to 2 tone presentations. Trace males have significantly higher freezing compared to CS only males and trace females have significantly higher freezing levels compared to CS only females. (*p < 0.05). *Abbreviations*: granular retrosplenial cortex (gRSC); postnatal day (PND); trace interval (TI); conditioned stimulus (CS).

Following acquisition and retrieval of trace fear memories (*Figure 20A*; *Figure 20B*), RSC slices were prepared for recordings. We observed no differences in intrinsic excitability of RS neurons in naive (*n = 10*), trace (*n = 5*), and CS only (*n = 18*) mid-adolescent males (*Figure 21A; Table 6*). Similarly, in females, intrinsic excitability was similar in naive (*n = 18*), trace (*n = 6*), and CS only (*n = 6*) groups (*Figure 21B; Table 6*). Three-way repeated measures ANOVA showed a significant effect of current ($F_{(12, 708)}$ = 161.55, p < 0.001), suggesting that RS neurons fire more APs in response to increasing current injections to the soma (*Figure 21A*; *Figure 21B*). There was no significant current by sex interaction ($F_{(12, 708)}$ = 0.27, p > 0.05), current by behavior interaction ($F_{(24, 708)}$ = 0.39, p > 0.05) or current by sex by behavior interaction ($F_{(24, 708)}$ = 0.99, p > 0.05;

Figure 21A; *Figure 21B*). Therefore, trace fear does not alter excitability of RS neurons in layer 5 of the male or female mid-adolescent rat gRSC.

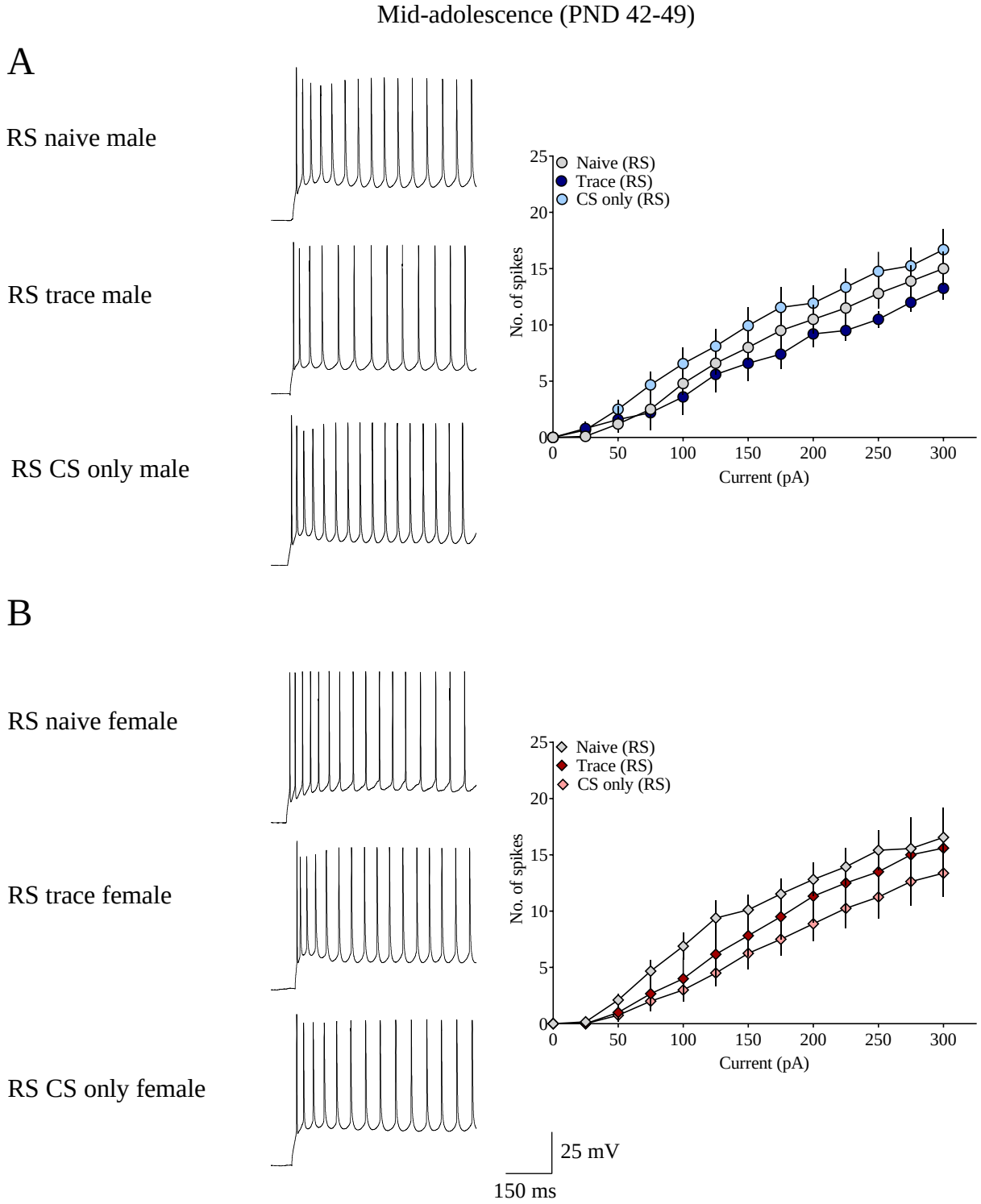


Figure 21. Retrieval of trace fear does not influence excitability of RS neurons in the mid-adolescent (PND 42-49) gRSC. Representative traces at 300 pA injections. (A) In males, intrinsic excitability of RS neurons in trace rats (dark blue circle) does not differ from naive (grey circle) or CS only (light blue circle) rats. (B) In females, excitability of RS neurons in trace (red diamond), naive (grey diamond), and CS only (pink diamond) does not differ across all three behavioral groups. *Abbreviations*: granular retrosplenial cortex (gRSC); postnatal day (PND); regular spiking (RS); conditioned stimulus (CS).

	Male			**Female**		
	Naive	**Trace**	**CS only**	**Naive**	**Trace**	**CS only**
	n = 11	*n = 5*	*n = 18*	*n = 18*	*n = 6*	*n = 8*
Membrane Properties						
RMP (mV)	-67.2 ± 0.3	-64.5 ± 0.7	-65.7 ± 0.4	-66.5 ± 0.5	- 67.63 ± 4.5	-66.2 ± 0.8
R_N (MΩ)	194.4 ± 37.5	202.0 ± 83.5	179.5 ± 18.5	193.2 ± 28.1	147.2 ± 23.5	134.8 ± 21.8
$AP_{threshold}$ (mV)	-35.9 ± 1.3	-39.3 ± 2.1	-39.2 ± 1.1	-37.6 ± 1.0	-37.8 ± 2.6	-36.3 ± 1.3
$AP_{amplitude}$ (mV)	71.3 ± 2.3	78.2 ± 4.0	68.3 ± 2.4*	71.1 ± 2.4	66.3 ± 4.9	64.5 ± 3.0
$AP_{half\text{-}width}$ (ms)	1.3 ± 0.0	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.4 ± 0.1
fAHP (mV)	-11.5 ± 1.1	-8.9 ± 1.2	-8.4 ± 0.9	-9.1 ± 0.7	-8.0 ± 0.8	-8.6 ± 1.3
mAHP (mV)	-3.5 ± 0.4	-4.6 ± 0.4	-3.8 ± 0.3	-3.2 ± 0.3	-3.7 ± 0.5	-3.8 ± 0.2
sAHP (mV)	-1.8 ± 0.2	-3.9 ± 2.0	-1.8 ± 0.2	-1.6 ± 0.2	-1.6 ± 0.2	-1.5 ± 0.2

Table 6. Effect of trace fear retrieval and CS exposure in membrane properties of RS neurons in the mid-adolescent (PND 42-49) gRSC. Significantly different from trace male (*, $p < 0.05$). *Abbreviations*: regular spiking (RS); regular spiking; postnatal day (PND); action potential (AP); afterdepolarization (ADP); fast afterhyperpolarization (fAHP); medium afterhyperpolarization (mAHP); slow afterhyperpolarization (sAHP); resting membrane potential (RMP); input resistance (R_N); granular retrosplenial cortex (gRSC); conditioned stimulus (CS).

Following acquisition (*Figure 20A*) and retrieval (*Figure 20B*) of trace fear, RSC slices from male and female mid-adolescent rats were prepared. Retrieval of trace fear reduced excitability of RS_{ADP} neurons in layer 5 of the mid-adolescent gRSC (*Figure 22A*; *Table 7*). In response to current injections, RS_{ADP} neurons from trace (*n = 20*) and CS only (*n = 23*) male rats fired significantly fewer APs compared to naive (*n = 22*) rats (*Figure 22A*; *Table 7*). However, in females, intrinsic excitability of RS_{ADP} neurons from naive (*n = 20*), trace (*n = 20*), and CS only (*n = 15*) rats were similar across all three groups (*Figure 22B*; *Table 7*). Three-way repeated measures ANOVA revealed a significant main effect of current ($F_{(18, 2052)} = 738.09$, $p < 0.001$), sex ($F_{(1, 114)} = 3.80$, $p = 0.054$), and behavior ($F_{(2, 114)} = 3.12$, $p < 0.05$; *Figure 22A*; *Figure 22B*).

There was also a significant sex by behavior interaction ($F_{(2, 114)} = 3.18$, $p < 0.05$), current by sex interaction ($F_{(18, 2052)} = 3.64$, $p < 0.05$), and a current by sex by behavior interaction ($F_{(36, 2052)} = 2.28$, $p < 0.05$; *Figure 22A*; *Figure 22B*). *Post hoc* analysis revealed that male RS_{ADP} neurons in trace rats fired a reduced number of APs compared to naive rats between current injections 150-450 pA ($p < 0.05$; *Figure 22A*). RS_{ADP} neurons in the male gRSC of CS only rats fired fewer APs compared to naive rats between current injections 125-450 pA ($p < 0.05$; *Figure 22A*). Overall, retrieval of trace fear memories does not alter excitability in the female mid-adolescent gRSC but substantially decreases excitability of RS_{ADP} neurons in trace and CS only males.

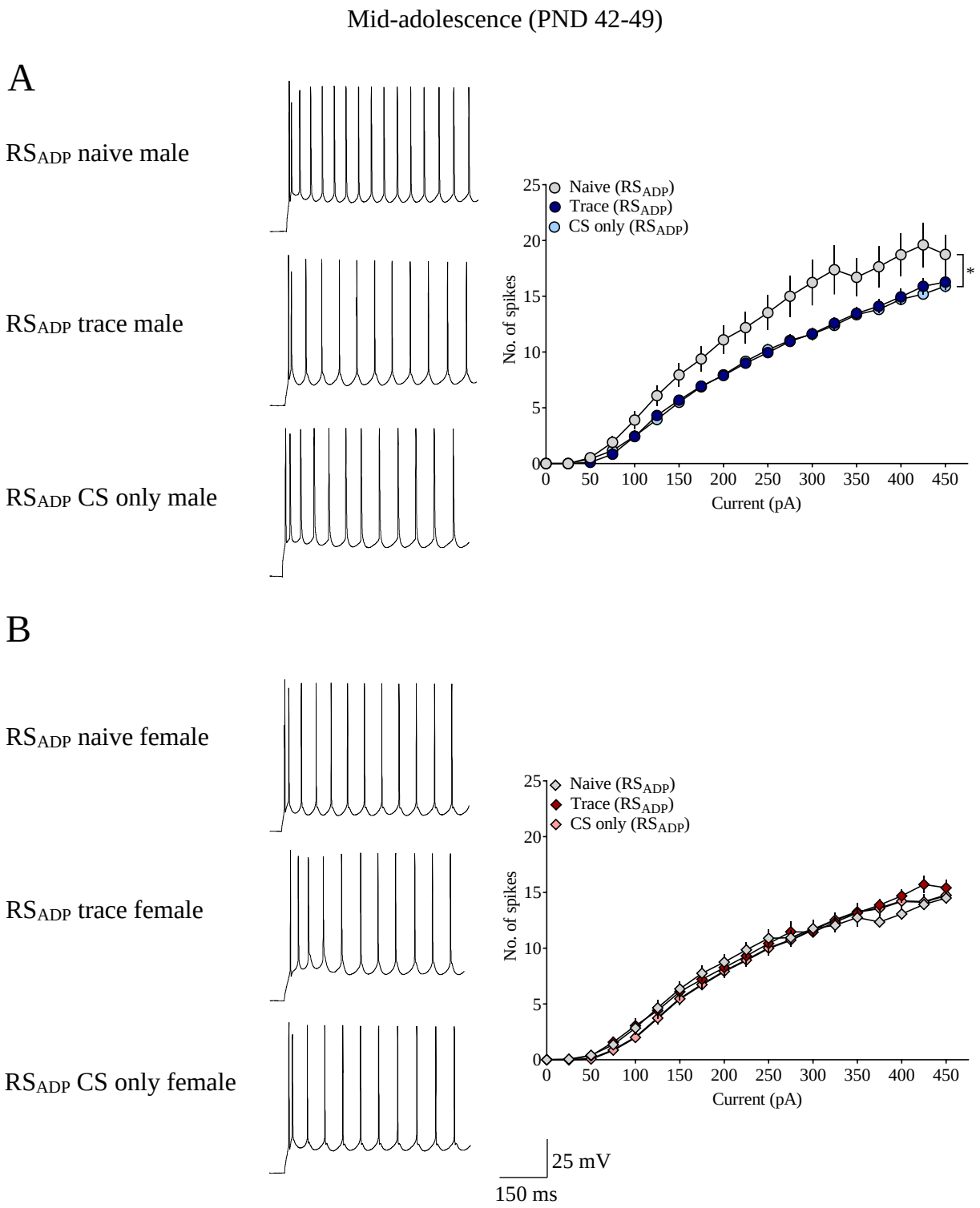


Figure 22. Retrieval of trace fear and CS exposure reduces excitability of male RS_{ADP} neurons in the mid-adolescent (PND 42-49) gRSC. Representative traces at 300 pA injections. (A) Retrieval of trace fear memories (dark blue circle) or CS alone (light blue circle) reduces intrinsic excitability of male RS_{ADP} neurons compared to those in naive (grey circle) rats. (B) Excitability of female RS_{ADP} neurons in trace (red diamond), naive (grey diamond), and CS only (pink diamond) are similar in all three groups. *Abbreviations*: granular retrosplenial cortex (gRSC); postnatal day (PND); regular spiking afterdepolarization (RS_{ADP}); conditioned stimulus (CS). (*$p < 0.05$).

We further examined the underlying mechanisms that may contribute to the experience-dependent changes in male mid-adolescent RS_{ADP} neurons by measuring basic membrane properties as well as the postburst AHP between the different behavioral groups (*Table 7*). Retrieval of trace fear memories or exposure to tone presentations modulated the mAHP in both male and female RS_{ADP} neurons. Two-way ANOVA revealed a significant effect of behavior ($F_{(2, 114)} = 3.28$, $p < 0.05$; *Figure 23*; *Table 7*). *Post hoc* analysis confirmed that trace fear learning significantly enhanced the mAHP in male RS_{ADP} neurons compared to the naive group ($p < 0.05$; *Figure 23*; *Table 7*). Interestingly, exposure to tone presentations (CS only group) significantly enhanced the mAHP in female RS_{ADP} neurons compared to naives ($p < 0.05$; Figure *23*). Thus, in addition to trace fear learning, novel stimuli can alter mAHP of RS_{ADP} neurons in the mid-adolescent gRSC.

	Male			**Female**		
	Naive	**Trace**	**CS only**	**Naive**	**Trace**	**CS only**
	n = 27	*n = 20*	*n = 23*	*n = 25*	*n = 20*	*n = 15*
Membrane Properties						
RMP (mV)	-66.1 ± 0.5	-66.0 ± 0.4	-65.8 ± 0.4	-66.3 ± 0.4	-66.8 ± 0.4	-66.5 ± 0.7
R_N (MΩ)	126.6 ± 8.0	90.6 ± 9.5	116.5 ± 15.4	116.0 ± 9.4	127.2 ± 15.7	96.3 ± 9.0
$AP_{threshold}$ (mV)	-38.1 ± 0.7	-39.0 ± 0.7	-37.7 ± 0.6	-37.4 ± 0.6	-37.7 ± 0.8	-37.7 ± 0.8
$AP_{amplitude}$ (mV)	75.0 ± 1.6	75.7 ± 1.5	72.9 ± 1.3	72.3 ± 1.6	73.9 ± 1.6	73.0 ± 2.3
$AP_{half\text{-}width}$ (ms)	1.2 ± 0.0	1.1 ± 0.0	1.1 ± 0.0	1.2 ± 0.0	1.2 ± 0.1	1.2 ± 0.0
$ADP_{amplitude}$ (mV)	1.2 ± 0.2	1.9 ± 0.3	1.6 ± 0.3	1.5 ± 0.3	1.2 ± 0.3	1.5 ± 0.3
fAHP (mV)	-9.2 ± 0.8	-9.6 ± 0.7	-9.6 ± 0.4	-8.8 ± 0.6	-9.7 ± 0.8	-8.7 ± 0.7
mAHP (mV)	-3.8 ± 0.3	-4.7 ± 0.3*	-4.2 ± 0.3	-3.6 ± 0.4	-4.0 ± 0.3	-4.9 ± 0.4#
sAHP (mV)	-1.6 ± 0.1	-1.6 ± 0.1	-1.7 ± 0.1	-1.6 ± 0.1	-1.7 ± 0.1	-1.9 ± 0.1

Table 7. Effect of trace fear retrieval and CS exposure in membrane properties of RS_{ADP} in the mid-adolescent (PND 42-49) gRSC. Significantly different from naive male (*, $p < 0.05$). Significantly different from naive female (#, $p < 0.05$). *Abbreviations*: regular spiking afterdepolarization (RS_{ADP}); postnatal day (PND); action potential (AP); afterdepolarization (ADP); fast afterhyperpolarization (fAHP); medium afterhyperpolarization (mAHP); slow

afterhyperpolarization (sAHP); resting membrane potential (RMP); input resistance (R_N); granular retrosplenial cortex (gRSC); conditioned stimulus (CS).

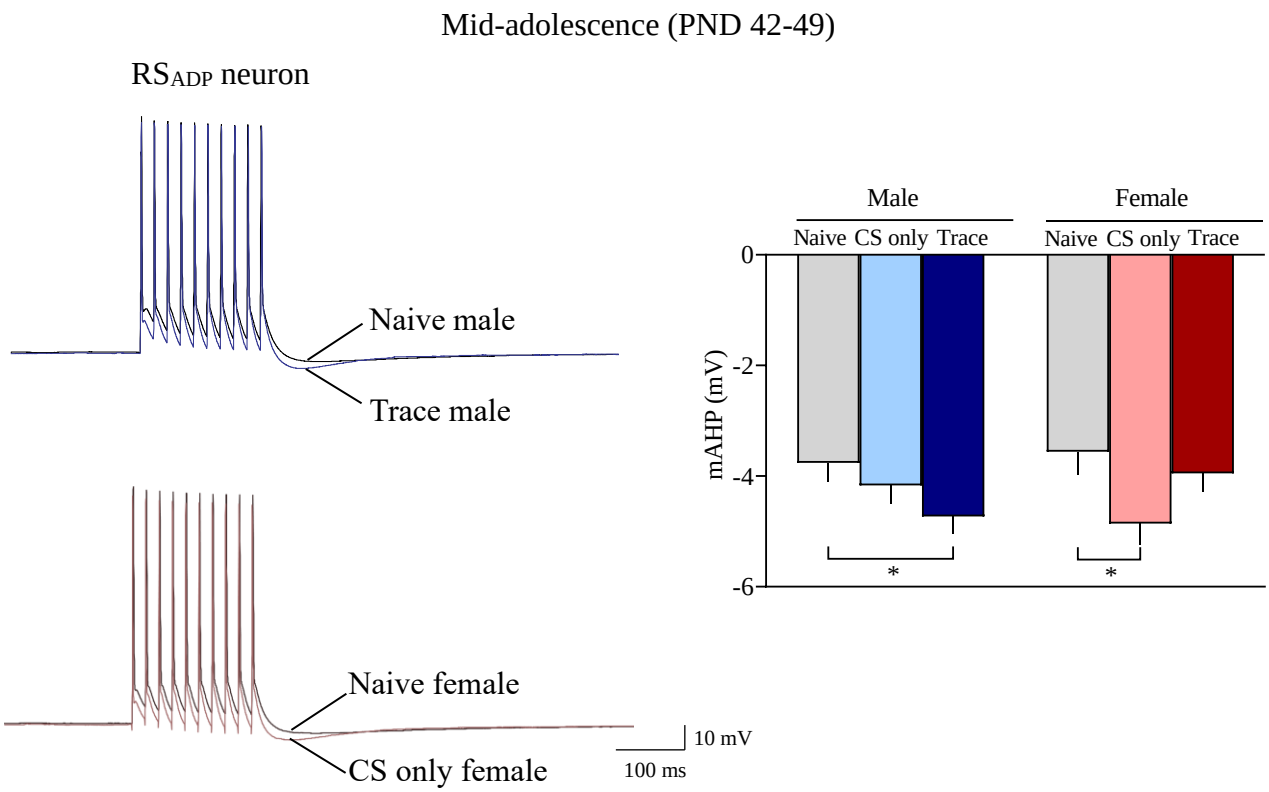


Figure 23. Retrieval of trace fear and CS exposure influence mAHP of RS_{ADP} neurons in the male and female mid-adolescent (PND 42-49) gRSC. *(Left)* Representative traces showing mAHP from RS_{ADP} neurons. (*Right*) Bar graphs representing mAHP of male RS_{ADP} neurons, which is enhanced following retrieval of trace fear memories (dark blue) compared to the naive homecage group (grey). mAHP of female RS_{ADP} neurons is increased in the group that only received tone presentations and no footshocks (pink) compared to the naive homecage group (grey). *Abbreviations*: postnatal day (PND); regular spiking afterdepolarization (RS_{ADP}); conditioned stimulus (CS); medium afterhyperpolarization (mAHP). (*$p < 0.05$).

Sex differences in experience-dependent intrinsic plasticity in the adult (PND > 60) gRSC

In the adult (PND > 60) gRSC, RS neurons are present in much smaller percentages in the male and female layer 5 gRSC compared to the mid-adolescent gRSC (~10%; *Figure 14*; *Table 1*). Intrinsic excitability of RS neurons did not differ between males ($n = 9$) and females ($n = 9$; *Figure 24A*; *Table 8*). Two-way repeated measures ANOVA demonstrated a significant effect of current ($F_{(12, 192)} = 42.78$, $p < 0.001$), indicating that the number of APs increased in response to increasing current injections (*Figure 24A*). There was no effect of sex ($F_{(1, 16)} = 1.60$, $p > 0.05$) or current by sex interaction ($F_{(12, 192)} = 1.91$, $p > 0.05$; *Figure 24A*).

RS_{ADP} neurons are the predominant firing type in the adult gRSC (~70%; *Figure 14; Table 1*). Naive male RS_{ADP} neurons (*n = 33*) had increased excitability compared to those in the female gRSC (*n = 30*; *Figure 24B*; *Table 8*). Two-way repeated measures ANOVA indicated a significant effect of current ($F_{(18, 1098)} = 473.06$, $p < 0.001$) and a significant effect of sex ($F_{(1, 61)} = 4.48$, $p < 0.05$) but not a significant current by sex interaction ($F_{(18, 1098)} = 1.46$, $p > 0.05$; *Figure 24B*). *Post hoc* analysis confirmed that at current injections higher than 350 pA, male RS_{ADP} neurons fire more APs compared to females ($p < 0.05$; *Figure 24B*). Thus, similar to the early- and mid-adolescent gRSC, sex differences are noted in intrinsic excitability of RS_{ADP} neurons in the adult gRSC.

In layer 5 of the adult gRSC, we observed that ~10% of the neuronal population were BS cells (*Figure 14*; *Table 1*). At higher current injections, female BS neurons (*n = 8*) fired more APs than males (*n = 6*; *Figure 24C*; *Table 8*). Two-way repeated measures ANOVA demonstrated a significant effect of current ($F_{(12, 144)} = 132.92$, $p < 0.001$), and a significant current by sex interaction ($F_{(12, 144)} = 3.37$, $p < 0.05$) but not a significant effect of sex ($F_{(1, 12)} = 1.81$, $p > 0.05$; *Figure 24C*). *Post hoc* analysis confirmed that at 300 pA, female BS neurons fired more APs compared to males ($p < 0.05$; *Figure 24C*). Together, sex differences in intrinsic excitability of layer 5 adult gRSC neurons occur in a cell type-specific manner.

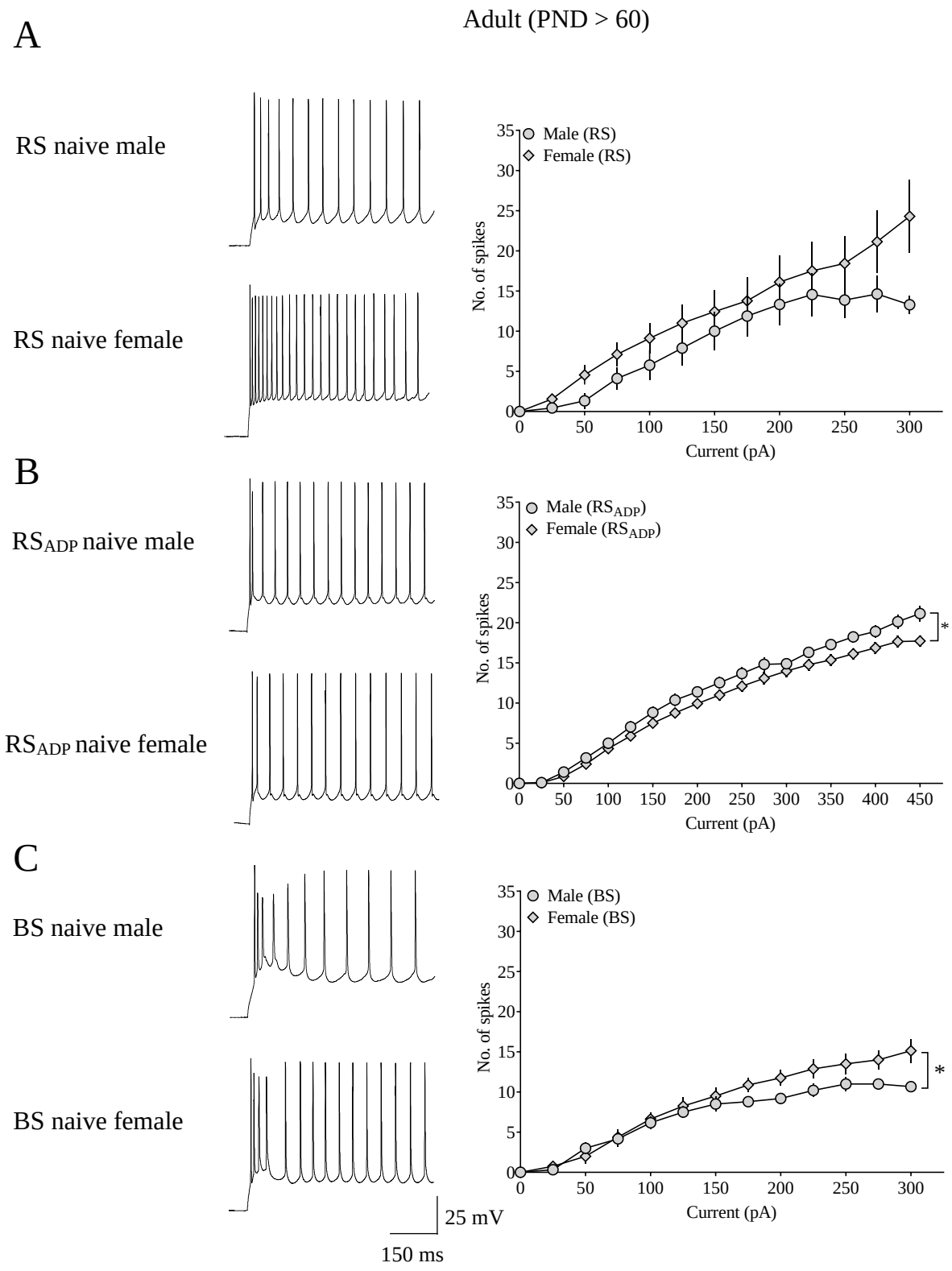

A
Adult (PND > 60)
RS naive male
RS naive female
Male (RS)
Female (RS)
No. of spikes
Current (pA)
B
RS$_{ADP}$ naive male
RS$_{ADP}$ naive female
Male (RS$_{ADP}$)
Female (RS$_{ADP}$)
No. of spikes
Current (pA)
*
C
BS naive male
BS naive female
Male (BS)
Female (BS)
No. of spikes
Current (pA)
*
25 mV
150 ms

Figure 24. Sex differences in intrinsic excitability of RS_{ADP} and BS neurons in the adult (PND > 60) gRSC. Representative traces at 300 pA injections. (A) RS neurons in the male (grey circle) and female (grey diamond) gRSC fire a similar number of APs in response to increasing somatic current injections. (B) At current injections higher than 250 pA, male RS_{ADP} neurons fire more APs compared to females. (C) At 300 pA, female BS neurons fire more APs compared to males. *Abbreviations*: granular retrosplenial cortex (gRSC); postnatal day (PND); regular spiking (RS), regular spiking afterdepolarization (RS_{ADP}); burst spiking (BS); action potential (AP). (*$p < 0.05$).

	RS		**RS_{ADP}**		**BS**	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**
	n = 9	*n = 10*	*n = 39*	*n = 35*	*n = 6*	*n = 8*
Membrane Properties						
RMP (mV)	-66.0 ± 1.3	-66.4 ± 0.5	-66.3 ± 0.4	-65.6 ± 0.3	-66.4 ± 0.8	-66.0 ± 1.0
R_N (MΩ)	212.3 ± 52.9*	284.3 ± 50.8#	141.6 ± 10.9	133.1 ± 9.9#	184.5 ± 14.9	135.3 ± 17.0
$AP_{threshold}$ (mV)	-38.5 ± 1.4	-39.7 ± 2.1	-39.1 ± 0.6	-37.8 ± 0.9	-38.3 ± 1.2	-39.4 ± 1.0
$AP_{amplitude}$(mV)	72.4 ± 4.1	67.2 ± 1.2	72.6 ± 1.8	71.2 ± 1.4	67.9 ± 6.9	77.3 ± 3.1
$AP_{half\text{-}width}$ (ms)	1.2 ± 0.1†	1.2 ± 0.1	1.0 ± 0.0†	1.1 ± 0.1	1.6 ± 0.2*	1.2 ± 0.1†
$ADP_{amplitude}$ (mV)	n/a	1.7 ± 0.2	n/a	1.4 ± 0.2	n/a	n/a
fAHP (mV)	-10.8 ± 1.6†	-9.1 ± 0.8	-10.2 ± 1.0†	-10.4 ± 0.8#	-2.8 ± 1.4	-5.2 ± 0.8
mAHP (mV)	-2.4 ± 0.6*	-2.6 ± 0.8	-3.9 ± 0.3	-3.9 ± 0.3	-1.1 ± 1.2#*	-3.3 ± 0.8
sAHP (mV)	-1.6 ± 0.4	-1.5 ± 0.4	-1.7 ± 0.2	-1.5 ± 0.2	-1.2 ± 0.4	-1.7 ± 0.3

Table 8. Sex differences in membrane properties of RS, RS_{ADP}, and BS neurons in the adult (PND > 60) gRSC. Significantly different from RS_{ADP} male (*, $p < 0.05$). Significantly different from BS male (†, $p < 0.05$). Significantly different from BS female (#, $p < 0.05$). *Abbreviations*: regular spiking (RS); regular spiking afterdepolarization (RS_{ADP}); burst firing (BS); postnatal day (PND); action potential (AP); afterdepolarization (ADP); fast afterhyperpolarization (fAHP); medium afterhyperpolarization (mAHP); slow afterhyperpolarization (sAHP); resting membrane potential (RMP); input resistance (R_N).

Adult male and female rats were conditioned with 6 tone-shock pairings separated by a trace interval. Two-way repeated measures ANOVA revealed a significant main effect of trial ($F_{(6, 156)} = 12.49$, $p < 0.001$), behavior ($F_{(1, 26)} = 15.81$, $p < 0.05$), trial by behavior interaction ($F_{(6, 156)} = 3.59$, $p < 0.05$), and trial by sex interaction ($F_{(6, 156)} = 2.16$, $p < 0.05$; *Figure 25A*). However, there was no significant trial by behavior by sex interaction ($F_{(6, 156)} = 0.03$, $p > 0.05$; *Figure 25A*). *Post hoc* analysis confirmed that female trace rats (*n = 7*) froze significantly more than CS only females (*n = 5*) during trial 4, whereas male trace rats (*n = 10*) froze more than CS only males (*n*

= *8*) during trials 2, 3, 4, and 5 ($p < 0.05$; *Figure 25A*). During retrieval, a two-way ANOVA showed a significant effect of behavior ($F_{(1, 26)} = 19.69$, $p < 0.001$), but not an effect of sex ($F_{(1, 26)} = 1.29$, $p > 0.05$) or behavior by sex interaction ($F_{(1, 26)} = 0.09$, $p > 0.05$; *Figure 23B*). *Post hoc* analysis confirmed that during a retrieval test, trace males and females froze more than their CS only counterparts ($p < 0.05$; *Figure 25B*), suggesting successful retrieval in adult trace rats.

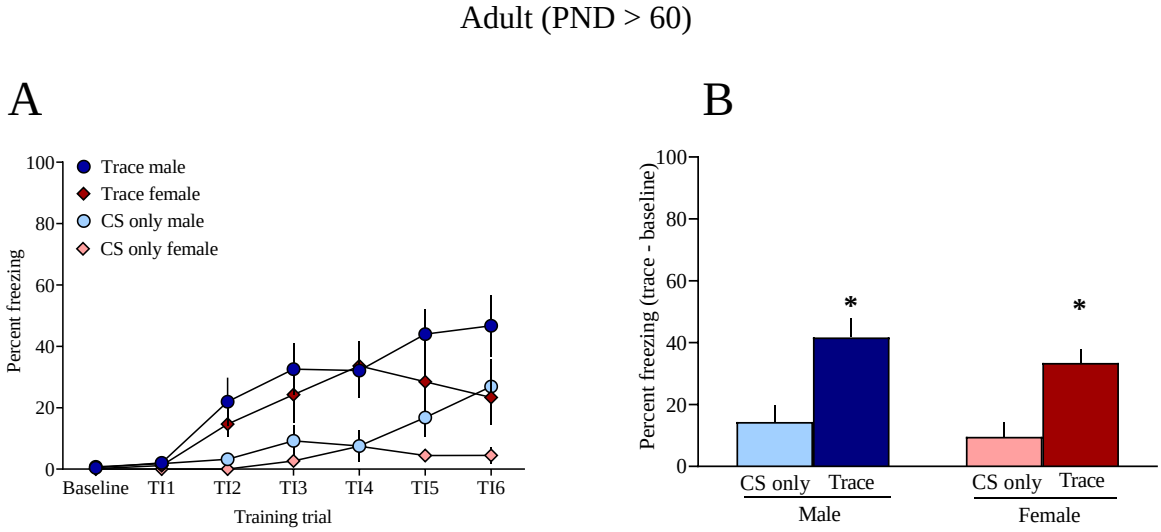


Figure 25. Acquisition and retrieval of trace fear in male and female adult (PND > 60) rats. (A) Male and female trace rats receive 6 tone-shock pairings that are separated by a 30 s interval. CS only rats receive 6 tone presentations and no footshocks. (B) Twenty-four hours later, trace and CS rats are brought to a novel environment and exposed to 2 tone presentations. Trace males freeze more than CS only males and trace females freeze more than CS only females. *Abbreviations*: granular retrosplenial cortex (gRSC); postnatal day (PND); trace interval (TI); conditioned stimulus (CS). (*$p < 0.05$).

Immediately after trace fear retrieval (*Figure 25B*), RSC slices were prepared for whole-cell patch-clamp recordings. We observed no differences in intrinsic excitability of RS neurons in naive (*n = 9*), trace (*n = 8*), and CS only (*n = 6*) adult males (*Figure 26A; Table 9*). Similarly, in females, intrinsic excitability was similar in naive (*n = 10*), trace (*n = 2*), and CS only (*n = 5*) groups (*Figure 26B; Table 9*). Three-way repeated measures ANOVA showed a significant effect of current ($F_{(12, 384)} = 72.28$, $p < 0.001$), suggesting that RS neurons fire more APs in response to increasing current injections to the soma (*Figure 26A; Figure 26B*). There was no significant current by sex interaction ($F_{(12, 384)} = 0.98$, $p > 0.05$), current by behavior interaction ($F_{(24, 384)} =$

0.20, $p > 0.05$) or current by sex by behavior interaction ($F_{(24, 384)} = 0.66$, $p > 0.05$; *Figure 26A; Figure 26B*). Therefore, trace fear does not influence RS neuronal excitability in layer 5 of the male or female adult rat gRSC.

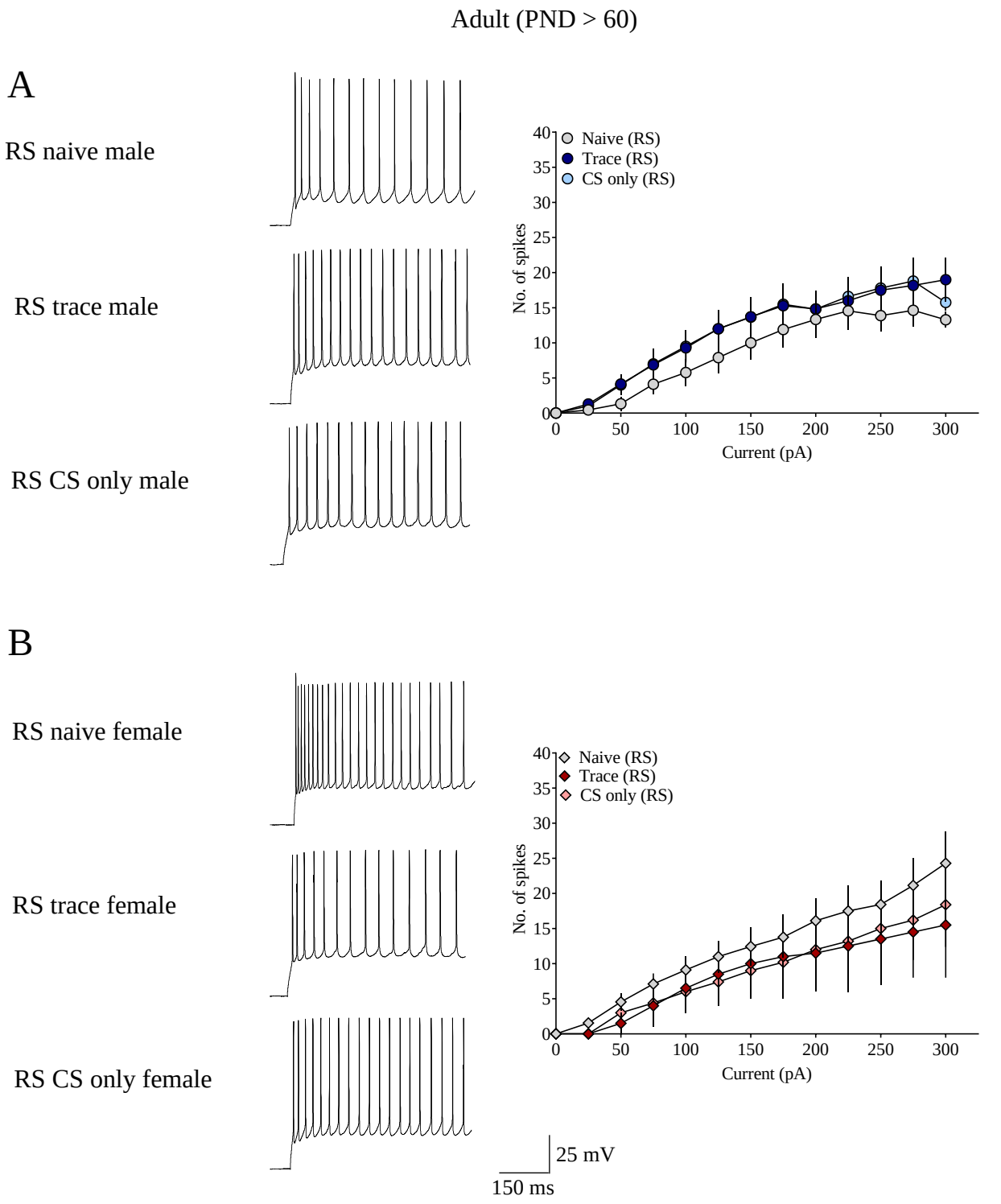


Figure 26. Retrieval of trace fear does not influence excitability of RS neurons in the adult (PND > 60) gRSC. Representative traces at 300 pA injections. (A) In males, intrinsic excitability of RS neurons in trace rats (dark blue circle) does not differ from naive (grey circle) or CS only (light blue circle) rats. (B) In females, excitability of RS neurons in trace (red diamond), naive (grey diamond), and CS only (pink diamond) does not differ across all three behavioral groups. *Abbreviations*: granular retrosplenial cortex (gRSC); postnatal day (PND); trace interval (TI); regular spiking (RS); conditioned stimulus (CS). (*$p < 0.05$).

	Male			Female		
	Naive	**Trace**	**CS only**	**Naive**	**Trace**	**CS only**
	n = 9	*n = 8*	*n = 6*	*n = 10*	*n = 2*	*n = 5*
Membrane Properties						
RMP (mV)	-66.0 ± 1.3	-67.0 ± 0.6	-67.1 ± 0.8	-66.4 ± 0.5	-65.5 ± 3.0	-65.8 ± 1.6
R_N (MΩ)	212.3 ± 52.9	285.3 ± 60.5	259.7 ± 62.7	284.3 ± 50.8	164.0 ± 21.0	185.6 ± 39.7
$AP_{threshold}$ (mV)	-38.5 ± 1.4	-39.3 ± 1.7	-39.7 ± 1.6	-39.7 ± 2.1	-42.0 ± 3.0	-39.0 ± 2.6
$AP_{amplitude}$ (mV)	72.4 ± 4.1	69.7 ± 1.6	70.3 ± 3.0	67.2 ± 1.2	66.3 ± 1.2	69.2 ± 3.6
$AP_{half\text{-}width}$ (ms)	1.2 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.3 ± 0.2	1.2 ± 0.1
fAHP (mV)	-10.8 ± 1.6	-10.4 ± 1.3	-11.7 ± 1.2	-9.1 ± 0.8*	-6.0 ± 1.6	-9.7 ± 2.9*
mAHP (mV)	-2.4 ± 0.6	-3.5 ± 0.5	-3.1 ± 0.5	-2.7 ± 0.8	-3.7 ± 1.0	-2.9 ± 0.5
sAHP (mV)	-1.6 ± 0.4	-1.7 ± 0.2	-1.8 ± 0.3	-1.5 ± 0.4	-1.8 ± 0.9	-1.4 ± 0.3

Table 9. Effect of trace fear retrieval and CS exposure in membrane properties of RS neurons in the adult (PND > 60) gRSC. Significantly different trace female (*, $p < 0.05$). *Abbreviations*: regular spiking (RS); postnatal day (PND); action potential (AP); afterdepolarization (ADP); fast afterhyperpolarization (fAHP); medium afterhyperpolarization (mAHP); slow afterhyperpolarization (sAHP); resting membrane potential (RMP); input resistance (R_N); granular retrosplenial cortex (gRSC); conditioned stimulus (CS).

Following acquisition (*Figure 25A*) and retrieval (*Figure 25B*) of trace fear learning, RSC slices from adult (PND > 60) rats were prepared. Exposure to the CS or trace fear learning reduced excitability of RS_{ADP} neurons in layer 5 of the adult gRSC (*Figure 27A*; *Table 10*). In response to current injections, RS_{ADP} neurons from trace (*n = 26*) and CS only (*n = 12*) male rats fired significantly fewer APs compared to naive (*n = 33*) rats (*Figure 27A*; *Table 10*). In females, at higher current injections, intrinsic excitability of RS_{ADP} neurons from trace (*n = 30*), and CS only (*n = 16*) rats was also reduced compared to naive (*n = 30*; *Figure 27B*; *Table 10*) rats. Three-way repeated measures ANOVA revealed a significant main effect of current ($F_{(18, 2502)} = 887.81$, $p < 0.001$), behavior ($F_{(2, 139)} = 5.23$, $p < 0.05$), current by sex interaction ($F_{(18, 2502)} = 3.55$, $p < 0.05$), current by behavior interaction ($F_{(36, 2502)} = 3.43$, $p < 0.05$) but not a significant current by sex by behavior interaction ($F_{(36, 2502)} = 1.09$, $p > 0.05$; *Figure 27A*; *Figure 27B*). *Post hoc* analysis

revealed that male RS_{ADP} neurons from trace and CS only rats had significantly reduced excitability compared to naive rats between 50-450 pA ($p < 0.05$; *Figure 27A*). Female RS_{ADP} neurons from trace rats had reduced excitability compared to CS only and naive rats at higher current injections of 375-450 pA ($p < 0.05$; *Figure 27B*). Overall, retrieval of trace memories or novel environment alters excitability of male RS_{ADP} neurons at lower current injections, whereas higher current injections are required to reduce excitability in female RS_{ADP} neurons from trace rats.

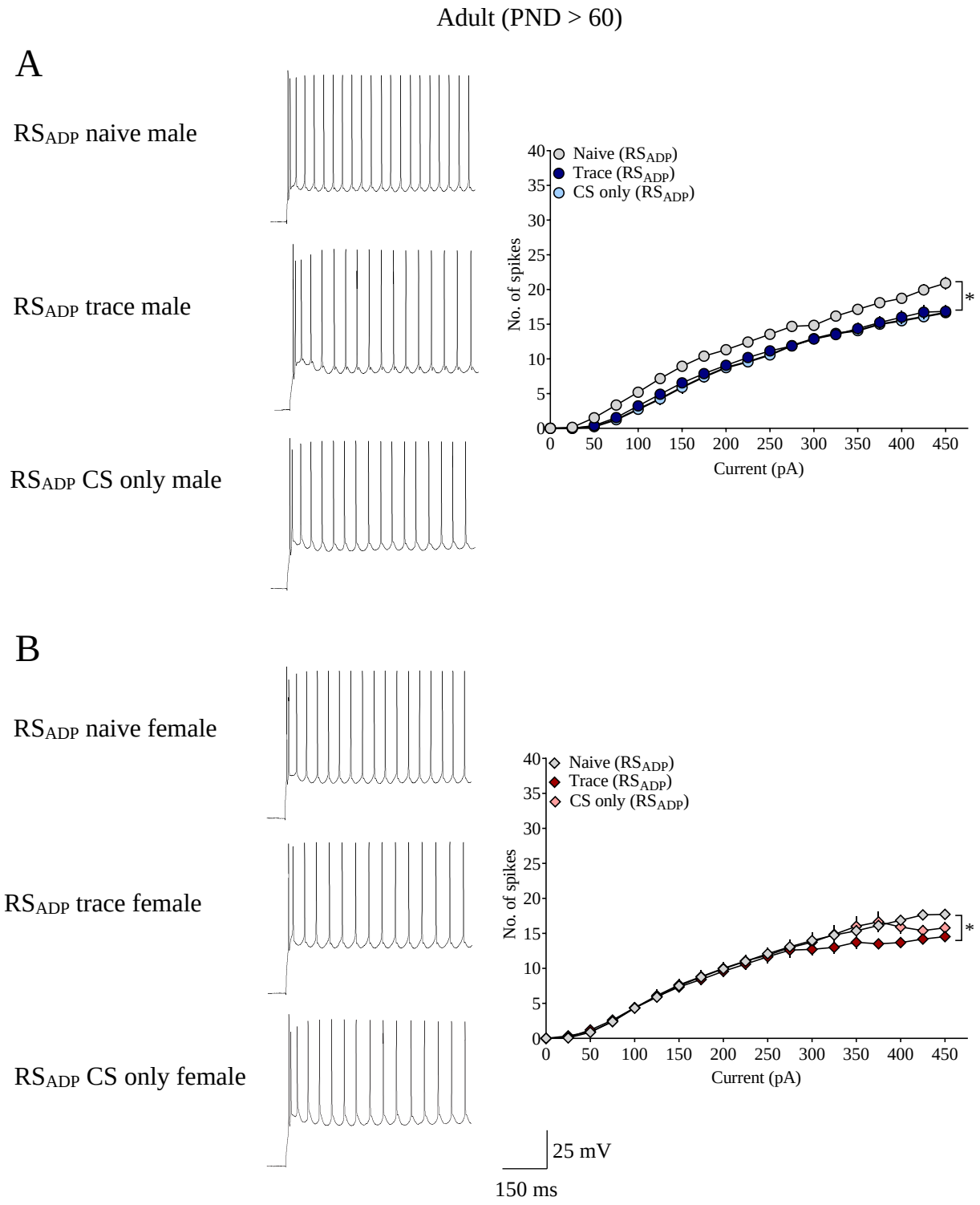


Figure 27. Behavioral training reduces excitability of male and female RS_{ADP} neurons in the adult (PND > 60) gRSC. Representative traces at 450 pA injections. (A) In males, retrieval of trace fear memories (dark blue) or exposure to the CS (light blue) reduces intrinsic excitability of RS_{ADP} neurons compared to those from the naive group (grey circle). (B) Excitability of female RS_{ADP} neurons from trace rats (red diamond) is reduced compared to RS_{ADP} neurons from naive (grey diamond), or CS only (pink diamond) rats. *Abbreviations*: granular retrosplenial cortex (gRSC); postnatal day (PND); regular spiking afterdepolarization (RS_{ADP}); conditioned stimulus (CS). (*$p < 0.05$).

By measuring basic membrane properties in the various behavioral groups, we identified underlying mechanisms that contribute to experience-related changes that occur in RS_{ADP} neurons in the male and female adult gRSC. Trace fear learning significantly enhances AP amplitude in both male and female RS_{ADP} neurons (*Figure 28*; *Table 10*). Two-way ANOVA revealed a significant effect of behavior ($F_{(2, 153)} = 5.00$, $p < 0.05$; *Figure 28*; *Table 10*). *Post hoc* analysis confirmed that AP amplitude of trace males and females were significantly higher compared to naive males and females respectively ($p < 0.05$; *Figure 28*).

	Male			**Female**		
	Naive	**Trace**	**CS only**	**Naive**	**Trace**	**CS only**
	n = 39	*n = 26*	*n = 12*	*n = 35*	*n = 30*	*n = 16*
Membrane Properties						
RMP (mV)	-66.3 ± 0.4	-66.0 ± 0.4	-66.2 ± 0.5	-65.6 ± 0.3	-66.3 ± 0.4	-65.6 ± 0.7
R_N (MΩ)	141.6 ± 10.9	111.7 ± 7.4	99.5 ± 11.8	133.1 ± 9.9	126.5 ± 13.1	122.9 ± 20.9
$AP_{threshold}$ (mV)	-39.1 ± 0.6	-37.2 ± 0.7	-38.2 ± 1.2	-37.8 ± 0.8	-38.3 ± 0.6	-38.8 ± 0.8
$AP_{amplitude}$ (mV)	72.6 ± 1.8	77.3 ± 1.6*	71.2 ± 2.9	71.2 ± 1.4	76.3 ± 1.5#	74.6 ± 1.8
$AP_{half\text{-}width}$ (ms)	1.0 ± 0.0	1.1 ± 0.03	1.1 ± 0.0	1.1 ± 0.1	1.1 ± 0.0	1.0 ± 0.0
$ADP_{amplitude}$ (mV)	1.7 ± 0.2	2.1 ± 0.3	2.3 ± 0.2	1.4 ± 0.2	1.5 ± 0.3	1.6 ± 0.3
fAHP (mV)	-10.2 ± 0.9	-10.9 ± 0.6	-11.1 ± 0.7	-10.4 ± 0.6	-9.9 ± 0.6	-10.0 ± 0.7
mAHP (mV)	-3.9 ± 0.3	-4.4 ± 0.3	-4.6 ± 0.4	-3.9 ± 0.3	-4.4 ± 0.3	-4.0 ± 0.3
sAHP (mV)	-1.7 ± 0.1	-2.0 ± 0.3	-1.5 ± 0.1	-1.5 ± 0.2	-1.8 ± 0.1	-1.5 ± 1.0

Table 10. Effect of trace fear retrieval and CS exposure in membrane properties of RS_{ADP} neurons in the adult (PND > 60) gRSC. Significantly different from naive male (*, $p < 0.05$). Significantly different from naive female (#, $p < 0.05$). *Abbreviations*: regular spiking afterdepolarization (RS_{ADP}); postnatal day (PND); action potential (AP); afterdepolarization (ADP); fast afterhyperpolarization (fAHP); medium afterhyperpolarization (mAHP); slow afterhyperpolarization (sAHP); resting membrane potential (RMP); input resistance (R_N); granular retrosplenial cortex (gRSC); conditioned stimulus (CS).

Adult (PND > 60)

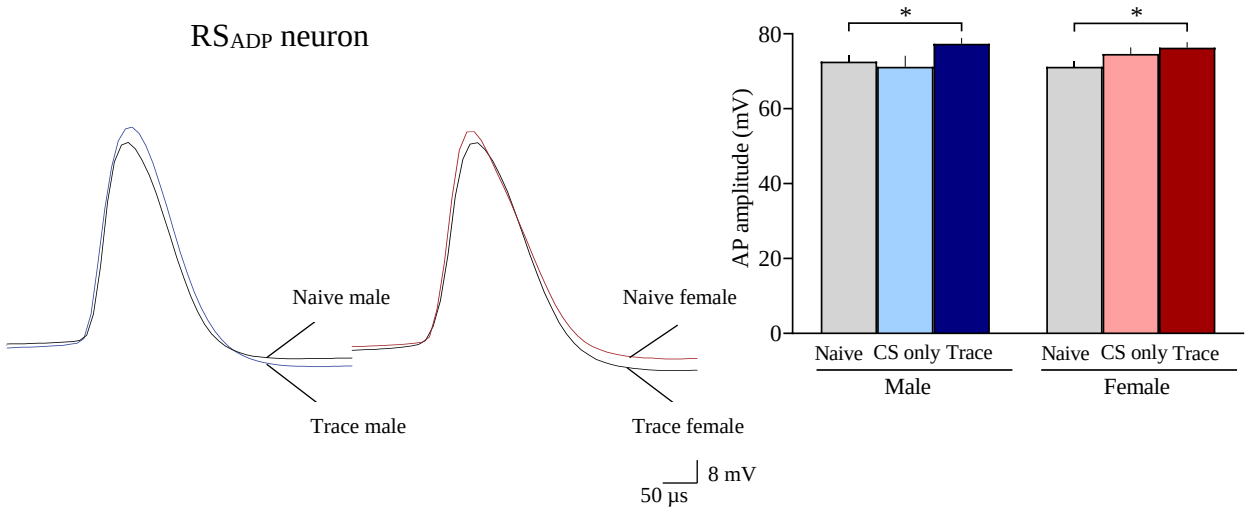


Figure 28. Retrieval of trace fear enhances AP amplitude in RS_{ADP} neurons of the adult (PND > 60) male and female gRSC. (*Left*) Representative traces show AP amplitude of male and female RS_{ADP} neurons. (*Right*) Bar graphs represent AP amplitude, which is increased in male RS_{ADP} neurons of trace (dark blue) compared to naive (grey) or CS only (light blue) groups. AP amplitude of female RS_{ADP} neurons is increased in trace (red) compared to naive (grey) or CS only (pink) groups. *Abbreviations*: regular spiking afterdepolarization (RS_{ADP}); postnatal day (PND); action potential (AP); granular retrosplenial cortex (gRSC); conditioned stimulus (CS); action potential (AP). (*$p < 0.05$).

We investigated whether retrieval of trace fear memories (*Figure 25A*; *Figure 25B*) influences adult BS neurons in layer 5 of the adult gRSC. BS neurons from male naive (*n = 6*), trace (*n = 4)*, and CS only (*n = 4*) had similar number of APs in response to increasing current injections (*Figure 29A*; *Table 11*). Likewise, female BS neurons from naive (*n = 8*), trace (*n = 9*), and CS only (*n = 5*) groups fired a similar number of APs (*Figure 29B*). Three-way repeated measures ANOVA showed a significant effect of current ($F_{(10, 300)} = 246.00$, $p < 0.05$) but not a significant current by behavior interaction ($F_{(20, 300)} = 0.37$, $p > 0.05$) or current by sex by behavior interaction ($F_{(20, 300)} = 1.80$, $p > 0.05$; *Figure 29A*; *Figure 29B*). Thus, adult BS neurons are unaffected by trace fear memories.

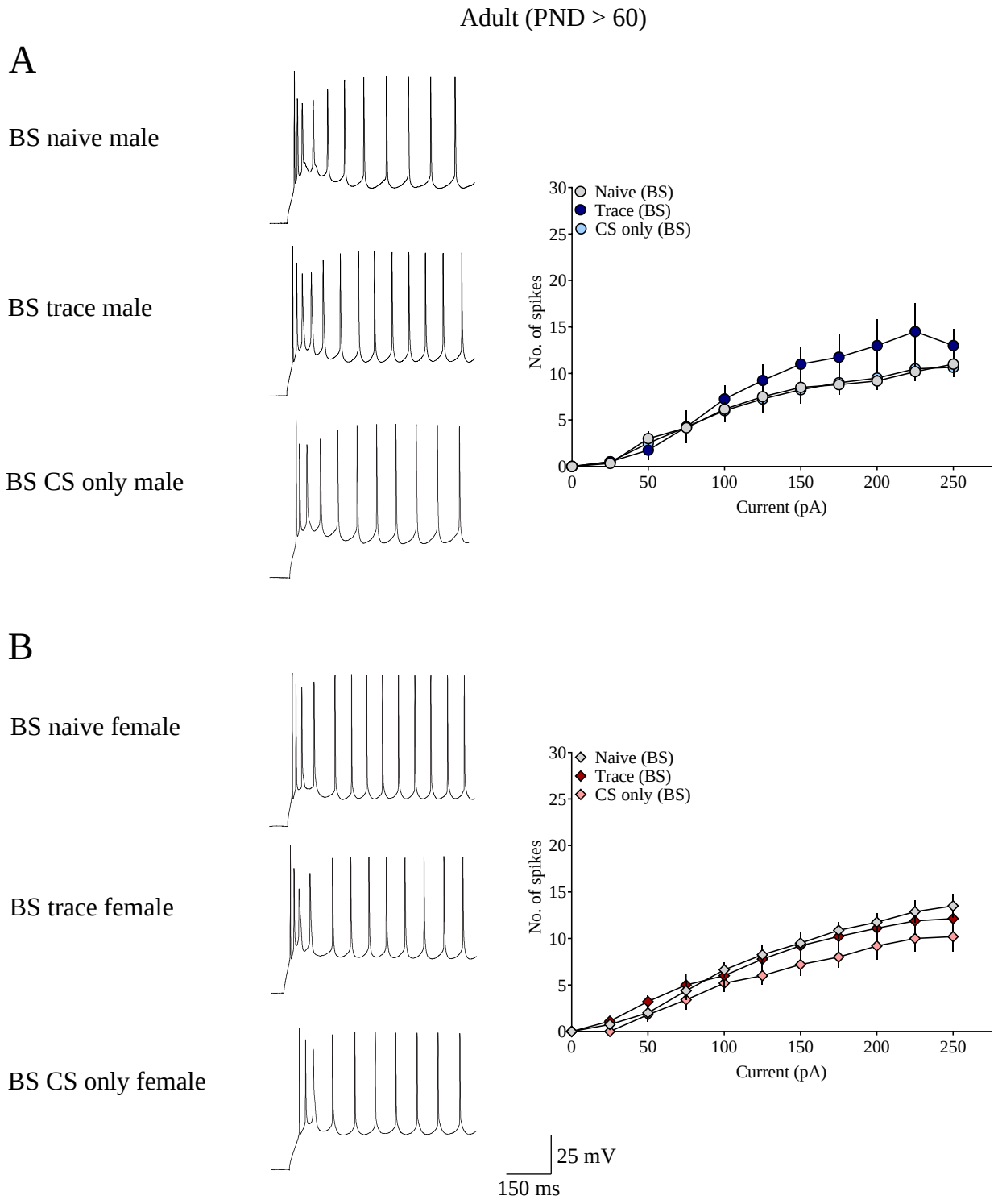


Figure 29. Retrieval of trace fear does not alter excitability of BS neurons in the adult (PND > 60) gRSC. Representative traces at 250 pA injections. (A) Excitability of male BS neurons in trace (dark blue circle), CS only (light blue circle), and naive (grey circle) rats are similar in all three groups. (B) Excitability of female BS neurons in trace (red diamond), naive (grey diamond), and CS only (pink diamond) are similar in all three groups. *Abbreviations*: granular retrosplenial cortex (gRSC); postnatal day (PND); burst spiking (BS); conditioned stimulus (CS). (*$p < 0.05$).

	Male			Female		
	Naive	**Trace**	**CS only**	**Naive**	**Trace**	**CS only**
	n = 6	*n = 4*	*n = 4*	*n = 8*	*n = 9*	*n = 5*
Membrane Properties						
RMP (mV)	-66.4 ± 0.8	-68.0 ± 0.7	-64.8 ± 0.8	-66.0 ± 1.0	-65.3 ± 0.6	-65.4 ± 0.7
R_N (MΩ)	184.5 ± 14.8	156.3 ± 23.1	167.5 ± 24.2	135.3 ± 17.0	1 65.1 ± 24.9	148.6 ± 22.7
$AP_{threshold}$ (mV)	-38.3 ± 1.3	-39.2 ± 1.7	-37.1 ± 1.4	-39.4 ± 1.0	-36.2 ± 1.4	-37.0 ± 0.7
$AP_{amplitude}$ (mV)	67.9 ± 6.9	74.3 ± 4.1	74.0 ± 5.0	77.3 ± 3.1	75.9 ± 3.5	67.2 ± 2.0
$AP_{half\text{-}width}$ (ms)	1.6 ± 0.2	1.3 ± 0.1	1.5 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.4 ± 0.1
fAHP (mV)	-2.8 ± 1.4	-5.6 ± 0.8	-5.7 ± 0.9	-5.3 ± 0.8	-4.8 ± 0.9	-4.9 ± 0.9
mAHP (mV)	-1.1 ± 1.2	-2.4 ± 0.7	-1.0 ± 0.7	-3.3 ± 0.8	-2.2 ± 0.9	-2.8 ± 0.5
sAHP (mV)	-1.2 ± 0.4	-1.6 ± 0.1	-1.3 ± 0.3	-1.7 ± 0.3	-1.6 ± 0.1	-1.7 ± 0.2

Table 11. Effect of trace fear retrieval and CS exposure in membrane properties of BS neurons in the adult (PND > 60) gRSC. Significantly different trace female (*, $p < 0.05$). *Abbreviations*: burst spiking (BS); postnatal day (PND); action potential (AP); afterdepolarization (ADP); fast afterhyperpolarization (fAHP); medium afterhyperpolarization (mAHP); slow afterhyperpolarization (sAHP); resting membrane potential (RMP); input resistance (R_N); granular retrosplenial cortex (gRSC); conditioned stimulus (CS).

Summary of sex-and age-differences on experience-dependent intrinsic excitability of RS and RS_{ADP} neurons of the gRSC

To capture a snapshot on how sex and age play a role in experience-dependent changes of different neuronal cell types, we measured number of APs at 300 pA. We chose this current injection as among most of the behavioral groups, firing plateaus at this point and neurons continue to fire at a more constant rate. We observed sex differences in RS neurons emerge in adulthood (*Figure 30A*). Two-way ANOVA revealed a significant effect of sex ($F_{(1, 86)} = 6.65$, $p < 0.05$), age ($F_{(2, 86)} = 9.20$, $p < 0.001$), and sex by age interaction ($F_{(2, 86)} = 4.96$, $p < 0.05$; *Figure 30A*). *Post hoc* analysis confirmed that the number of spikes in female RS neurons increases progressively from early adolescence to adulthood ($p < 0.05$; *Figure 30A*). Further, sex differences in RS neurons

emerge in adulthood where female RS neurons have an increased number of APs compared to male RS neurons ($p < 0.05$; *Figure 30A*).

Next, we summarized how learning influences excitability of RS neurons as a function of sex and age (*Figure 30B*; *Figure 30C*). Three-way ANOVA demonstrated a significant effect of age ($F_{(2, 230)} = 12.41$, $p < 0.001$), but not a significant effect of sex ($F_{(1, 230)} = 1.44$, $p > 0.05$), or behavior ($F_{(2, 230)} = 0.25$, $p > 0.05$) or sex by behavior by age interaction ($F_{(4, 230)} = 2.00$, $p > 0.05$). *Post hoc* analysis confirmed that learning or novel environments do not change male RS neuronal excitability at any age ($p > 0.05$; *Figure 30B*). However, in adult females, naive RS neurons have significantly enhanced excitability compared to those from trace or CS only groups ($p < 0.05$; *Figure 30C*). Thus, sex differences in RS neurons emerge in adulthood and learning or novel stimuli only reduces excitability in adult female RS neurons of the gRSC.

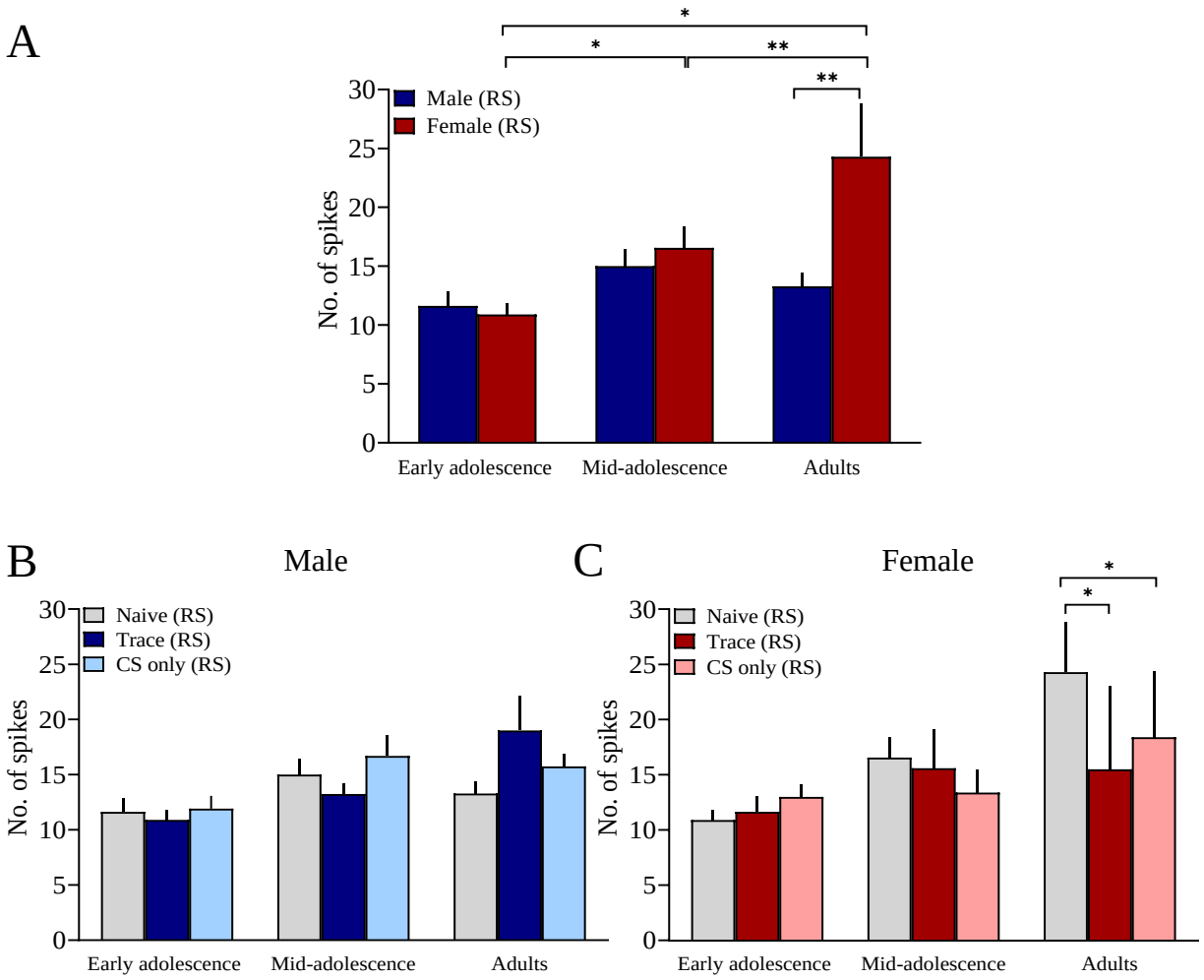


Figure 30. Summary of male and female RS neuronal excitability following retrieval in three different age groups. Bar graphs representing excitability of RS neurons in the gRSC at 300 pA current injections. (A) Sex differences in excitability of naive RS neurons emerge in adulthood. Adult female RS neurons have increased excitability compared to adult males. Excitability of female RS neurons increases from early adolescence to adulthood. (B) In males, trace fear memories do not alter excitability of RS neurons at any age. (C) In females, retrieval of trace fear memories or CS presentations alone reduces excitability of RS neurons only in adulthood. *Abbreviations*: granular retrosplenial cortex (gRSC); regular spiking (RS); conditioned stimulus (CS). (*$p < 0.05$; **$p < 0.01$).

At 450 pA current injection, sex differences favoring males in excitability of RS_{ADP} neurons are present during early adolescence and are continued into adulthood (*Figure 31A*). We chose this current injection as it highlights sex differences and experience-dependent changes at higher current injections. Two-way ANOVA revealed a significant effect of sex ($F_{(1, 112)} = 13.98$, $p < 0.001$), age ($F_{(2, 112)} = 4.45$, $p < 0.05$), but not a significant sex by age interaction ($F_{(2, 112)} = 0.34$, $p > 0.05$; *Figure 31A*). *Post hoc* analysis showed that the number of APs in early adolescent male RS_{ADP} neurons was higher ($p = 0.06$) compared to females, and significantly higher in male

mid-adolescents and adults than females ($p < 0.05$; *Figure 31A*). Female adult RS_{ADP} neurons fired more APs compared to female early adolescent ($p = 0.07$) and mid-adolescent neurons ($p < 0.05$; *Figure 31A*).

Sex differences in learning-induced changes in RS_{ADP} neurons were observed as a three-way ANOVA revealed a significant effect of sex ($F_{(1, 284)} = 5.39$, $p < 0.05$), age ($F_{(2, 284)} = 5.35$, $p < 0.01$), behavior ($F_{(2, 284)} = 5.35$, $p < 0.001$), sex by behavior ($F_{(2, 284)} = 6.28$, $p < 0.01$), but not a significant effect sex by age by behavior interaction ($F_{(4, 284)} = 1.25$, $p > 0.05$; *Figure 31B*; *Figure 31C*). *Post hoc* analysis confirmed that trace learning decreased excitability of RS_{ADP} neurons in early- and mid-adolescent, and adult males ($p < 0.05$), and exposure to CS reduced exitability in mid-adolescent and adult males ($p < 0.05$; *Figure 31B*). In females, trace fear only reduced excitability of RS_{ADP} neurons in adults ($p < 0.05$; *Figure 31C*). Together, these data summarize that sex differences favoring females in RS neurons emerge in adulthood and learning only influences excitability of this firing type in female adults. Sex differences in RS_{ADP} neurons exist in all three ages and learning alters excitability of these neurons in males of all age groups but only in adult females.

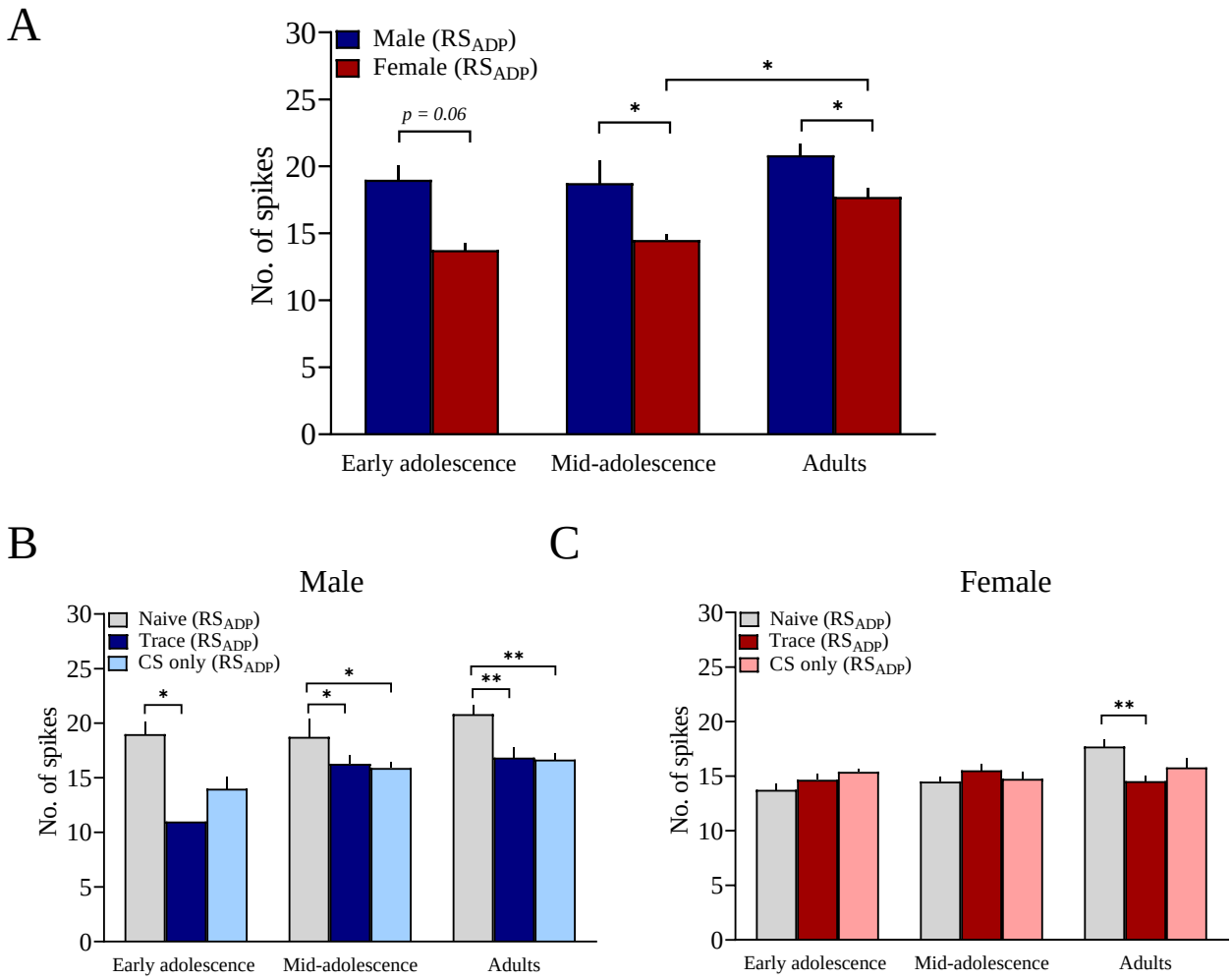


Figure 31. Summary of male and female RS$_{ADP}$ neuronal excitability following retrieval in three different age groups. Bar graphs representing excitability of RS$_{ADP}$ neurons in the gRSC at 450 pA current injections. (A) Excitability of naive male RS$_{ADP}$ neurons is increased compared to females during all 3 ages. Excitability of adult female RS$_{ADP}$ neurons is enhanced compared to neurons from early- and mid- adolescent females (B) Retrieval of trace memories or CS presentations reduces excitability of male RS$_{ADP}$ neurons in all three ages. (C) Trace fear reduces excitability of female RS$_{ADP}$ neurons only during adulthood. *Abbreviations*: granular retrosplenial cortex (gRSC); regular spiking afterdepolarization (RS$_{ADP}$); conditioned stimulus (CS). (*$p < 0.05$; **$p < 0.01$).

Discussion

We first examined that retrieval of trace fear memories changes as a consequence of sex and age. Sex differences in trace fear retrieval were only observed during early adolescence and not during mid-adolescence or adulthood. Across ages, males retrieve trace memories in a nonlinear manner, where mid-adolescent males freeze less than early adolescent or adult male rats. In females, freezing progressively increases across ages, suggesting sex differences in developmental age-related changes in fear behavior. Next, we investigated cellular mechanisms underlying age- and sex-related alterations in the RSC. Intrinsic excitability of male RS_{ADP} neurons is increased compared to females and this sex difference appears in early adolescence and continues until adulthood. Sex differences in excitability of RS neurons emerge in adulthood where female neurons have higher excitability compared to males. In males, trace fear memory formation and CS exposure suppresses RS_{ADP} neuronal excitability at all ages, however, retrieval of trace fear and CS exposure reduces excitability of both RS and RS_{ADP} neurons only in adult females. We found no experience-related alterations in adult BS neurons. Together, our data are the first to reveal that sex differences in the gRSC can occur during early adolescence or adulthood depending on the cell type (RS versus RS_{ADP}). Further, trace memory formation and CS exposure reduces excitability in both RS and RS_{ADP} neurons in a sex- and age-specific way.

Age- and sex-dependent changes in retrieval of trace fear memories

No differences in acquisition of trace fear learning between the 6 groups (male and female early adolescent, mid-adolescent, and adults) were found. However, during retrieval of trace fear, female rats had a positive linear trend in freezing behaviors from early adolescence into adulthood, whereas male mid-adolescent rats exhibit lower freezing levels compared to male early adolescents and adults. This nonlinear developmental trajectory in male rodents has been noted previously, in

which one study directly compared delay and trace fear conditioning in three different ages of male rats: early adolescence (PND 23), mid-adolescence (PND 35), and adults (PND 90; Den and Richardson, 2013). During the retrieval test, delay fear conditioned rats showed similar freezing levels across all three ages, however, trace fear conditioned mid-adolescent rats (PND 35) showed the strongest retrieval of trace fear compared to early adolescent and adult rats (Den and Richardson, 2013). The pattern of freezing behavior across three ages in this study (Den and Richardson, 2013) is opposite to what we observe in our current study where we see depressed freezing in male mid-adolescent rats. One reason for the discrepancy in behavioral patterns is that the study by Den and Richardson, 2013 measure retrieval of freezing during the CS tone and we measure during the 30 s 'trace' interval following the CS. Thus, distinct behaviors can be expressed during the auditory cue or the subsequent interval following the CS.

In another study, during a test of context retrieval, male mice between PND 23-27 exhibit high levels of freezing, which drop during mid-adolescence (PND 29-39; Pattwell et al., 2011). Freezing behaviors return after PND 39 and remain elevated into adulthood (Pattwell et al., 2011). Learning impairment during mid-adolescence is not due to lack of context fear acquisition but an inability to retrieve fear memories (Pattwell et al., 2011). Our results parallel to those of Pattwell et al., 2011 as trace fear conditioning promotes more contextual processing compared to delay conditioning (Marlin, 1981). The deficit in learning during PND 29-39 is associated with reduced synaptic potentiation in the amygdala, and decreased signaling of phosphoinositide 3-kinases (PI3Ks) and mitogen-activated protein kinase (MAPK) in the hippocampus (Pattwell et al., 2011). Interestingly, in the RS_{ADP} neurons that dominate the mid-adolescent gRSC have substantially reduced dendritic complexity compared to RS neurons predominantly found in the early adolescent gRSC (*Figure 9*). In females, there is no difference in dendritic complexity between RS and RS_{ADP}

neurons (*Figure 11*). One possible reason for the suppressed freezing during mid-adolescence may be that developmental processes such as synaptic pruning, and reorganization of the fear-related circuitry may disrupt successful retrieval of trace or context fear memories.

In addition to age-dependent changes in retrieval of fear behavior, we observe sex differences favoring males during early adolescence (prior to puberty). Generally, puberty occurs approximately at PND 32 in female and PND 42 in male rats (Koss et al., 2015; Juraska and Willing, 2017; McCormick et al., 2017). Our results show that following puberty, sex differences are absent in mid-adolescent and adult rats. Sex differences in tests of learning and memory may occur before or after puberty due to the organizational and/or activational effects of sex steroid hormones (Dalla and Shors, 2009; Juraska and Willing, 2017). For example, sex differences in RSC-dependent spatial and contextual fear memories occur before puberty (Newhouse et al., 2007; Colon et al., 2018; Arakawa, 2019), suggesting that the organizational effects of hormones may occur earlier in development. Further, early-life stress (before puberty) influences expression of fear in female adolescent (after puberty) but not in male adolescent rats (Toledo-Rodriguez and Sandi, 2007), suggesting that external factors may play a role in shaping sex-specific fear behaviors later in development. Consistent with behavioral patterns, sex differences in neuronal and morphological properties of prefrontal cortex and BLA emerge before puberty (Koss et al., 2014; Rubinow and Juraska, 2015; Willing and Juraska, 2015). Specifically, sex differences in the RS-dominant early adolescent gRSC are prevalent where male RS neurons have significantly more dendritic branching compared to females (*Figure 9*; *Figure 11*), and sex differences in dendritic branching are absent in the RS_{ADP}-dominant mid-adolescent and adult gRSC (*Figure 9*; *Figure 11*). These data might suggest that the early adolescent fear circuit may be processing fear memories differently than the mid-adolescent and adult.

Role of age and sex in experience-related plasticity of the gRSC

In the gRSC, naive male RS_{ADP} neurons fire a higher number of APs compared to females, and this sex difference was present from early adolescence until adulthood. Conversely, female RS neurons exhibit higher excitability compared to males but this sex differences does not emerge until adulthood. Distinct cell types (RS versus RS_{ADP}) may form different fear circuits (Gao and Zheng, 2004; Le Be et al., 2007) to promote flexible processes required for memory formation. Further, the reversed pattern of sex differences in RS and RS_{ADP} neurons may suggest that the male and female RSCs may be projecting to different fear-related brain structures, and development of fear circuits may occur at different stages of development. For example, stimulation of the developing BLA induces LTP in the mPFC of rats (Caballero et al., 2014), and this BLA-mPFC connectivity is enabled by approximately PND 30, which remains steady into adulthood (Caballero et al., 2014). However, other projections such as ventral hippocampus to mPFC emerge later in development (PND 55; Caballero et al., 2014). Thus, developmental changes in plasticity and connectivity can occur at different time points, and may be sex- and region-specific.

Within the fear circuit, fear learning increases excitability of several brain regions including the amygdala (Sehgal et al., 2014), hippocampus (Kaczorowski and Disterhoft, 2009; McKay et al., 2009; Song et al., 2012; Dunn et al., 2018), and mPFC (Santini et al., 2008; Santini and Porter, 2010; Sepulveda-Orengo et al., 2013; Soler-Cedeno et al., 2016). We expected to see a similar enhancement in excitability in the gRSC following retrieval of trace fear, however, we observed a experience-related decrease in this brain region. Decreased excitability as a result of associative learning has also been noted in other limbic structures including the BLA (Motanis et al., 2014) and mPFC (Song et al., 2015; Whitaker et al., 2017). A experience-related suppression in intrinsic excitability may be due to a homeostatic mechanism, which counterbalances increased

excitatory synaptic inputs (Hayton et al., 2010, 2011) onto gRSC neurons. Decreased excitability may act as a negative feedback mechanism to regulate neurons within their physiological firing rate (Zhang and Linden, 2003; Turrigiano, 2008). This regulatory feedback mechanism is prevalent in the mPFC where learning elevates AMPA/NMDA receptor ratios and reduces intrinsic excitability (Hayton et al., 2010, 2011). Whether gRSC neurons are counterbalancing enhanced glutamatergic input from the hippocampus or other fear-related brain regions remains to be investigated.

Retrieval of trace fear memories reduces excitiability of gRSC neurons, however, exposure to the CS tone during training and testing also suppresses excitability of RS and RS_{ADP} neurons. Similarly, exposure to a novel context (without an aversive stimuli) increases excitability of RS neurons in the subiculum (Dunn et al., 2018). Novel context exposure induces a larger percentage of RS cells and decreases the percentage of BS cells in the subiculum (Dunn et al., 2018). Given that the RSC has unique features to process spatial information (Chen et al., 1994; Cho and Sharp, 2001; Smith et al., 2012), we suspect that novel environments induce plastic changes within this region.

In the male adult gRSC, RS_{ADP} neurons from trace or CS only rats express experience-related decreases in excitability at lower current injections (~100 pA). In contrast, compared to males, female RS and RS_{ADP} neurons from adult behaviorally trained rats respond only to higher current injections (~350 pA), suggesting that male and female neurons require different current intensities to drive learning-induced plasticity. Similar to studies of intrinsic excitability, LTP induction protocols such as theta-burst stimulations (4 pulses at 100 Hz with brief pauses) or high-frequency stimulations (~1 s burst of equally spaced pulses at 100 Hz) play a role in driving sex differences in rodent hippocampal slices (Yang et al., 2004; Qi et al., 2016). In general, the early

phase of LTP, which is dependent on pre-existing protein modification can be induced by high frequency stimulation (Huang and Kandel, 2005). Theta burst stimulations drive the late phase of LTP, and this phase requires gene transcription and new protein synthesis in the postsynaptic cell (Huang and Kandel, 2005). In the Schaffer-commissural projections (Yang et al., 2004) and the temporoammonic pathway (Qi et al., 2016) of the rat hippocampus, sex differences in LTP magnitude are driven by theta burst stiumulations but not high frequency stimulations, suggesting sex differences in the late stage of LTP versus the early stage. Sex differences within an LTP induction protocol have also been noted where 5 pairs of theta bursts produces a significant LTP in males, however, this stimulation protocol was not sufficient to induce an LTP in the female hippocampus (Wang et al., 2018). One reason for the sex differences in LTP magnitude is that the different tetanic stimulations may lead to differential activation of Ca^{2+} channels and intracellular postsynaptic Ca^{2+} release (Grover and Teyler, 1990; Regehr and Tank, 1992; Jager et al., 1998; Morgan and Teyler, 1999). Similar to synaptic plasticity, intrinsic excitability induces long-term changes by activating second messenger systems and synthesizing protein (Xu et al., 2005; Grabauskas et al., 2007; Oh et al., 2009; Cohen-Matsliah et al., 2010). Therefore, we speculate that different current intensities in our studies of intrinsic excitability might be activating distinct signaling pathways in a sex- or age- dependent way.

In conclusion, we find that intrinsic plasticity in neurons of the gRSC are different between males and females across three different firing types. The RSC is recruited in trace fear learning in both sexes, however, the male gRSC is part of the trace fear circuit early in development, whereas the female gRSC joins the circuit during adulthood. The female RSC's participation in adulthood raises the question regarding its role as an integral part of the trace fear circuitry or whether it plays a supplementary role in encoding complex associative fear memories.

Understanding sex and age differences in the RSC can reveal the developmental stage when females become more susceptible to maladaptive memory formation that underlie anxiety disorders.

CHAPTER FOUR: General discussion and future directions

The RSC is an integral node in the trace fear circuit and several structures in this circuit including the hippocampus (Maren et al., 1994; Maren, 1995; Wang et al., 2018), mPFC (Urban and Valentino, 2017), and amygdala (Blume et al., 2017) undergo sex-dependent plasticity. However, the majority of studies examining the role of RSC in learning and memory have solely used male subjects. Thus, the overarching question of this dissertation is whether sex differences are prevalent in the RSC like other brain structures of the fear circuit? We combine several different techniques including Pavlovian fear conditioning, whole-cell patch-clamp electrophysiology, pharmacological manipulations, and morphological analysis to answer questions related to developmental mechanisms of the RSC and how it encodes trace fear memory formation. Previously, we have established that the neuronal distribution of the RS-dominant early adolescent gRSC dramatically changes to a RS_{ADP}-dominant mid-adolescent and adult gRSC (Yousuf et al., 2020b). The ADP plays a role during development of the gRSC, however, the function of the ADP in other fear-related regions has been to promote excitability and plasticity (Wu et al., 2004; Thomas et al., 1998; Pike et al., 1999), which are requirements of learning. Therefore, the first aim of this dissertation was to understand sex-specific factors including learning, NMDA receptor activity, or dendritic morpholoy that regulate the ADP, an intrinsic property key for RSC development. In chapter two (Aim 1) of this dissertation, we first report that learning does not increase the percentage of RS_{ADP} neurons of the gRSC in either sex. Second, we found that NMDA receptor activity mediates the ADP in female but not in male RS_{ADP} neurons.

Third, male RS_{ADP} neurons have decreased dendritic complexity and branching compared to male RS neurons, and this difference in morphology between RS and RS_{ADP} neurons is absent in females. Together, we conclude that NMDA receptor activity plays a larger role in the ADP of females, whereas dendritic morphology may regulate the ADP in males.

Since we observed sex-dependent physiological changes in the developing gRSC in chapter 2 (Aim 1), chapter 3 (Aim 2) of this dissertation focused on two questions: 1) how learning changes as a function of age and sex, and 2) how learning changes the physiology of the RSC in males and females across development. We report that males follow a nonlinear trajectory, in which male mid-adolescent rats had suppressed retrieval of trace fear memories compared to early adolescent and adult males. A positive linear trend in freezing behaviors was noted in females across different ages. Our behavioral data indicate that the male fear circuit may go through a drastic reorganization during mid-adolescence that may not occur in females.

After examining behavioral changes, chapter 3 (Aim 2) also aimed at understanding how different neuronal firing types in the RSC are altered as a consequence of learning. In the gRSC, we observed sex differences in three different firing types, RS, RS_{ADP}, and BS, however, when these sex differences emerge is dependent on the developmental age. For example, sex differences in excitability of RS_{ADP} neurons favoring males are noted during early adolescence and last until adulthood. In contrast, sex differences in excitability of RS neurons favoring females appear during adulthood. Similar to RS neurons, excitability in adult female BS neurons is heightened compared to those of males. We were unable to identify the developmental stage that sex differences occur in BS neurons because they are prevalent in smaller percentages in layer 5 of the gRSC. Once we established sex differences in intrinsic excitability in the RSC at basal levels, we next examined how trace fear memory formation influences intrinsic plasticity of the male and female gRSC. Our

data demonstrate that retrieval of trace fear and CS exposure decreases intrinsic excitability of both RS and RS_{ADP} neurons at different stages of the life span. In all three male age groups, retrieval of trace fear and CS exposure suppresses excitability of RS_{ADP} neurons but does not affect RS neurons. This pattern was not seen in females because trace fear retrieval and CS exposure reduces excitability of RS and RS_{ADP} neurons only in adults. Further, BS neurons in both adult male and female gRSC remain unaffected by learning. These data indicate that the male RSC participates in the trace fear circuitry early in development, and much later in development in females.

In summary, the mechanisms that mediate the drastic developmental changes in the gRSC differ between males and females, and RSC-dependent fear learning developmental trajectories are also sex-specific. At basal levels, sex differences emerge during early adolescence depending on the neuronal firing type. Finally, learning or novel experiences alter gRSC plasticity differently in males at all ages but only during adulthood in females. Our data raise important questions about the male and female fear circuitry, and how factors such as sex steroid hormones or molecular signaling pathways may play a role in shaping fear regulation in males and females.

Role of sex steroid hormones in sex-dependent mechanisms of learning

Factors that may influence sex-dependent plasticity are sex steroid hormones such as estrogens. Specifically, the potent form of estrogen,17β-estradiol (E_2) can lower the threshold for LTP and increase LTP amplitude in both the male and female hippocampus of rodents (Teyler et al., 1980; Montoya and Carrer, 1997; Foy et al., 1999; Gu et al., 1999; Fugger et al., 2001; Sharrow et al., 2002; Smith and McMahon, 2006; Kramár et al., 2009; Smejkalova and Woolley, 2010; Jain et al., 2019), however, E_2-induced potentiation activates different glutamatergic signaling mechanisms in the hippocampus of females and males. E_2-mediated responses occur via GluN2B subunits of NMDA receptors in female rodents, and through AMPA receptors in males (Romeo et

al., 2005; Smith and McMahon, 2005; Kramár et al., 2009). E_2 differentially enhances presynaptic glutamate release via estrogen receptor (ER) β in female rats but through ERα in males (Oberlander and Woolley, 2016). Further, E_2 promotes postsynaptic glutamate sensitivity through ERβ in male and G protein coupled ERs in female rats (Oberlander and Woolley, 2016). Sex differences are also observed in E_2-mediated Ca^{2+} signaling in the rat hippocampus (Jain et al., 2019). In female rats, both L-type Ca^{2+} channels and Ca^{2+} release from internal stores are required for E_2-induced LTP, whereas in males, either L-type Ca^{2+} channels or Ca^{2+} from internal stores is required for potentiation (Jain et al., 2019). Thus, this is another example of the intricate differences in neural mechanisms between sexes, which may lead to the same outcome.

Sex differences in molecular mechanisms in fear-related brain regions

Intrinsic excitability and strong excitatory synaptic inputs promote intracellular Ca^{2+} in the postsynaptic neurons, which then trigger multiple signaling cascades necessary for memory formation. These Ca^{2+} dependent signaling molecules include calmodulin (CaM), Ca^{2+}/CaM-dependent kinases, phosphatases such as CaMKII and calcineurin (Lee et al., 2009; Fujii et al., 2013; Chang et al., 2017), ERK (Sananbenesi et al., 2002; Shalin et al., 2004; Sweatt, 2004), protein kinase C, and protein kinase M zeta (Sacktor, 2011). These signaling cascades transduce the synaptic signals to the nucleus, which then go on to activate transcription factors such as cAMP Responsive Element Binding Protein (CREB; Kogan et al., 1997; Pittenger et al., 2002; Josselyn et al., 2004; Alberini, 2009). Activated CREB binds to cAMP response element sites, which then regulate immediate early genes (IEGs) such as cFos (Radulovic et al., 1998), early growth response protein 1, activity-regulated cytoskeleton-associated protein (Arc; Guzowski et al., 2000).

Consistent with the idea that sex differences are evident in Ca^{2+} signaling (Wilmott and Thompson, 2013; Jain et al., 2019), similar differences are reported in calcium calmodulin kinase

kinase α (CAMKKα), which is required for contextual fear in mice (Mizuno et al., 2006, 2007). CAMKKα knockout male mice have impaired contextual fear memories compared to wild-type males, however, CAMKKα knockout females do not show any memory impairments compared to wild-type females (Mizuno et al., 2006). Similarly, following delay fear conditioning, ERK phosphorylation is significantly increased in the male but not female ventral hippocampus of rats (Gresack et al., 2009). Since both ERK and CAMKKα signaling cascades regulate the expression of plasticity-associated genes through activation of CREB, these studies suggest that activation of CREB could occur in a sex-specific manner. Indeed, one study demonstrated that context fear conditioning promoted higher levels of CREB phosphorylation in the male versus the female hippocampus of rats (Kudo et al., 2004). CREB targets IEGs (e.g. cFos), and sex-dependent changes in cFos activity have been reported, in which retrieval of context fear memories enhances cFos expression in the CA1, CA3, and dentate gyrus of the mouse hippocampus in males but not females (Keiser et al., 2017). Generalization of context fear also leads to a similar increase in cFos only in the male but not female mouse hippocampus (Keiser et al., 2017). Both retrieval of context fear and generalization promoted cFos levels in the amygdala of female but not in male mice (Keiser et al., 2017). Further, increased cFos and Arc expression in the female RSC are observed following remote context fear memory retrieval, and this increase in learning-related activity is not seen in male mice (Keiser, 2018). Thus, recruitment of different brain regions in fear memories is consistent with the concept that males and females use different strategies for spatial and fear learning. Future work is required to understand the precise mechanisms underlying distinct fear circuits in males and females.

Future directions

Mechanisms underlying development of the gRSC

Several regions of the fear circuit go through developmental changes in intrinsic and morphological properties (Ehrlich et al., 2012; Ryan et al., 2016; Kroon et al., 2019), required for connectivity and circuit formation. The emergence of the ADP may be unique to RSC development, therefore, understanding the components that shape the ADP will also give insight into how this region is primed for learning-related plasticity. Questions that have been left unanswered is whether new RS_{ADP} neurons replace RS neurons or do RS neurons eventually turn into RS_{ADP} neurons during gRSC development? Since an ADP can be induced with synaptic stimulations in the hippocampus (Wu et al., 2004), we speculate that as the gRSC matures, synaptic inputs from other brain regions may induce an ADP on RS neurons to stabilize the fear circuit. Novel techniques that combine whole-cell patch-clamp electrophysiology and mRNA sequencing of cell contents following electrophysiological recordings could provide answers regarding the overlap in gene expression of RS and RS_{ADP} neurons.

In addition to understanding whether RS_{ADP} neurons originate from RS neurons, much more work is required to investigate the mechanisms that regulate the ADP. Although we provide indirect evidence that the ADP may reduce dendritic complexity in male gRSC neurons, techniques using laser ablation to severe dendrites will give more direct evidence regarding the relationship between ADP and dendritic morphology. Further, another factor that needs to be elucidated in the gRSC is the role of Na^+ and Ca^{2+} dendritic conductances that shape the ADP (Magee and Carruth, 1999). Finally, a more comprehensive understanding of the components that mediate the ADP is required. So far, we have identified NMDA receptors and mGluRs (Yousuf et al., 2020b), however, these receptors only partially regulate the ADP, which leads to follow-up questions regarding the contribution of ion channels and other receptors. Future work related to

the mechanisms of the ADP in the gRSC and other fear-related brain regions can not only give insight into developmental patterns but also how neurons undergo intrinsic and synaptic plasticity.

Distinct roles of RSC subregions

The RSC plays an integral role in spatial and fear learning, however, only more recently it has become a focus of research (Todd et al., 2019). The RSC is subdivided into the gRSC and dysgranular region, which are morphologically and functionally distinct (Van Groen and Wyss, 1990; Pothuizen et al., 2010; Sugar et al., 2011). The gRSC forms connections with the anterior thalamic nuclei, whereas the dysgranular RSC is interconnected with the visual cortex (Vogt and Miller, 1983; Vann et al., 2009). We have shown age and sex differences in the physiology of one subregion (anterior gRSC), however, the physiology of the dysgranular region remains unknown. Additionally, along the rostro-caudal axis of the RSC, the anterior and posterior regions of the RSC are functionally distinct (Trask et al., 2021). A recent study optogenetically inhibited the male aRSC or pRSC during CS-US pairings of a trace fear conditioning task. Twenty-four hours later, when introduced to a novel environment with CS presentations, rats that have optogenetic inhibition of the anterior RSC during training freeze significantly less to the tone than control rats (Trask et al., 2021). Interestingly, optogenetic inhibition of the aRSC does not affect freezing to the acquisition context, suggesting that the aRSC plays a role in encoding event-related information or the "what" pathway (Trask et al., 2021). Further, rats with inhibition of the pRSC freeze significantly less to the acquisition context compared to controls (Trask et al., 2021), suggesting that the pRSC processes context-related information or the "where" pathway (Trask et al., 2021). All our recordings were from the anterior section of the gRSC and we found that during memory formation, females and males may encode event-related information differently. Since sex differences are observed in context fear memories (Maren et al., 1994; Kosten et al., 2006;

Gresack et al., 2009; Mizuno et al., 2012; Sase et al., 2019), future work is required to examine the physiology of the pRSC and how this subregion changes following retrieval of trace fear in males and females. These data highlight that the RSC is a large cortical structure with several subregions that may have unique physiologies and roles in tests of learning and memory.

Sex and age-specific role of the RSC in the fear circuit

We and others (Pattwell et al., 2011; Den and Richardson, 2013) have shown that the male fear circuitry goes through a change during mid-adolescence as fear behavior at this time point is different than other developmental stages. In contrast, we report a positive linear trend in fear behavior in females across the lifespan. For mid-adolescent rats, we chose the ages PND 42-49 as during this time, both males and females reach puberty. Choosing this age group minimizes variability in our experiments since the rats are age-matched, however, we realize that it does not capture the time right after puberty for both males and females as typically male rats reach puberty at PND 42 and females at PND 32 (Koss et al., 2015; Juraska and Willing, 2017; McCormick et al., 2017). Thus, by using the age group of PND 42-49, we may have missed the window immediately following puberty in females, when the fear circuit may go through a shift. Given the epidemiological evidence that after puberty, females are twice as likely to develop anxiety disorders compared to their male counterparts (Angold and Costello, 2006), more preclinical studies need to focus on how fear behavior and circuits change in females during and following puberty. Since the fear circuitry may go through changes across development, another important question is examining the male and female circuits that are established in the adult rat brain. Previous work has shown that retrieval of fear memories increases cfos expression in the male hippocampus but not in the female (Keiser et al., 2017). Conversely, fear memory formation increases cfos activity in the amygdala of females but not males (Keiser et al., 2017). Studies of

retrosplenial connectivity to the hippocampus have solely focused on male subjects (Cooper and Mizumori, 2001; Miyashita and Rockland, 2007; Opalka and Wang, 2020), thus, leaving a large gap in the fear circuitry literature.

Conclusion

Nearly half a century of preclinical neuroscientific research has primarily used male rodents as the default model organism (Shansky and Murphy, 2021). About a decade ago, an evaluation of neuroscience and biomedical research showed that male subjects were 6 times more often used compared to females in neuroscience research (Beery and Zucker, 2011). Unfortunately, a more recent evaluation of 2017 found that this disparity in the use of male subjects has barely improved (Mamlouk et al., 2020; Woitowich et al., 2020). Sex differences are prevalent in a wide range of psychiatric and neurological disorders including attention deficit hyperactivity disorder, autism, Alzheimer's disease, and eating disorders (Volkmar et al., 1993; Kessler et al., 1995; Biederman et al., 2002; Stein et al., 2002; Kessler, 2003; Holden, 2005; Steiner et al., 2005; Hyde et al., 2008; Sotiropoulos et al., 2008). Due to the focus of using male subjects in preclinical studies, women suffer from misdiagnosis in a range of medical conditions (Hinshaw et al., 2012; Newman-Toker et al., 2014; Wu et al., 2018) as well as side effects from medication that are developed based on the male physiology (Anderson, 2008). Specifically, women are three times more likely to develop posttraumatic stress disorder (Kessler et al., 1995, 2012), and this sex difference may appear early in development or due to puberty (Angold and Costello, 2006). Understanding when and why sex differences occur in maladaptive fear learning can lead to tailored sex-specific therapeutic interventions and improve the quality of lives of many women.

References

Adhikari A, Topiwala MA, Gordon JA (2011) Single units in the medial prefrontal cortex with anxiety-related firing patterns are preferentially influenced by ventral hippocampal activity. Neuron 71:898-910.

Aggleton JP, Wright NF, Vann SD, Saunders RC (2012) Medial temporal lobe projections to the retrosplenial cortex of the macaque monkey. Hippocampus 22:1883-1900.

Agster KL, Burwell RD (2009) Cortical efferents of the perirhinal, postrhinal, and entorhinal cortices of the rat. Hippocampus 19:1159-1186.

Akers KG, Arruda-Carvalho M, Josselyn SA, Frankland PW (2012) Ontogeny of contextual fear memory formation, specificity, and persistence in mice. Learning & memory 19:598-604.

Alberini CM (2009) Transcription factors in long-term memory and synaptic plasticity. Physiological reviews 89:121-145.

Anderson GD (2008) Chapter 1 gender differences in pharmacological response. International Review of Neurobiology 83:1-10.

Andreasen M, Lambert J (1995) Regenerative properties of pyramidal cell dendrites in area CA1 of the rat hippocampus. The Journal of Physiology 483:421-441.

Angold A, Costello EJ (2006) Puberty and depression. Child and Adolescent Psychiatric Clinics 15:919-937.

Arakawa H (2019) Age and sex differences in the innate defensive behaviors of C57BL/6 mice exhibited in a fear conditioning paradigm and upon exposure to a predatory odor. Physiology & behavior 204:264-274.

Arruda-Carvalho M, Wu W-C, Cummings KA, Clem RL (2017) Optogenetic examination of prefrontal-amygdala synaptic development. Journal of Neuroscience 37:2976-2985.

Asok A, Kandel ER, Rayman JB (2018) The neurobiology of fear generalization. Frontiers in behavioral neuroscience 12.

Azouz R, Jensen MS, Yaari Y (1996) Ionic basis of spike after-depolarization and burst generation in adult rat hippocampal CA1 pyramidal cells. The Journal of Physiology 492:211-223.

Barnet RC, Hunt PS (2005) Trace and long-delay fear conditioning in the developing rat. Learning & behavior 33:437-443.

Bean BP (2007) The action potential in mammalian central neurons. Nature Reviews Neuroscience 8:451.

Beery AK, Zucker I (2011) Sex bias in neuroscience and biomedical research. Neuroscience Biobehavior Reviews *35*: 565-572.

Bekkers JM, Häusser M (2007) Targeted dendrotomy reveals active and passive contributions of the dendritic tree to synaptic integration and neuronal output. Proceedings of the National Academy of Sciences 104:11447-11452.

Berger-Sweeney J, Arnold A, Gabeau D, Mills J (1995) Sex differences in learning and memory in mice: effects of sequence of testing and cholinergic blockade. Behavioral Neuroscience 109:859.

Beurrier C, Congar P, Bioulac B, Hammond C (1999) Subthalamic nucleus neurons switch from single-spike activity to burst-firing mode. Journal of Neuroscience 19:599-609.

Beylin AV, Gandhi CC, Wood GE, Talk AC, Matzel LD, Shors TJ (2001) The role of the hippocampus in trace conditioning: temporal discontinuity or task difficulty? Neurobiol Learn Mem 76:447-461.

Biederman J, Mick E, Faraone SV, Braaten E, Doyle A, Spencer T, Wilens TE, Frazier E, Johnson MA (2002) Influence of gender on attention deficit hyperactivity disorder in children referred to a psychiatric clinic. American Journal of psychiatry 159:36-42.

Blanchard RJ, Blanchard DC (1969) Crouching as an index of fear. Journal of Comparative and Physiological Psychology 67:370.

Bliss TV, Lomo T (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. Journal of Physiology 232:331-356.

Blume SR, Freedberg M, Vantrease JE, Chan R, Padival M, Record MJ, DeJoseph MR, Urban JH, Rosenkranz JA (2017) Sex-and estrus-dependent differences in rat basolateral amygdala. Journal of Neuroscience 37:10567-10586.

Brown JT, Randall AD (2009) Activity-dependent depression of the spike after-depolarization generates long-lasting intrinsic plasticity in hippocampal CA3 pyramidal neurons. The Journal of Physiology 587:1265-1281.

Brennan EKW, Sudhakar SK, Jedrasiak-Cape I, John TT, Ahmed OJ (2020) Hyperexcitable Neurons Enable Precise and Persistent Information Encoding in the Superficial Retrosplenial Cortex. Cell Rep 30:1598-1612.e1598.

Caballero A, Flores-Barrera E, Cass DK, Tseng KY (2014) Differential regulation of parvalbumin and calretinin interneurons in the prefrontal cortex during adolescence. Brain Structure and Function 219:395-406.

Caeser M, Brown DA, Gähwiler BH, Knöpfel T (1993) Characterization of a calcium-dependent current generating a slow afterdepolarization of CA3 pyramidal cells in rat hippocampal slice cultures. European Journal of Neuroscience 5:560-569.

Chang J-Y, Parra-Bueno P, Laviv T, Szatmari EM, Lee S-JR, Yasuda R (2017) CaMKII autophosphorylation is necessary for optimal integration of Ca^{2+} signals during LTP induction, but not maintenance. Neuron 94:800-808. e804.

Chapman PF, Kairiss EW, Keenan CL, Brown TH (1990) Long-term synaptic potentiation in the amygdala. Synapse 6:271-278.

Chen LL, Lin L-H, Green EJ, Barnes CA, McNaughton BL (1994) Head-direction cells in the rat posterior cortex. Experimental Brain Research 101:8-23.

Chen S, Yaari Y (2008) Spike Ca^{2+} influx upmodulates the spike afterdepolarization and bursting via intracellular inhibition of KV7/M channels. The Journal of Physiology 586:1351-1363.

Cho J, Sharp PE (2001) Head direction, place, and movement correlates for cells in the rat retrosplenial cortex. Behavioral Neuroscience 115:3.

Chocyk A, Przyborowska A, Makuch W, Majcher-Maślanka I, Dudys D, Wędzony K (2014) The effects of early-life adversity on fear memories in adolescent rats and their persistence into adulthood. Behavioural Brain Research 264:161-172.

Cohen-Matsliah SI, Motanis H, Rosenblum K, Barkai E (2010) A novel role for protein synthesis in long-term neuronal plasticity: maintaining reduced postburst afterhyperpolarization. Journal of Neuroscience 30:4338-4342.

Colon L, Odynocki N, Santarelli A, Poulos AM (2018) Sexual differentiation of contextual fear responses. Learning & Memory 25:230-240.

Cooper BG, Mizumori SJ (2001) Temporary inactivation of the retrosplenial cortex causes a transient reorganization of spatial coding in the hippocampus. Journal of Neuroscience 21:3986-4001.

Corcoran KA, Yamawaki N, Leaderbrand K, Radulovic J (2018) Role of retrosplenial cortex in processing stress-related context memories. Behavioral Neuroscience 132:388-395.

Corcoran KA, Donnan MD, Tronson NC, Guzman YF, Gao C, Jovasevic V, Guedea AL, Radulovic J (2011) NMDA receptors in retrosplenial cortex are necessary for retrieval of recent and remote context fear memory. Journal of Neuroscience 31:11655-11659.

Cowansage KK, Shuman T, Dillingham BC, Chang A, Golshani P, Mayford M (2014) Direct reactivation of a coherent neocortical memory of context. Neuron 84:432-441.

Dachtler J, Fox KD, Good MA (2011) Gender specific requirement of GluR1 receptors in contextual conditioning but not spatial learning. Neurobiology of Learning and Memory 96:461-467.

Dalla C, Shors TJ (2009) Sex differences in learning processes of classical and operant conditioning. Physiology & Behavior 97:229-238.

Dalla C, Bangasser DA, Edgecomb C, Shors TJ (2007) Neurogenesis and learning: acquisition and asymptotic performance predict how many new cells survive in the hippocampus. Neurobiology of Learning and Memory 88:143-148.

Dalla C, Papachristos EB, Whetstone AS, Shors TJ (2009) Female rats learn trace memories better than male rats and consequently retain a greater proportion of new neurons in their hippocampi. Proceedings of the National Academy Sciences U S A 106:2927-2932.

Daoudal G, Debanne D (2003) Long-term plasticity of intrinsic excitability: learning rules and mechanisms. Learning & Memory 10:456-465.

Daoudal G, Hanada Y, Debanne D (2002) Bidirectional plasticity of excitatory postsynaptic potential (EPSP)-spike coupling in CA1 hippocampal pyramidal neurons. Proceedings of the National Academy of Sciences 99:14512-14517.

de Olmos S, Bueno A, Bender C, Lorenzo A, de Olmos J (2008) Sex differences and influence of gonadal hormones on MK801-induced neuronal degeneration in the granular retrosplenial cortex of the rat. Brain Structure and Function 213:229.

de Sousa AF, Cowansage KK, Zutshi I, Cardozo LM, Yoo EJ, Leutgeb S, Mayford M (2019) Optogenetic reactivation of memory ensembles in the retrosplenial cortex induces systems consolidation. Proceedings of the National Academy of Science 116:8576-8581.

Den ML, Richardson R (2013) Enhanced sensitivity to learning fearful associations during adolescence. Neurobiology of Learning and Memory 104:92-102.

Detert JA, Kampa ND, Moyer JR, Jr. (2008) Differential effects of training intertrial interval on acquisition of trace and long-delay fear conditioning in rats. Behavioral Neuroscience 122:1318.

Disterhoft JF, Coulter DA, Alkon DL (1986) Conditioning-specific membrane changes of rabbit hippocampal neurons measured in vitro. Proceedings of the National Academy of Sciences 83:2733-2737.

Disterhoft JF, Golden DT, Read HL, Coulter DA, Alkon DL (1988) AHP reductions in rabbit hippocampal neurons during conditioning correlate with acquisition of the learned response. Brain research 462:118-125.

Disterhoft JF, Oh MM (2006) Learning, aging and intrinsic neuronal plasticity. Trends in Neurosciences 29:587-599.

Disterhoft JF, Oh MM (2007) Alterations in intrinsic neuronal excitability during normal aging. Aging Cell 6: 327-336.

Dumas TC, Rudy JW (2010) Development of the hippocampal memory system: Creating networks and modifiable synapses.

Dunn AR, Kaczorowski CC (2019) Regulation of intrinsic excitability: Roles for learning and memory, aging and Alzheimer's disease, and genetic diversity. Neurobiology of Learning and Memory 164:107069.

Dunn AR, Neuner SM, Ding S, Hope KA, O'Connell KMS, Kaczorowski CC (2018) Cell-type-specific changes in intrinsic excitability in the subiculum following learning and exposure to novel environmental contexts. eNeuro 5.

Dursun N, Bayar Y, Tan B, Süer C, Altuntaş H (2018) Sex differences in hippocampal long-term depression and the N-Methyl-D-Aspartate Receptor in rats-Positive correlation between LTD and Glun2b subunit. Journal of Neurology and Neuroscience 9:267.

Ehrlich D, Ryan S, Rainnie D (2012) Postnatal development of electrophysiological properties of principal neurons in the rat basolateral amygdala. The Journal of Physiology 590:4819-4838.

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

Esclassan F, Coutureau E, Di Scala G, Marchand AR (2009b) A cholinergic-dependent role for the entorhinal cortex in trace fear conditioning. Journal of Neuroscience 29:8087-8093.

Esteban JA, Shi SH, Wilson C, Nuriya M, Huganir RL, Malinow R (2003) PKA phosphorylation of AMPA receptor subunits controls synaptic trafficking underlying plasticity. Nature Neuroscience 6:136-143.

Fanselow MS (2000) Contextual fear, gestalt memories, and the hippocampus. Behavior Brain Research 110:73-81.

Fanselow MS, Sterlace SR (2014) Pavlovian fear conditioning. The Wiley Blackwell handbook of operant and classical conditioning:117-141.

Fidzinski P, Shor O, Behr J (2008) Target-cell-specific bidirectional synaptic plasticity at hippocampal output synapses. European Journal of Neuroscience 27:1111-1118.

Fowler MA, Sidiropoulou K, Ozkan ED, Phillips CW, Cooper DC (2007) Corticolimbic expression of TRPC4 and TRPC5 channels in the rodent brain. PLoS One 2:e573.

Foy M, Xu J, Xie X, Brinton R, Thompson R, Berger T (1999) 17β-estradiol enhances NMDA receptor-mediated EPSPs and long-term potentiation. Journal of Neurophysiology 81:925-929.

Fugger HN, Kumar A, Lubahn DB, Korach KS, Foster TC (2001) Examination of estradiol effects on the rapid estradiol mediated increase in hippocampal synaptic transmission in estrogen receptor α knockout mice. Neuroscience Letters 309:207-209.

Fujii H, Inoue M, Okuno H, Sano Y, Takemoto-Kimura S, Kitamura K, Kano M, Bito H (2013) Nonlinear decoding and asymmetric representation of neuronal input information by CaMKIIα and calcineurin. Cell Reports 3:978-987.

Gao WJ, Zheng ZH (2004) Target-specific differences in somatodendritic morphology of layer V pyramidal neurons in rat motor cortex. Journal of Comparative Neurology 476:174-185.

Garden DL, Massey PV, Caruana DA, Johnson B, Warburton EC, Aggleton JP, Bashir ZI (2009) Anterior thalamic lesions stop synaptic plasticity in retrosplenial cortex slices: expanding the pathology of diencephalic amnesia. Brain 132:1847-1857.

Gentet LJ, Stuart GJ, Clements JD (2000) Direct measurement of specific membrane capacitance in neurons. Biophysical Journal 79:314-320.

Gilmartin MR, Helmstetter FJ (2010) Trace and contextual fear conditioning require neural activity and NMDA receptor-dependent transmission in the medial prefrontal cortex. Learning and Memory 17:289-296.

Gilmartin MR, Kwapis JL, Helmstetter FJ (2012) Trace and contextual fear conditioning are impaired following unilateral microinjection of muscimol in the ventral hippocampus or amygdala, but not the medial prefrontal cortex. Neurobiology of Learning and Memory 97:452-464.

Goode TD, Maren S (2017) Role of the bed nucleus of the stria terminalis in aversive learning and memory. Learning & Memory 24:480-491.

Grabauskas G, Lancaster B, O'Connor V, Wheal HV (2007) Protein kinase signalling requirements for metabotropic action of kainate receptors in rat CA1 pyramidal neurones. Journal of Physiology 579:363-373.

Greene CC, Schwindt PC, Crill WE (1994) Properties and ionic mechanisms of a metabotropic glutamate receptor-mediated slow afterdepolarization in neocortical neurons. Journal of Neurophysiology 72:693-704.

Gresack JE, Schafe GE, Orr PT, Frick KM (2009) Sex differences in contextual fear conditioning are associated with differential ventral hippocampal extracellular signal-regulated kinase activation. Neuroscience 159:451-467.

Grienberger C, Chen X, Konnerth A (2014) NMDA receptor-dependent multidendrite Ca(2+) spikes required for hippocampal burst firing in vivo. Neuron 81:1274-1281.

Grover LM, Teyler TJ (1990) Two components of long-term potentiation induced by different patterns of afferent activation. Nature 347:477-479.

Gruene TM, Flick K, Stefano A, Shea SD, Shansky RM (2015) Sexually divergent expression of active and passive conditioned fear responses in rats. Elife 4:e11352.

Gu Q, Korach KS, Moss RL (1999) Rapid action of 17β-estradiol on kainate-induced currents in hippocampal neurons lacking intracellular estrogen receptors. Endocrinology 140:660-666.

Guatteo E, Mercuri NB, Bernardi G, Knöpfel T (1999) Group I metabotropic glutamate receptors mediate an inward current in rat substantia nigra dopamine neurons that is independent from calcium mobilization. Journal of Neurophysiology 82:1974-1981.

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

Haj-Dahmane S, Andrade R (1997) Calcium-activated cation nonselective current contributes to the fast afterdepolarization in rat prefrontal cortex neurons. Journal of Neurophysiology 78:1983-1989.

Haj-Dahmane S, Andrade R (1998) Ionic mechanism of the slow afterdepolarization induced by muscarinic receptor activation in rat prefrontal cortex. Journal of Neurophysiology 80:1197-1210.

Häusser M, Stuart G, Racca C, Sakmann B (1995) Axonal initiation and active dendritic propagation of action potentials in substantia nigra neurons. Neuron 15:637-647.

Hayashi Y, Shi SH, Esteban JA, Piccini A, Poncer JC, Malinow R (2000) Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction. Science 287:2262-2267.

Hayton SJ, Olmstead MC, Dumont EC (2011) Shift in the intrinsic excitability of medial prefrontal cortex neurons following training in impulse control and cued-responding tasks. PLoS One 6:e23885.

Hayton SJ, Lovett-Barron M, Dumont EC, Olmstead MC (2010) Target-specific encoding of response inhibition: increased contribution of AMPA to NMDA receptors at excitatory synapses in the prefrontal cortex. Journal of Neuroscience 30:11493-11500.

Herring BE, Nicoll RA (2016) Long-Term Potentiation: From CaMKII to AMPA Receptor Trafficking. Annual Reviews of Physiology 78:351-365.

Herry C, Ciocchi S, Senn V, Demmou L, Müller C, Lüthi A (2008) Switching on and off fear by distinct neuronal circuits. Nature 454:600.

Hinshaw SP, Owens EB, Zalecki C, Huggins SP, Montenegro-Nevado AJ, Schrodek E, Swanson, EN (2012) Prospective follow-up of girls with attention-deficit/hyperactivity disorder into early adulthood: continuing impairment includes elevated risk for suicide attempts and self-injury. Journal of Consulting and Clinical Psychology 80:1041-1051.

Hoffman DA, Magee JC, Colbert CM, Johnston D (1997) K+ channel regulation of signal propagation in dendrites of hippocampal pyramidal neurons. Nature 387:869.

Holden C (2005) Sex and the suffering brain. In: American Association for the Advancement of Science.

Holland PC, Gallagher M (1999) Amygdala circuitry in attentional and representational processes. Trends in Cognitive Sciences 3:65-73.

Huang YY, Kandel ER (2005) Theta frequency stimulation induces a local form of late phase LTP in the CA1 region of the hippocampus. Learning & Memory 12:587-593.

Hunt PS, Barnet RC (2016) Adolescent and adult rats differ in the amnesic effects of acute ethanol in two hippocampus-dependent tasks: Trace and contextual fear conditioning. Behavioral Brain Research 298:78-87.

Hunt PS, Burk JA, Barnet RC (2016) Adolescent transitions in reflexive and non-reflexive behavior: review of fear conditioning and impulse control in rodent models. Neuroscience & Biobehavioral Reviews 70:33-45.

Hunt PS, Barnet RC, Shea ME, Baker EM (2006) Cholinergic modulation of trace conditioning trained in serial compound: A developmental analysis. Neurobiology of Learning and Memory 86:311-321.

Huttenlocher PR (1979) Synaptic density in human frontal cortex-developmental changes and effects of aging. Brain Research 163:195-205.

Hyde JS, Mezulis AH, Abramson LY (2008) The ABCs of depression: integrating affective, biological, and cognitive models to explain the emergence of the gender difference in depression. Psychological Review 115:291.

Jäger T, Reymann KG, Behnisch T (1998) Analysis of the tetanic and post-tetanic components of intradendritic Ca2+ signals in hippocampal CA1 neurons. Neuroscience 86:423-429.

Jain A, Huang GZ, Woolley CS (2019) Latent sex differences in molecular signaling that underlies excitatory synaptic potentiation in the hippocampus. Journal of Neuroscience 39:1552-1565.

Jensen MS, Azouz R, Yaari Y (1996) Spike after-depolarization and burst generation in adult rat hippocampal CA1 pyramidal cells. The Journal of Physiology 492:199-210.

Jones B, Groenewegen H, Witter M (2005) Intrinsic connections of the cingulate cortex in the rat suggest the existence of multiple functionally segregated networks. Neuroscience 133:193-207.

Josselyn S, Kida S, Silva A (2004) Inducible repression of CREB function disrupts amygdala-dependent memory. Neurobiology of Learning and Memory 82:159-163.

Jovasevic V, Corcoran KA, Leaderbrand K, Yamawaki N, Guedea AL, Chen HJ, Shepherd GM, Radulovic J (2015) GABAergic mechanisms regulated by miR-33 encode state-dependent fear. Nat Neurosci 18:1265-1271.

Juraska JM, Willing J (2017) Pubertal onset as a critical transition for neural development and cognition. Brain Research 1654:87-94.

Kaczorowski CC, Disterhoft JF (2009) Memory deficits are associated with impaired ability to modulate neuronal excitability in middle-aged mice. Learning & Memory 16:362-366.

Kaczorowski CC, Disterhoft J, Spruston N (2007) Stability and plasticity of intrinsic membrane properties in hippocampal CA1 pyramidal neurons: effects of internal anions. The Journal of Physiology 578:799-818.

Kaczorowski CC, Davis SJ, Moyer JR Jr. (2012) Aging redistributes medial prefrontal neuronal excitability and impedes extinction of trace fear conditioning. Neurobiology of Aging 33:1744-1757.

Kalmbach BE, Chitwood RA, Dembrow NC, Johnston D (2013) Dendritic generation of mGluR-mediated slow afterdepolarization in layer 5 neurons of prefrontal cortex. Journal of Neuroscience 33:13518-13532.

Keene CS, Bucci DJ (2008a) Neurotoxic lesions of retrosplenial cortex disrupt signaled and unsignaled contextual fear conditioning. Behavioral Neuroscience 122:1070.

Keene CS, Bucci DJ (2008b) Contributions of the retrosplenial and posterior parietal cortices to cue-specific and contextual fear conditioning. Behavioral Neuroscience 122:89.

Keene CS, Bucci DJ (2008c) Involvement of the retrosplenial cortex in processing multiple conditioned stimuli. Behavioral Neuroscience 122:651.

Keiser AA, Turnbull LM, Darian MA, Feldman DE, Song I, Tronson NC (2017) Sex differences in context fear generalization and recruitment of hippocampus and amygdala during retrieval. Neuropsychopharmacology 42:397.

Keiser AA (2018) Sex Differences in Retrieval of Context Fear: Behavioral and Neural Mechanisms. University of Michigan Ann Arbor.

Kessler RC (2003) Epidemiology of women and depression. Journal of Affective Disorders 74:5-13.

Kessler RC, Sonnega A, Bromet E, Hughes M, Nelson CB (1995) Posttraumatic stress disorder in the National Comorbidity Survey. Archives of General Psychiatry 52:1048-1060.

Kessler RC, Petukhova M, Sampson NA, Zaslavsky AM, Wittchen HU (2012) Twelve-month and lifetime prevalence and lifetime morbid risk of anxiety and mood disorders in the United States. International Journal of Methods in Psychiatric Research 21:169-184.

Kitamura T, Macdonald CJ, Tonegawa S (2015) Entorhinal-hippocampal neuronal circuits bridge temporally discontiguous events. Learning and Memory 22:438-443.

Knapska E, Macias M, Mikosz M, Nowak A, Owczarek D, Wawrzyniak M, Pieprzyk M, Cymerman IA, Werka T, Sheng M, Maren S, Jaworski J, Kaczmarek L (2012) Functional anatomy of neural circuits regulating fear and extinction. Proceedings of National Academy of Science U S A 109:17093-17098.

Knight DC, Cheng DT, Smith CN, Stein EA, Helmstetter FJ (2004) Neural substrates mediating human delay and trace fear conditioning. Journal of Neuroscience 24:218-228.

Kogan JH, Frankland PW, Blendy JA, Coblentz J, Marowitz Z, Schütz G, Silva AJ (1997) Spaced training induces normal long-term memory in CREB mutant mice. Current Biology 7:1-11.

Koss WA, Belden CE, Hristov AD, Juraska JM (2014) Dendritic remodeling in the adolescent medial prefrontal cortex and the basolateral amygdala of male and female rats. Synapse 68:61-72.

Koss WA, Lloyd MM, Sadowski RN, Wise LM, Juraska JM (2015) Gonadectomy before puberty increases the number of neurons and glia in the medial prefrontal cortex of female, but not male, rats. Developmental Psychobiology 57:305-312.

Kosten TA, Lee HJ, Kim JJ (2006) Early life stress impairs fear conditioning in adult male and female rats. Brain Research 1087:142-150.

Kowalska M, Disterhoft JF (1994) Relation of nimodipine dose and serum concentration to learning enhancement in aging rabbits. Experimental Neurology 127:159-166.

Kramár EA, Chen LY, Brandon NJ, Rex CS, Liu F, Gall CM, Lynch G (2009) Cytoskeletal changes underlie estrogen's acute effects on synaptic transmission and plasticity. Journal of Neuroscience 29:12982-12993.

Krasne FB, Glanzman DL (1995) What we can learn from invertebrate learning. Annual Review of Psychology 46:585-624.

Krieg WJ (1946) Connections of the cerebral cortex. I. The albino rat. B. Structure of the cortical areas. Journal of Comparative Neurology 84:277-323.

Kroon T, van Hugte E, van Linge L, Mansvelder HD, Meredith RM (2019) Early postnatal development of pyramidal neurons across layers of the mouse medial prefrontal cortex. Scientific Reports 9:5037.

Kudo K, Qiao C-X, Kanba S, Arita J (2004) A selective increase in phosphorylation of cyclic AMP response element-binding protein in hippocampal CA1 region of male, but not female, rats following contextual fear and passive avoidance conditioning. Brain Research 1024:233-243.

Kuehl-Kovarik M, Partin K, Handa R, Dudek F (2005) Spike-dependent depolarizing afterpotentials contribute to endogenous bursting in gonadotropin releasing hormone neurons. Neuroscience 134:295-300.

Kumar A (2011) Long-Term Potentiation at CA3-CA1 Hippocampal Synapses with Special Emphasis on Aging, Disease, and Stress. Frontiers in Aging Neuroscience 3:7.

Kurotani T, Miyashita T, Wintzer M, Konishi T, Sakai K, Ichinohe N, Rockland KS (2013) Pyramidal neurons in the superficial layers of rat retrosplenial cortex exhibit a late-spiking firing property. Brain Structure and Function 218:239-254.

Kwapis JL, Jarome TJ, Helmstetter FJ (2014) The role of the medial prefrontal cortex in trace fear extinction. Learning and Memory 22:39-46.

Kwapis JL, Jarome TJ, Schiff JC, Helmstetter FJ (2011) Memory consolidation in both trace and delay fear conditioning is disrupted by intra-amygdala infusion of the protein synthesis inhibitor anisomycin. Learning and Memory 18:728-732.

Kwapis JL, Jarome TJ, Lee JL, Helmstetter FJ (2015) The retrosplenial cortex is involved in the formation of memory for context and trace fear conditioning. Neurobiology of Learning and Memory 123:110-116.

Laroche S, Jay TM, Thierry AM (1990) Long-term potentiation in the prefrontal cortex following stimulation of the hippocampal CA1/subicular region. Neuroscience Letters 114:184-190.

Leaderbrand K, Chen HJ, Corcoran KA, Guedea AL, Jovasevic V, Wess J, Radulovic J (2016) Muscarinic acetylcholine receptors act in synergy to facilitate learning and memory. Learn Mem 23:631-638.

Le Be JV, Silberberg G, Wang Y, Markram H (2007) Morphological, electrophysiological, and synaptic properties of corticocallosal pyramidal cells in the neonatal rat neocortex. Cerebral Cortex 17:2204-2213.

Lebron-Milad K, Milad MR (2012) Sex differences, gonadal hormones and the fear extinction network: implications for anxiety disorders. Biology of Mood & Anxiety Disorders 2:3.

LeDoux JE (2000) Emotion circuits in the brain. Annual Reviews of Neuroscience 23:155-184.

Lee S-JR, Escobedo-Lozoya Y, Szatmari EM, Yasuda R (2009) Activation of CaMKII in single dendritic spines during long-term potentiation. Nature 458:299.

Legendre P, Poulain D (1992) Intrinsic mechanisms involved in the electrophysiological properties of the vasopressin-releasing neurons of the hypothalamus. Progress in Neurobiology 38:1-17.

Lei Y-T, Thuault SJ, Launay P, Margolskee RF, Kandel ER, Siegelbaum SA (2014) Differential contribution of TRPM4 and TRPM5 nonselective cation channels to the slow afterdepolarization in mouse prefrontal cortex neurons. Frontiers in Cellular Neuroscience 8:267.

Li SH, Graham BM (2017) Why are women so vulnerable to anxiety, trauma-related and stress-related disorders? The potential role of sex hormones. The Lancet Psychiatry 4:73-82.

Lisman J, Yasuda R, Raghavachari S (2012) Mechanisms of CaMKII action in long-term potentiation. Nature Reviews of Neuroscience 13:169-182.

Liu SQ, Cull-Candy SG (2000) Synaptic activity at calcium-permeable AMPA receptors induces a switch in receptor subtype. Nature 405:454-458.

Lüscher C, Malenka RC (2012) NMDA receptor-dependent long-term potentiation and long-term depression (LTP/LTD). Cold Spring Harbor Perspectives Biology 4.

Luscher C, Nicoll RA, Malenka RC, Muller D (2000) Synaptic plasticity and dynamic modulation of the postsynaptic membrane. Nature Neuroscience 3:545-550.

Lynch GS, Dunwiddie T, Gribkoff V (1977) Heterosynaptic depression: a postsynaptic correlate of long-term potentiation. Nature 266:737-739.

Lynch J, Cullen PK, Jasnow AM, Riccio DC (2013) Sex differences in the generalization of fear as a function of retention intervals. Learning & Memory 20:628-632.

Lynch MA (2004) Long-term potentiation and memory. Physiological Reviews 84:87-136.

Magee JC, Carruth M (1999) Dendritic voltage-gated ion channels regulate the action potential firing mode of hippocampal CA1 pyramidal neurons. Journal of Neurophysiology 82:1895-1901.

Mainen ZF, Sejnowski TJ (1996) Influence of dendritic structure on firing pattern in model neocortical neurons. Nature 382:363.

Mamlouk GM, Dorris DM, Barrett LR, Meitzen J (2020) Sex bias and omission in neuroscience research is influenced by research model and journal, but not reported NIH funding. Frontiers in Neuroendocrinology 57:100835.

Man HY, Sekine-Aizawa Y, Huganir RL (2007) Regulation of {alpha}-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor trafficking through PKA phosphorylation of the Glu receptor 1 subunit. Proceedings of National Academy of Science U S A 104:3579-3584.

Maren S (1995) Sexually dimorphic perforant path long-term potentiation (LTP) in urethane-anesthetized rats. Neuroscience Letters 196:177-180.

Maren S (2001) Neurobiology of Pavlovian fear conditioning. Annual Review of Neuroscience 24:897-931.

Maren S, De Oca B, Fanselow MS (1994) Sex differences in hippocampal long-term potentiation (LTP) and Pavlovian fear conditioning in rats: positive correlation between LTP and contextual learning. Brain Research 661:25-34.

Marlin NA (1981) Contextual associations in trace conditioning. Animal Learning & Behavior 9:519-523.

Mayford M, Siegelbaum SA, Kandel ER (2012) Synapses and memory storage. Cold Spring Harb Perspect Biol 4.

McCormick CM, Green MR, Simone JJ (2017) Translational relevance of rodent models of hypothalamic-pituitary-adrenal function and stressors in adolescence. Neurobiology of Stress 6:31-43.

McCutcheon JE, Marinelli M (2009) Age matters. European Journal of Neuroscience 29:997-1014.

McKay BM, Matthews EA, Oliveira FA, Disterhoft JF (2009) Intrinsic neuronal excitability is reversibly altered by a single experience in fear conditioning. Journal of Neurophysiology 102:2763-2770.

McLean CP, Asnaani A, Litz BT, Hofmann SG (2011) Gender differences in anxiety disorders: prevalence, course of illness, comorbidity and burden of illness. Journal of Psychiatric Research 45:1027-1035.

Metz AE, Jarsky T, Martina M, Spruston N (2005) R-type calcium channels contribute to afterdepolarization and bursting in hippocampal CA1 pyramidal neurons. Journal of Neuroscience 25:5763-5773.

Metz AE, Spruston N, Martina M (2007) Dendritic D-type potassium currents inhibit the spike afterdepolarization in rat hippocampal CA1 pyramidal neurons. Journal of Physiology 581:175-187.

Miles OW, Maren S (2019) Role of the bed nucleus of the stria terminalis in PTSD: Insights from preclinical models. Frontiers in Behavioral Neuroscience 13.

Miyashita T, Rockland KS (2007) GABAergic projections from the hippocampus to the retrosplenial cortex in the rat. European Journal of Neuroscience 26:1193-1204.

Mizuno K, Dempster E, Mill J, Giese K (2012) Long-lasting regulation of hippocampal Bdnf gene transcription after contextual fear conditioning. Genes, Brain and Behavior 11:651-659.

Mizuno K, Ris L, Sánchez-Capelo A, Godaux E, Giese KP (2006) Ca^{2+}/calmodulin kinase kinase α is dispensable for brain development but is required for distinct memories in male, though not in female, mice. Molecular and Cellular Biology 26:9094-9104.

Mizuno K, Antunes-Martins A, Ris L, Peters M, Godaux E, Giese K (2007) Calcium/calmodulin kinase kinase β has a male-specific role in memory formation. Neuroscience 145:393-402.

Morgan SL, Teyler TJ (1999) VDCCs and NMDARs underlie two forms of LTP in CA1 hippocampus in vivo. Journal of Neurophysiology 82:736-740.

Monfort P, Gomez-Gimenez B, Llansola M, Felipo V (2015) Gender differences in spatial learning, synaptic activity, and long-term potentiation in the hippocampus in rats: molecular mechanisms. ACS Chemical Neuroscience 6:1420-1427.

Montoya DC, Carrer H (1997) Estrogen facilitates induction of long term potentiation in the hippocampus of awake rats. Brain Research 778:430-438.

Moore MD, Cushman J, Chandra D, Homanics GE, Olsen RW, Fanselow MS (2010) Trace and contextual fear conditioning is enhanced in mice lacking the alpha4 subunit of the GABA(A) receptor. Neurobiology of Learning and Memory 93:383-387.

Morris R, Pandya DN, Petrides M (1999) Fiber system linking the mid-dorsolateral frontal cortex with the retrosplenial/presubicular region in the rhesus monkey. Journal of Comparative Neurology 407:183-192.

Motanis H, Maroun M, Barkai E (2014) Learning-induced bidirectional plasticity of intrinsic neuronal excitability reflects the valence of the outcome. Cerebral Cortex 24:1075-1087.

Moye TB, Rudy JW (1987) Ontogenesis of trace conditioning in young rats: dissociation of associative and memory processes. Developmental Psychobiology: The Journal of the International Society for Developmental Psychobiology 20:405-414.

Moyer JR, Thompson LT, Black JP, Disterhoft JF (1992) Nimodipine increases excitability of rabbit CA1 pyramidal neurons in an age- and concentration-dependent manner. Journal of Neurophysiology 68:2100-2109.

Moyer JR Jr., Thompson LT, Disterhoft JF (1996) Trace eyeblink conditioning increases CA1 excitability in a transient and learning-specific manner. Journal of Neuroscience 16:5536-5546.

Moyer JR Jr., Brown TH (1998) Methods for whole-cell recording from visually preselected neurons of perirhinal cortex in brain slices from young and aging rats. Journal of Neuroscience Methods 86:35-54.

Mozzachiodi R, Byrne JH (2010) More than synaptic plasticity: role of nonsynaptic plasticity in learning and memory. Trends in Neurosciences 33:17-26.

Newhouse P, Newhouse C, Astur RS (2007) Sex differences in visual-spatial learning using a virtual water maze in pre-pubertal children. Behavior Brain Research 183:1-7.

Newman-Toker DE, Moy E, Valente E, Coffey R, Hines AL (2014) Missed diagnosis of stroke in the emergency department: a cross-sectional analysis of a large population-based sample. Diagnosis (Berl) 1:155-166.

Oberlander JG, Woolley CS (2016) 17β-Estradiol acutely potentiates glutamatergic synaptic transmission in the hippocampus through distinct mechanisms in males and females. The Journal of Neuroscience 36:2677.

Oh MM, Power JM, Thompson LT, Moriearty PL, Disterhoft JF (1999) Metrifonate increases neuronal excitability in CA1 pyramidal neurons from both young and aging rabbit hippocampus. Journal of Neuroscience 19:1814-1823.

Oh MC, Derkach VA, Guire ES, Soderling TR (2006) Extrasynaptic membrane trafficking regulated by GluR1 serine 845 phosphorylation primes AMPA receptors for long-term potentiation. Journal of Biological Chemistry 281:752-758.

Oh MM, McKay BM, Power JM, Disterhoft JF (2009) Learning-related postburst afterhyperpolarization reduction in CA1 pyramidal neurons is mediated by protein kinase A. Proceedings of the National Academy of Science U S A 106:1620-1625.

Oh MM, Oliveira FA, Disterhoft JF (2010) Learning and aging related changes in intrinsic neuronal excitability. Frontiers in Aging Neuroscience 2:2.

Opalka AN, Wang DV (2020) Hippocampal efferents to retrosplenial cortex and lateral septum are required for memory acquisition. Learning & Memory 27:310-318.

Orsini CA, Kim JH, Knapska E, Maren S (2011) Hippocampal and prefrontal projections to the basal amygdala mediate contextual regulation of fear after extinction. Journal of Neuroscience 31:17269-17277.

Park J-Y, Remy S, Varela J, Cooper DC, Chung S, Kang H-W, Lee J-H, Spruston N (2010) A post-burst afterdepolarization is mediated by group I metabotropic glutamate receptor-dependent upregulation of Cav2. 3 R-type calcium channels in CA1 pyramidal neurons. PLoS biology 8:e1000534.

Pattwell SS, Lee FS, Casey B (2013) Fear learning and memory across adolescent development: Hormones and Behavior Special Issue: Puberty and Adolescence. Hormones and Behavior 64:380-389.

Pattwell SS, Bath KG, Casey B, Ninan I, Lee FS (2011) Selective early-acquired fear memories undergo temporary suppression during adolescence. Proceedings of the National Academy of Sciences 108:1182-1187.

Pavlov IP (1927) Conditioned reflexes: an investigation of the physiological activity of the cerebral cortex: Translated and edited by Anrep, GV (Oxford University Press, London, 1927).

Petanjek Z, Judaš M, Šimić G, Rašin MR, Uylings HB, Rakic P, Kostović I (2011) Extraordinary neoteny of synaptic spines in the human prefrontal cortex. Proceedings of the National Academy of Sciences 108:13281-13286.

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

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

Pike FG, Meredith RM, Olding AW, Paulsen O (1999) Postsynaptic bursting is essential for 'Hebbian'induction of associative long-term potentiation at excitatory synapses in rat hippocampus. The Journal of Physiology 518:571-576.

Pittenger C, Huang YY, Paletzki RF, Bourtchouladze R, Scanlin H, Vronskaya S, Kandel ER (2002) Reversible inhibition of CREB/ATF transcription factors in region CA1 of the dorsal hippocampus disrupts hippocampus-dependent spatial memory. Neuron 34:447-462.

Pothuizen HH, Davies M, Aggleton JP, Vann SD (2010) Effects of selective granular retrosplenial cortex lesions on spatial working memory in rats. Behavioural Brain Research 208:566-575.

Pothuizen HH, Davies M, Albasser MM, Aggleton JP, Vann SD (2009) Granular and dysgranular retrosplenial cortices provide qualitatively different contributions to spatial working

memory: evidence from immediate-early gene imaging in rats. European Journal of Neuroscience 30:877-888.

Power JM, Oh MM, Disterhoft JF (2001) Metrifonate decreases sI(AHP) in CA1 pyramidal neurons in vitro. Journal of Neurophysiology 85:319-322.

Pryce CR, Lehmann J, Feldon J (1999) Effect of sex on fear conditioning is similar for context and discrete CS in Wistar, Lewis and Fischer rat strains. Pharmacological Biochemistry Behavior 64:753-759.

Qi X, Zhang K, Xu T, Yamaki VN, Wei Z, Huang M, Rose GM, Cai X (2016) Sex Differences in Long-Term Potentiation at Temporoammonic-CA1 Synapses: Potential Implications for Memory Consolidation. PLoS One 11:e0165891.

Quirk GJ, Likhtik E, Pelletier JG, Paré D (2003) Stimulation of medial prefrontal cortex decreases the responsiveness of central amygdala output neurons. Journal of Neuroscience 23:8800-8807.

Racine RJ, Wilson DA, Gingell R, Sunderland D (1986) Long-term potentiation in the interpositus and vestibular nuclei in the rat. Experimental Brain Research 63:158-162.

Radulovic J, Kammermeier J, Spiess J (1998) Relationship between fos production and classical fear conditioning: effects of novelty, latent inhibition, and unconditioned stimulus preexposure. Journal of Neuroscience 18:7452-7461.

Raineki C, Holman PJ, Debiec J, Bugg M, Beasley A, Sullivan RM (2010) Functional emergence of the hippocampus in context fear learning in infant rats. Hippocampus 20:1037-1046.

Regehr WG, Tank DW (1992) Calcium concentration dynamics produced by synaptic activation of CA1 hippocampal pyramidal cells. Journal of Neuroscience 12:4202-4223.

Richardson R, Hunt PS (2010) Ontogeny of fear conditioning. Oxford handbook of developmental behavioral neuroscience:527-545.

Ris L, Angelo M, Plattner F, Capron B, Errington M, Bliss T, Godaux E, Giese K (2005) Sexual dimorphisms in the effect of low-level p25 expression on synaptic plasticity and memory. European Journal of Neuroscience 21:3023-3033.

Roberts C, O'Boyle M, Suter K (2009) Dendrites determine the contribution of after depolarization potentials (ADPs) to generation of repetitive action potentials in hypothalamic gonadotropin releasing-hormone (GnRH) neurons. Journal of Computational Neuroscience 26:39.

Robinson S, Keene CS, Iaccarino HF, Duan D, Bucci DJ (2011). Involvement of retrosplenial cortex in forming associations between multiple sensory stimuli. Behavioral Neuroscience 125: 578.

Robinson S, Adelman JS, Mogul AS, Ihle PC, Davino GM (2018). Putting fear in context: Elucidating the role of the retrosplenial cortex in context discrimination in rats. Neurobiology of Learning and Memory 148: 50-59.

Romeo RD, McCarthy JB, Wang A, Milner TA, McEwen BS (2005) Sex differences in hippocampal estradiol-induced N-methyl-D-aspartic acid binding and ultrastructural localization of estrogen receptor-alpha. Neuroendocrinology 81:391-399.

Rosenkranz JA, Grace AA (2002) Dopamine-mediated modulation of odour-evoked amygdala potentials during pavlovian conditioning. Nature 417:282-287.

Rubinow MJ, Juraska JM (2009) Neuron and glia numbers in the basolateral nucleus of the amygdala from preweaning through old age in male and female rats: a stereological study. Journal of Compartive Neurology 512:717-725.

Rudy JW (1993) Contextual conditioning and auditory cue conditioning dissociate during development. Behavioral Neuroscience 107:887.

Rudy JW, Morledge P (1994) Ontogeny of contextual fear conditioning in rats: implications for consolidation, infantile amnesia, and hippocampal system function. Behavioral Neuroscience 108:227.

Ryan SJ, Ehrlich DE, Rainnie DG (2016) Morphology and dendritic maturation of developing principal neurons in the rat basolateral amygdala. Brain Structure and Function 221:839-854.

Sacktor TC (2011) How does PKMζ maintain long-term memory? Nature Reviews Neuroscience 12:9.

Sah P, Bekkers JM (1996) Apical dendritic location of slow afterhyperpolarization current in hippocampal pyramidal neurons: implications for the integration of long-term potentiation. Journal of Neuroscience 16:4537-4542.

Sanabria ER, Su H, Yaari Y (2001) Initiation of network bursts by Ca^{2+}-dependent intrinsic bursting in the rat pilocarpine model of temporal lobe epilepsy. The Journal of Physiology 532:205-216.

Sananbenesi F, Fischer A, Schrick C, Spiess J, Radulovic J (2002) Phosphorylation of hippocampal Erk-1/2, Elk-1, and p90-Rsk-1 during contextual fear conditioning: interactions between Erk-1/2 and Elk-1. Molecular and Cellular Neuroscience 21:463-476.

Sans N, Vissel B, Petralia RS, Wang Y-X, Chang K, Royle GA, Wang C-Y, O'Gorman S, Heinemann SF, Wenthold RJ (2003) Aberrant formation of glutamate receptor complexes

in hippocampal neurons of mice lacking the GluR2 AMPA receptor subunit. Journal of Neuroscience 23:9367-9373.

Santarelli AJ, Khan AM, Poulos AM (2018) Contextual fear retrieval-induced Fos expression across early development in the rat: An analysis using established nervous system nomenclature ontology. Neurobiology of Learning and Memory 155:42-49.

Santini E, Porter JT (2010) M-type potassium channels modulate the intrinsic excitability of infralimbic neurons and regulate fear expression and extinction. Journal of Neuroscience 30:12379-12386.

Santini E, Quirk GJ, Porter JT (2008) Fear conditioning and extinction differentially modify the intrinsic excitability of infralimbic neurons. Journal of Neuroscience 28:4028-4036.

Sase AS, Lombroso SI, Santhumayor BA, Wood RR, Lim CJ, Neve RL, Heller EA (2019) Sex-specific regulation of fear memory by targeted epigenetic editing of Cdk5. Biological Psychiatry 85:623-634.

Schreiber WB, Hunt PS (2013) Deficits in trace fear conditioning induced by neonatal alcohol persist into adulthood in female rats. Developmental Psychobiology 55:352-360.

Schwartzkroin PA (1975) Characteristics of CA1 neurons recorded intracellularly in the hippocampalin vitro slice preparation. Brain Research 85:423-436.

Sehgal M, Ehlers VL, Moyer JR Jr. (2014) Learning enhances intrinsic excitability in a subset of lateral amygdala neurons. Learning & Memory 21:161-170.

Sehgal M, Song C, Ehlers VL, Moyer JR Jr. (2013) Learning to learn–intrinsic plasticity as a metaplasticity mechanism for memory formation. Neurobiology of Learning and Memory 105:186-199.

Sepulveda-Orengo MT, Lopez AV, Soler-Cedeno O, Porter JT (2013) Fear extinction induces mGluR5-mediated synaptic and intrinsic plasticity in infralimbic neurons. Journal of Neuroscience 33:7184-7193.

Shalin SC, Zirrgiebel U, Honsa KJ, Julien JP, Miller FD, Kaplan DR, Sweatt JD (2004) Neuronal MEK is important for normal fear conditioning in mice. Journal of Neuroscience Research 75:760-770.

Shansky RM, Woolley CS (2016) Considering sex as a biological variable will be valuable for neuroscience research. Journal of Neuroscience 36:11817-11822.

Shansky RM, Murphy AZ (2021) Considering sex as a biological variable will require a global shift in science culture. Nature Neuroscience.

Sharrow KM, Kumar A, Foster TC (2002) Calcineurin as a potential contributor in estradiol regulation of hippocampal synaptic function. Neuroscience 113:89-97.

Shi SH, Hayashi Y, Petralia RS, Zaman SH, Wenthold RJ, Svoboda K, Malinow R (1999) Rapid spine delivery and redistribution of AMPA receptors after synaptic NMDA receptor activation. Science 284:1811-1816.

Shor OL, Fidzinski P, Behr J (2009) Muscarinic acetylcholine receptors and voltage-gated calcium channels contribute to bidirectional synaptic plasticity at CA1-subiculum synapses. Neuroscience Letters 449:220-223.

Shors TJ, Beylin AV, Wood GE, Gould E (2000) The modulation of Pavlovian memory. Behavioural Brain Research 110:39-52.

Šišková Z, Justus D, Kaneko H, Friedrichs D, Henneberg N, Beutel T, Pitsch J, Schoch S, Becker A, Kammer H, Remy S (2014) Dendritic structural degeneration is functionally linked to cellular hyperexcitability in a mouse model of Alzheimer's disease. Neuron 5:1023-1033.

Smejkalova T, Woolley CS (2010) Estradiol acutely potentiates hippocampal excitatory synaptic transmission through a presynaptic mechanism. Journal of Neuroscience 30:16137-16148.

Smith CC, McMahon LL (2005) Estrogen-induced increase in the magnitude of long-term potentiation occurs only when the ratio of NMDA transmission to AMPA transmission is increased. Journal of Neuroscience 25:7780-7791.

Smith CC, McMahon LL (2006) Estradiol-induced increase in the magnitude of long-term potentiation is prevented by blocking NR2B-containing receptors. Journal of Neuroscience 26:8517-8522.

Smith DM, Barredo J, Mizumori SJ (2012) Complimentary roles of the hippocampus and retrosplenial cortex in behavioral context discrimination. Hippocampus 22:1121-1133.

Smithers HE, Terry JR, Brown JT, Randall AD (2019) Sex-associated differences in excitability within the bed nucleus of the stria terminalis are reflective of cell-type. Neurobiology of Stress 10:100143.

Soler-Cedeno O, Cruz E, Criado-Marrero M, Porter JT (2016) Contextual fear conditioning depresses infralimbic excitability. Neurobiology of Learning and Memory 130:77-82.

Song C, Moyer JR Jr. (2017) Layer-and subregion-specific differences in the neurophysiological properties of rat medial prefrontal cortex pyramidal neurons. Journal of Neurophysiology 119:177-191.

Song C, Ehlers VL, Moyer JR Jr. (2015) Trace fear conditioning differentially modulates intrinsic excitability of medial prefrontal cortex–basolateral complex of amygdala projection neurons in infralimbic and prelimbic cortices. Journal of Neuroscience 35:13511-13524.

Song C, Detert JA, Sehgal M, Moyer JR, Jr. (2012) Trace fear conditioning enhances synaptic and intrinsic plasticity in rat hippocampus. Journal of Neurophysiology 107:3397-3408.

Sotiropoulos I, Cerqueira JJ, Catania C, Takashima A, Sousa N, Almeida O (2008) Stress and glucocorticoid footprints in the brain—the path from depression to Alzheimer's disease. Neuroscience & Biobehavioral Reviews 32:1161-1173.

Sotres-Bayon F, Sierra-Mercado D, Pardilla-Delgado E, Quirk GJ (2012) Gating of fear in prelimbic cortex by hippocampal and amygdala inputs. Neuron 76:804-812.

Spruston N (2008) Pyramidal neurons: dendritic structure and synaptic integration. Nature Reviews Neuroscience 9:206.

Spruston N, Stuart G, Häusser M (2016) Principles of dendritic integration. Dendrites 351:597.

Stein MB, Jang KL, Taylor S, Vernon PA, Livesley WJ (2002) Genetic and environmental influences on trauma exposure and posttraumatic stress disorder symptoms: a twin study. American Journal of Psychiatry 159:1675-1681.

Steiner M, Allgulander C, Ravindran A, Kosar H, Burt T, Austin C (2005) Gender differences in clinical presentation and response to sertraline treatment of generalized anxiety disorder. Human Psychopharmacology: Clinical and Experimental 20:3-13.

Storm J (1987) Action potential repolarization and a fast after-hyperpolarization in rat hippocampal pyramidal cells. The Journal of Physiology 385:733-759.

Storm JF (1988) Temporal integration by a slowly inactivating K+ current in hippocampal neurons. Nature 336:379.

Storm JF (1989) An after-hyperpolarization of medium duration in rat hippocampal pyramidal cells. The Journal of Physiology 409:171-190.

Stuart GJ, Häusser M (2001) Dendritic coincidence detection of EPSPs and action potentials. Nature Neuroscience 4:63.

Su H, Alroy G, Kirson ED, Yaari Y (2001) Extracellular calcium modulates persistent sodium current-dependent burst-firing in hippocampal pyramidal neurons. Journal of Neuroscience 21:4173-4182.

Sugar J, Witter MP, van Strien N, Cappaert N (2011) The retrosplenial cortex: intrinsic connectivity and connections with the (para) hippocampal region in the rat. An interactive connectome. Frontiers in Neuroinformatics 5:7.

Sugita S, Tanaka E, North R (1993) Membrane properties and synaptic potentials of three types of neurone in rat lateral amygdala. The Journal of Physiology 460:705-718.

Sweatt JD (2004) Mitogen-activated protein kinases in synaptic plasticity and memory. Current Opinion in Neurobiology 14:311-317.

Tanaka H, Grooms SY, Bennett MV, Zukin RS (2000) The AMPAR subunit GluR2: still front and center-stage. Brain Research 886:190-207.

Tayler KK, Tanaka KZ, Reijmers LG, Wiltgen BJ (2013) Reactivation of neural ensembles during the retrieval of recent and remote memory. Current Biology 23:99-106.

Teyler TJ, Vardaris RM, Lewis D, Rawitch AB (1980) Gonadal steroids: effects on excitability of hippocampal pyramidal cells. Science 209:1017-1018.

Thomas MJ, Watabe AM, Moody TD, Makhinson M, O'dell TJ (1998) Postsynaptic complex spike bursting enables the induction of LTP by theta frequency synaptic stimulation. Journal of Neuroscience 18:7118-7126.

Thompson RF, Krupa DJ (1994) Organization of memory traces in the mammalian brain. Annual Review of Neuroscience 17:519-549.

Todd TP, Bucci DJ (2015) Retrosplenial cortex and long-term memory: molecules to behavior. Neural plasticity 2015.

Todd TP, Fournier DI, Bucci DJ (2019) Retrosplenial cortex and its role in cue-specific learning and memory. Neuroscience & Biobehavioral Reviews.

Todd TP, Jiang MY, DeAngeli NE, Bucci DJ (2017) Intact renewal after extinction of conditioned suppression with lesions of either the retrosplenial cortex or dorsal hippocampus. Behavioural Brain Research 320:143-153.

Todd TP, Mehlman ML, Keene CS, DeAngeli NE, Bucci DJ (2016) Retrosplenial cortex is required for the retrieval of remote memory for auditory cues. Learning & Memory 23:278-288.

Toledo-Rodriguez M, Sandi C (2007) Stress before puberty exerts a sex-and age-related impact on auditory and contextual fear conditioning in the rat. Neural plasticity 2007.

Trask S, Pullins SE, Ferrara NC, Helmstetter FJ (2021) The anterior retrosplenial cortex encodes event-related information and the posterior retrosplenial cortex encodes context-related information during memory formation. Neuropsychopharmacology.

Tronson NC, Keiser AA (2019) A dynamic memory systems framework for sex differences in fear memory. Trends in Neurosciences.

Tsubokawa H, Ross WN (1996) IPSPs modulate spike backpropagation and associated [Ca^{2+}] i changes in the dendrites of hippocampal CA1 pyramidal neurons. Journal of Neurophysiology 76:2896-2906.

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

Urban KR, Valentino RJ (2017) Age- and Sex-Dependent Impact of Repeated Social Stress on Intrinsic and Synaptic Excitability of the Rat Prefrontal Cortex. Cerebral Cortex 27:244-253.

van Groen T, Wyss J (1990) Connections of the retrosplenial granular a cortex in the rat. Journal of Comparative Neurology 300:593-606.

van Groen T, Wyss JM (1992) Connections of the retrosplenial dysgranular cortex in the rat. Journal of Comparative Neurology 315:200-216.

van Groen T, Kadish I, Wyss JM (2004) Retrosplenial cortex lesions of area Rgb (but not of area Rga) impair spatial learning and memory in the rat. Behavioural Brain Research 154:483-491.

Vann SD, Aggleton JP, Maguire EA (2009) What does the retrosplenial cortex do? Nature Reviews Neuroscience 10:792.

Vasilyev DV, Barish ME (2002) Postnatal development of the hyperpolarization-activated excitatory current I h in mouse hippocampal pyramidal neurons. Journal of Neuroscience 22:8992-9004.

Vigil P, Del Río JP, Carrera B, ArÁnguiz FC, Rioseco H, Cortés ME (2016) Influence of sex steroid hormones on the adolescent brain and behavior: An update. Linacre Q 83:308-329.

Vogt BA, Peters A (1981) Form and distribution of neurons in rat cingulate cortex: areas 32, 24, and 29. Journal of Comparative Neurology 195:603-625.

Vogt BA, Miller MW (1983) Cortical connections between rat cingulate cortex and visual, motor, and postsubicular cortices. Journal of Comparative Neurology 216:192-210.

Volkmar FR, Szatmari P, Sparrow SS (1993) Sex differences in pervasive developmental disorders. Journal of Autism and Developmental Disorders 23:579-591.

Wang W, Le AA, Hou B, Lauterborn JC, Cox CD, Levin ER, Lynch G, Gall CM (2018) Memory-related synaptic plasticity is sexually dimorphic in rodent hippocampus. Journal of Neuroscience 38:7935-7951.

Watson JB, Rayner R (1920) Conditioned emotional reactions. Journal of Experimental Psychology 3:1.

Weible AP, McEchron MD, Disterhoft JF (2000) Cortical involvement in acquisition and extinction of trace eyeblink conditioning. Behavioral Neuroscience 114:1058-1067.

Whitaker LR, Warren BL, Venniro M, Harte TC, McPherson KB, Beidel J, Bossert JM, Shaham Y, Bonci A, Hope BT (2017) Bidirectional Modulation of Intrinsic Excitability in Rat

Prelimbic Cortex Neuronal Ensembles and Non-Ensembles after Operant Learning. Journal of Neuroscience 37:8845-8856.

Williams SR, Stuarty GJ (1999) Mechanisms and consequences of action potential burst firing in rat neocortical pyramidal neurons. The Journal of Physiology 521:467-482.

Willing J, Juraska JM (2015) The timing of neuronal loss across adolescence in the medial prefrontal cortex of male and female rats. Neuroscience 301:268-275.

Wilmott LA, Thompson LT (2013) Sex-and dose-dependent effects of post-trial calcium channel blockade by magnesium chloride on memory for inhibitory avoidance conditioning. Behavioural Brain Research 257:49-53.

Wiltgen BJ, Sanders MJ, Behne NS, Fanselow MS (2001) Sex differences, context preexposure, and the immediate shock deficit in Pavlovian context conditioning with mice. Behavioral Neuroscience 115:26-32.

Woitowich NC, Beery A, Woodruff T (2020) A 10-year follow-up study of sex inclusion in the biological sciences. Elife, 9.

Wu WW, Chan CS, Disterhoft JF (2004) Slow afterhyperpolarization governs the development of NMDA receptor-dependent afterdepolarization in CA1 pyramidal neurons during synaptic stimulation. Journal of Neurophysiology 92:2346-2356.

Xu J, Kang N, Jiang L, Nedergaard M, Kang J (2005) Activity-dependent long-term potentiation of intrinsic excitability in hippocampal CA1 pyramidal neurons. Journal of Neuroscience 25:1750-1760.

Yan H-D, Villalobos C, Andrade R (2009) TRPC channels mediate a muscarinic receptor-induced afterdepolarization in cerebral cortex. Journal of Neuroscience 29:10038-10046.

Yang D-W, Pan B, Han T-Z, Xie W (2004) Sexual dimorphism in the induction of LTP: critical role of tetanizing stimulation. Life sciences 75:119-127.

Young SR, Chuang SC, Wong RK (2004) Modulation of afterpotentials and firing pattern in guinea pig CA3 neurones by group I metabotropic glutamate receptors. The Journal of Physiology 554:371-385.

Yousuf H, Ehlers VL, Sehgal M, Song,C, Moyer JR Jr (2020a) Modulation of intrinsic excitability as a function of learning within the fear conditioning circuit. Neurobiology of Learning and Memory, 107132.

Yousuf H, Nye AN, Moyer JR Jr (2020b) Heterogeneity of neuronal firing type and morphology in rat retrosplenial cortex (in preparation).

Yue C, Yaari Y (2004) KCNQ/M channels control spike afterdepolarization and burst generation in hippocampal neurons. Journal of Neuroscience 24:4614-4624.

Yue C, Remy S, Su H, Beck H, Yaari Y (2005) Proximal persistent Na+ channels drive spike afterdepolarizations and associated bursting in adult CA1 pyramidal cells. Journal of Neuroscience 25:9704-9720.

Zhang D, Wang X, Wang B, Garza JC, Fang X, Wang J, Scherer PE, Brenner R, Zhang W, Lu XY (2017) Adiponectin regulates contextual fear extinction and intrinsic excitability of dentate gyrus granule neurons through AdipoR2 receptors. Molecular Psychiatry 22:1044-1055.

Zhang W, Linden DJ (2003) The other side of the engram: experience-driven changes in neuronal intrinsic excitability. Nature Reviews Neuroscience 4:885.

Zimmermann KS, Richardson R, Baker KD (2019) Maturational changes in prefrontal and amygdala circuits in adolescence: implications for understanding fear inhibition during a vulnerable period of development. Brain Sciences 9:65.

Hanna Yousuf
Curriculum Vitae

EDUCATION

Ph.D. candidate, 2017 – present
Thesis: *Sex- and age-associated effects on the retrosplenial cortex following trace fear learning*
Department of Psychology
University of Wisconsin-Milwaukee, Milwaukee WI

M.S. Experimental Psychology, 2016
Thesis: *The role of BDNF in 17β-estradiol-induced facilitation of extinction of cocaine seeking*
Department of Psychology
University of Wisconsin-Milwaukee, Milwaukee WI

B.A. Neuroscience, 2012
Neuroscience Program
College of Wooster, Wooster OH

AWARDS AND HONORS

2018-21: Advanced Opportunity Fellowship (University of Wisconsin-Milwaukee)

2018: Three Minute Thesis Finalist (University of Wisconsin-Milwaukee)

2015-2017: Graduate Student Travel Award (University of Wisconsin-Milwaukee)

2016: Summer Fellowship Research Award (University of Wisconsin-Milwaukee)

2012: Galpin Scholarship (College of Wooster)

2011: Dean's List (College of Wooster)

2011: Henry J. Copeland Fund for Senior Thesis Research (College of Wooster)

2011: Women's Advisory Board Scholarship for Outstanding Student of the Year (College of Wooster)

PUBLICATIONS

Yousuf, H., Nye, A.N., Moyer, J.R., Jr. Heterogeneity of neuronal firing type and morphology in male rat retrosplenial cortex. *Journal of Neurophysiology*, 2020, 123: 1849-1863.

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

Yousuf, H. Role of estrogens in addiction-related learning. In Estrogens and Memory: Basic research and clinical implications, ed. K.M. Frick, Oxford University Press, New York, NY, 2019, pp. 456-467.

Yousuf, H., Smies, C.W., Hafenbreidel, M., Tuscher, J.J., Fortress, A.M., Frick, K.M., Mueller, D. Infralimbic estradiol enhances neuronal excitability and facilitates extinction of cocaine seeking in female rats via a BDNF/TrkB mechanism. *Frontiers of Behavioral Neuroscience*, 2019, 13:168.

Otis, J.M., Fitzgerald, M.K., **Yousuf, H.**, Burkard, J., Drake, M., and Mueller, D. Prefrontal neuronal excitability maintains cocaine-associated memory during retrieval. *Frontiers of Behavioral Neuroscience* 2018, 12:119.

Ferreira, A.N., **Yousuf, H.**, Dalton, S., Sheets, P.L. Highly differentiated cellular and circuit properties of infralimbic pyramidal neurons projecting to the periaqueductal gray and amygdala. *Frontiers of Cellular Neuroscience* 2015, 9:161.

ABSTRACTS

Yousuf, H., Moyer, J. R., Jr. (2019). Developmental emergence of sex differences in the intrinsic membrane properties of retrosplenial cortical neurons.
Poster presentation at the Society for Neuroscience meeting, Chicago, IL.

Yousuf, H., Moyer, J. R., Jr. (2019). Developmental emergence of sex differences in the intrinsic membrane properties of retrosplenial cortical neurons.
Poster presentation at the Pavlovian Society, Vancouver, British Columbia.

Yousuf, H., Moyer, J. R., Jr. (2019). Developmental emergence of sex differences in the intrinsic membrane properties of retrosplenial cortical neurons.
Poster presentation at the Organization for the Study of Sex Differences, Washington D.C.

Yousuf, H., Moyer, J. R., Jr. (2019). Developmental emergence of sex differences in the intrinsic membrane properties of retrosplenial cortical neurons.
Poster presentation at the Association for Graduate Students in Psychology, University of Wisconsin-Milwaukee, WI.

Yousuf, H., Moyer, J. R., Jr. (2018). Intrinsic excitability of retrosplenial cortical neurons varies as a function of sex and age.
Poster presentation at the Society for Neuroscience, San Diego, CA.

Yousuf, H., Moyer, J. R., Jr. (2017). The role of BDNF in 17β-estradiol-induced facilitation of extinction of cocaine seeking.
Poster presentation at the Association for Graduate Students in Psychology, University of Wisconsin-Milwaukee, WI.

Yousuf, H., Moyer, J. R., Jr. (2016). The role of BDNF in 17β-estradiol-induced facilitation of extinction of cocaine seeking.
Poster presentation at the Society for Neuroscience, San Diego, CA.

Yousuf, H., Otis J.M., Fitzgerald M.K., Mueller, D. (2015). PKA mediates prelimbic neuronal excitability underlying cocaine-associated memory retrieval.
Poster presentation at the Society for Neuroscience, San Diego, CA.

TEACHING EXPERIENCE

University of Wisconsin – Milwaukee

Spring 2020	Associate lecturer, Psychology of Aging (online)
Spring 2018	Associate lecturer, Neuropsychology
Fall 2017	Associate lecturer, Advanced Physiological Psychology (online)
Spring 2017	Grader, Advanced Physiological Psychology
Fall 2015 – Fall 2016	Teaching Assistant, Physiological Psychology

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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