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Sex-dependent effects of acute stress on hippocampal synaptic plasticity

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RÉSUMÉ

Essentiel à la survie, le stress est une expérience connue de tous les organismes. Son excès, tout comme son manque, peut cependant induire des conséquences néfastes pour la santé. Ainsi, un stress aigu peut engendrer des déficits au niveau des fonctions cognitives via l'activation de récepteurs aux glucocorticoïdes (GRs). L'activation de ces-derniers peut perturber les fonctions neuronales et induire des altérations du comportement et même de la physiologie neuronale. À ce jour, très peu d'information est disponible quant aux effets précis de l'activation des GRs sur la plasticité et la fonction synaptique; d'autant moins lorsque les différences sexuelles sont prises en compte. De plus, la manière dont la signalisation GR dans les types de cellules non-neuronales contribue au dysfonctionnement synaptique associé au stress reste encore moins claire. Ainsi, notre but était de caractériser les effets du stress aigu sur la fonction synaptique de l'hippocampe chez les souris mâles et femelles afin de mettre en évidence le rôle de la signalisation aux glucocorticoïde au sein des cellules non-neuronales. À cet effet, des souris ont été soumises à un test de nage forcée (*acute swim stress*), puis des tranches d'hippocampe ont été préparées *in-vitro* pour l'étude électrophysiologique. Les souris mâles ont exprimé une réponse neuroendocrine plus prononcée au stress aigu, alors que cette dernière est demeurée absente chez les femelles. Dans cet ordre d'idées, les déficits de potentialisation à long-terme (LTP) obtenus en réponse au stress ont aussi été observés exclusivement chez les mâles. Finalement, les enregistrements électrophysiologiques en cellule-attachée ont montré qu'un stress aigu augmente l'excitabilité intrinsèque dans CA1 chez les deux sexes, mais que des modifications aux afférences excitatrices de CA1 sont observés seulement chez les mâles.

Mots-clés : Stress, hippocampe, LTP, *paired-pulse ratio*, astrocytes, glucocorticoïdes, électrophysiologie, différences sexuelles, corticostérone, excitabilité.

Summary

Stress is a global experience across all organisms, and although important for our survival, stress can have detrimental effects on brain health. More specifically, acute stress induces an intense deficit in cognitive function via the activation of glucocorticoid receptors (GRs). The activation of GRs can modify neuronal function and structure to promote lasting changes in behaviour and physiology. Despite this, the effects and precise mechanisms of stress and GR activation on synaptic function and plasticity in male and female mice remain unclear. Furthermore, how GR signalling in non-neuronal cell types contributes to the synaptic dysfunction associated with stress remains even less clear. Thus, we aimed to conduct a detailed characterization of the effects of acute stress on hippocampal synaptic function in male and female mice and highlight the role of GR signalling in non-neuronal cell types in governing these effects. To accomplish this, mice were subjected to an acute swim stress and hippocampal brain slices were prepared for in-vitro electrophysiology. We found that male mice have a pronounced neuroendocrine response to acute stress, accompanied by an increase in astrocyte GR signalling. However, these changes were absent in female mice. In line with this, we have also found that stress-induced impairments of hippocampal long-term potentiation (LTP) are specific to males. Finally, whole-cell patch clamp recordings demonstrate that acute stress increases the intrinsic excitability of CA1 neurons in male and female mice; however, only male mice have changes in the excitatory inputs of CA1 neurons. Overall, our results demonstrate a sexually dimorphic response to an acute swim stress.

Keywords: Stress, hippocampus, LTP, paired-pulse ratio, astrocytes, glucocorticoids, electrophysiology, sex differences, corticosterone, excitation.

LIST OF ABBREVIATIONS

aCSF	Artificial Cerebrospinal Fluid
ACTH	Adrenocorticotrophic hormone
AHP	After Hyperpolarization
AMPA	a-amino-3-hydroxy-5-methyl-4-isoxazolprepionic acid
AR	Androgen receptor
BDNF	Brain-derived neurotrophic factor
BSA	Bovine Serum Albumin
CaMK	Calcium-calmodulin protein kinase
CaMKII	Calcium-calmodulin protein kinase II
CaMKIV	Calcium-calmodulin protein kinase IV
cAMP	Cyclic adenosine 3`,5`- monophosphate
CCAC	Canadian Council on Animal Care
CIPA	Institutional Committee for the Protection of Animals
CORT	Corticosterone
CREB	cAMP response element binding protein
CRF	Corticotropin-releasing factor
CRFR	Corticotropin-releasing factor receptor
CRH	Corticotropin-releasing hormone
CSDS	Chronic social defeat stress
DEX	Dexamethasone
DG	Dentate Gyrus
DHT	Dihydrotestosterone
E2	17β - Estradiol

EPSP	Excitatory Postsynaptic Potential
ER	Estrogen Receptor
ELS	Early-life stress
fEPSP	Field Excitatory Postsynaptic Potential
GABA	Gamma-aminobutyric acid
GFAP	Glial fibrillary acidic protein
GLT-1	Glutamate transporter 1
GPCR	G-protein coupled receptor
GR	Glucocorticoid receptor
GRE	Glucocorticoid response element
GSK3	Glycogen synthetase kinase 3
HPA	Hypothalamic – pituitary – adrenal
IntDen	Integrated Density
IPSP	Inhibitory Postsynaptic Potential
Kcnab1	Potassium channel subunit beta-1
LTD	Long-term Depression
LTP	Long-term Potentiation
MLCK	Myosin light chain kinase
mPFC	medial Prefrontal Cortex
MR	Mineralocorticoid receptor
MRE	Mineralocorticoid response element
NMDA	N-methyl-D-aspartate
NMDG	N-methyl-D-glucamine

OCT	Optimal Cutting Temperature
PBS	Phosphate Buffer Saline
PFA	Paraformaldehyde
PKA	Protein Kinase A
PP1	Protein Phosphatases 1
PPR	Paired pulse ratio
PTP	Post-tetanic potentiation
PV	Parvalbumin
RMP	Resting membrane potential
sAP	Spontaneous Action Potential
sEPSC	Spontaneous excitatory postsynaptic current
sIPSC	Spontaneous inhibitory postsynaptic current
TMT	Trimethyl thiazoline

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1.0. Introduction

1.1. The hippocampus:

The hippocampus, located in the medial temporal lobe of the brain, plays a critical role in the formation of episodic and spatial memories (Milner et al., 1998). The hippocampus also plays a prominent role in facilitating the consolidation of long-term memories for permanent storage in the neocortex. These connections with the cortex are important for awareness about our conscious knowledge (Anand and Dhikav, 2012). In the context of spatial memories, the hippocampus has specific neurons known as place cells, which are neurons that are activated when rodents enter a specific region in their local environment (O'Keefe, 1976). These place cells provide rodents with the capacity to create an internal representation of their spatial awareness, otherwise known as a "cognitive map" (Moser et al., 2015). In addition to the cognitive functions the hippocampus plays, it also associates with several subcortical and cortical structures including the mamillary bodies, anterior thalamic nuclei, septal nuclei of the basal forebrain, retrosplenial cortex, and the parahippocampal gyrus (Thierry et al., 2000). These connections allow the hippocampus to play non-cognitive roles in the brain such as regulation of motor behaviour (Burman, 2019), hypothalamic function (Bang et al., 2022), and emotional behaviour (Toyoda et al., 2011) (Anand and Dhikav, 2012).

The hippocampus has an elaborate circuitry to accommodate the diverse roles it plays across the brain. The major input to the hippocampus is the entorhinal cortex, and once information has been received it can be processed along a unidirectional path consisting of three major synaptic connections, commonly referred to as the trisynaptic circuit. Axonal projections from the entorhinal cortex synapse on the granule cells of the dentate gyrus (DG), forming the perforant pathway. Granule cells can further project via mossy fiber axons to the pyramidal cells of the

CA3 region of the hippocampus. Axonal projections from the CA3 regions provide input to the pyramidal cells of the CA1 regions of the hippocampus, via the Schaffer collaterals (Anand and Dhikav, 2012). (**Figure 1**). The circuitry that contributes to the consolidation and retrieval of memory (the learning and memory loop) can be subdivided into the polysynaptic and monosynaptic pathways. The polysynaptic pathway projects into the hippocampus from the parietal, temporal and, occipital areas of the brain via the entorhinal cortex then travels along the trisynaptic circuity of the hippocampus (Morgado-Bernal, 2011). These tracts of the polysynaptic pathway pass ultimately to the posterior cingulated cortex and facilitate the consolidation of semantic memories (i.e. long-term memories involving recall of words, concepts or numbers) (Morgado-Bernal, 2011). The direct, intrahippocampal pathway projects from the perirhinal and entorhinal area of the cortex directly to CA1. From there, these projections travel via the subiculum to the inferior temporal cortex and prefrontal cortex. The monosynaptic pathway plays an important role in episodic and spatial memories (Morgado-Bernal, 2011; Anand and Dhikav, 2012).

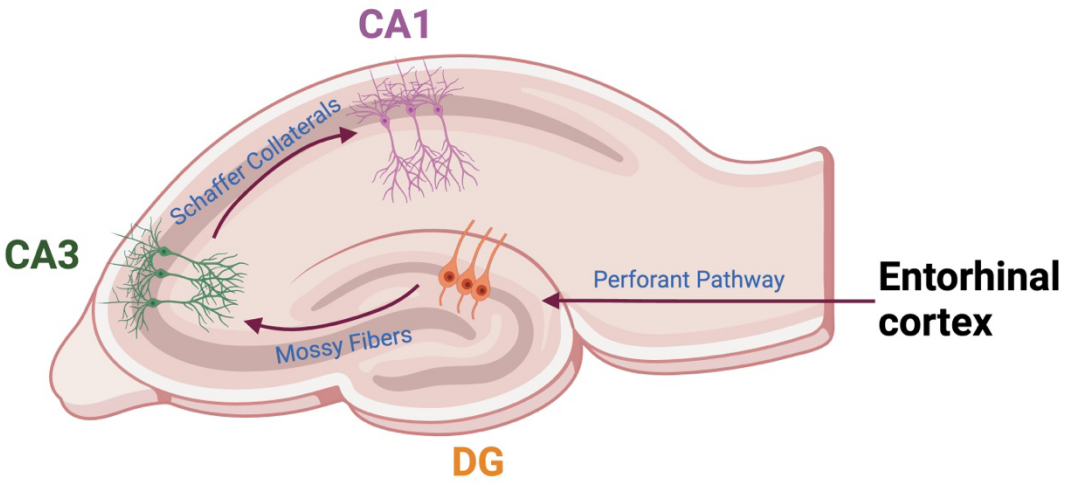


Figure 1: Trisynaptic circuit of the hippocampus. An illustration of the synaptic connections within the hippocampus. Input to the hippocampus from the entorhinal cortex synapses onto granular cells of the dentate gyrus (perforant pathway). Granular cells of the dentate gyrus project to the CA3 region of the hippocampus (mossy fiber pathway). CA3 pyramidal cells project to CA1 pyramidal cells to form the Schaffer-collateral pathway. Adapted from: Altered properties of hippocampal neuronal networks in reeler mice, Kowalski (2010). Created with BioRender.com

1.1.2. Hippocampal synaptic transmission

In the hippocampus, synaptic transmission allows communication between interconnected subregions. Hippocampal neurons communicate with their neighbouring neurons via electrochemical signalling. Following excitatory inputs to the dendrites and soma of the presynaptic neurons, an influx of positively charged sodium ions results in the depolarization of the plasma membrane. These signals will travel to the axon hillock region of the neuron to generate an action potential. The arrival of action potentials to the presynaptic axon terminals will allow for the activation of voltage-gated Ca^{2+} channels localized to the plasma membrane of the presynaptic terminals (Holz and Fisher, 1999). These channels can be identified with an alphabetic nomenclature and include P/Q-type channels, N-type, and R-type channels (Catterall, 2011). However, P/Q-type and N-type channels initiate neurotransmitter release at most fast synapses (Iwasaki and Takahashi, 1998; Ishikawa et al., 2005). Their activation will allow for specialized vesicles containing neurotransmitters to fuse will the presynaptic membrane to release neurotransmitters. More specifically, these synaptic vesicles are tagged with synaptobrevin that interact with syntaxin proteins on the membrane of the axon terminal to form a SNARE complex. The rise of calcium activates synaptotagmin (calcium sensor located on

synaptic vesicles) to catalyze the fusion of the vesicular and plasma membranes and initiate the exocytosis of neurotransmitters into the synaptic cleft. Neurotransmitters released into the synaptic cleft can then exert their functions by binding to postsynaptic receptors (i.e., ionotropic or metabotropic receptors) to have a diverse function on their postsynaptic targets (Holz and Fisher, 1999). In addition to action potential-induced neurotransmitter release, synaptic terminals can release neurotransmitters via spontaneous vesicle fusion, which occurs without a presynaptic action potential and recruits a separate pool of specialized vesicles. The separate pool of vesicles is spontaneously released via unique SNARE proteins such as VAMP7 and VAMP4 (Bal et al., 2013; Lin et al., 2020a). Spontaneous transmission of excitatory and inhibitory neurotransmitters allows homeostasis of synaptic communication and plays a significant role in neuronal processes such as: axonal growth (Young and Poo, 1983; McAllister et al., 1996), neuronal morphology (McKinney et al., 1999) and postnatal development of postsynaptic receptors such as N-methyl-D-aspartate (NMDA) receptors (Rajan et al., 1999).

The hippocampus contains largely excitatory (granular DG and pyramidal CA3 and CA1 neurons) and inhibitory interneurons (e.g. paravalbumin (PV) and somatostatin neurons). The predominant neurons in the hippocampus are excitatory pyramidal neurons which release glutamate and synapse with neighbouring pyramidal neurons (Anand and Dhikav, 2012). Once released into the synapse, glutamate can bind to ionotropic glutamate receptors (a-amino-3-hydroxy-5-methyl-4-isoxazolprepionic acid (AMPA), kainite or NMDA). Activation of these receptors results in an excitatory postsynaptic potential (EPSP), a transient depolarization of the postsynaptic membrane and an increased probability of a neuron firing an action potential. Inhibitory interneurons of the hippocampus work in a similar manner, however, they release gamma-aminobutyric acid (GABA) from their presynaptic terminals to act on ionotropic $GABA_A$

or GABA-rho (formerly known as $GABA_c$ receptors (results in Cl- influx), G-protein coupled $GABA_B$ receptors (open potassium channels to allow K+ efflux) (Pelkey et al., 2017) GABAergic signalling will result in an inhibitory postsynaptic potential (IPSP) that will result in a transient hyperpolarization to reduce the probability of action potential firing **(Figure 2)**

To ensure optimal functioning of the hippocampus, a balance between the excitatory and inhibitory neurotransmission must be maintained. The dense number of excitatory and inhibitory neurons creates particular interest in different methods to record excitatory/inhibitory balance. In brain slices, spontaneous current recordings from individual hippocampal neurons allow for a measurement of the output of these neurons without any artificial stimulation. Thus, recordings of spontaneous excitatory and inhibitory currents can provide insight into global hippocampal function, and whether any perturbation to an organism can have consequences on hippocampal output. Using whole-cell patch clamp, spontaneous excitatory postsynaptic currents (sEPSCs) and spontaneous inhibitory postsynaptic currents (sIPSCs) can be recorded from neurons to determine whether there are changes in the balance of excitation and inhibition. Increased frequency or size of sEPSCs or sIPSCs will give insight into whether the intrinsic properties of the cell are altered, and whether these are due to changes in excitation (changes in sEPSC frequency or amplitude) or inhibition (changes in sIPSC frequency or amplitude). An intricate balance between sEPSCs and sIPSCs allows for optimal function of the brain region being studied (Glasgow et al., 2019).

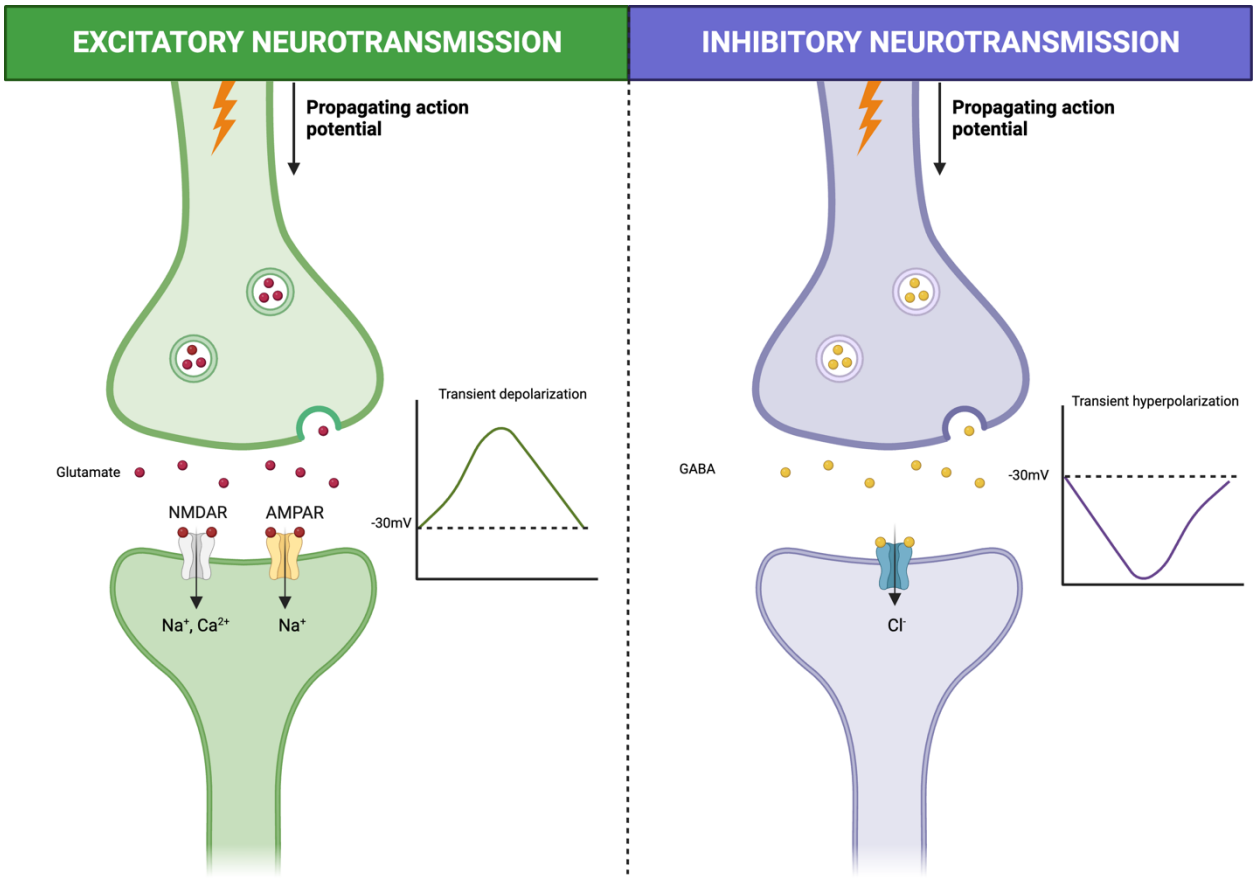


Figure 2: Simplified diagram of excitatory and inhibitory neurotransmission. Excitatory synapses (left; green) release glutamate and promote sodium influx into postsynaptic neurons to result in EPSPs. Inhibitory synapses (right; purple) release GABA to promote chloride influx to result in IPSPs. Adapted from: https://ib.bioninja.com.au/options/option-a-neurobiology-and/a5-neuropharmacology/synaptic-transmission.html. Created with BioRender.com

1.2. Synaptic plasticity

Synaptic plasticity, the activity-dependent strengthening or weakening of the synaptic connections, represents the neurological underpinnings of cognitive function. These modifications of synaptic efficacy can exist in multiple forms and can last on a variety of timescales. Short-term plasticity exists on a temporal scale of seconds to minutes, while long-

term plasticity can last for hours to days (Citri and Malenka, 2008). Both forms of synaptic plasticity have independent mechanisms of induction and expression; however, in the Schaffer-collateral pathway of the hippocampus (the most extensive and common pathway for hippocampal LTP studies) the induction of short-term and long-term plasticity are dependent on glutamatergic signalling.

1.2.1. Short-term synaptic plasticity

Short-term plasticity is a transient change in synaptic strength and efficacy and is thought to play an important role in our ability to adapt to external stimuli and short-term memory (Zucker and Regehr, 2002; Regehr, 2012). These transient modifications in synaptic strength and efficacy are the result of an accumulation of presynaptic calcium (Katz and Miledi, 1968). The large amounts of calcium directly modulate the probability of neurotransmitters being released from the presynaptic terminal (Oleskevich et al., 2000). A common technique for *ex-vivo* recordings of short-term plasticity entails delivering two stimuli within a short interval, whereby the response to the second stimulus can undergo facilitation (enhanced response) or depression (depressed response) relative to the first response. The direction of plasticity can be determined by the time interval between the paired stimuli. Typically, shorter interstimulus intervals (less than 20ms) will promote paired-pulse depression, while longer intervals (20-500ms) will promote facilitation. Longer forms of short-term plasticity also exist following repetitive stimulations of synapses applied at high frequencies (100-200 Hz) (Citri and Malenka, 2008). Following stimulation paradigms for LTP, a transient enhancement of neurotransmitter release results in a post-tetanic potentiation (PTP; a form of short-term plasticity following LTP induction) (Xue and Wu, 2010). PTP is the result of calcium build-up in the presynaptic terminal during the train of high-frequency stimulation and leads to the activation of calcium-calmodulin

(CaMK) to then activate myosin light chain kinase (MLCK) (Xue and Wu, 2010). MLCK can activate myosin II, allowing the translocation of synaptic vesicles containing glutamate to the readily release pool of vesicles, and once released, the glutamate will potentiate the postsynaptic response (Xue and Wu, 2010).

In the mammalian brain, short-term plasticity plays a prominent role in visual processing in retinal ganglion neurons (Nikolaev et al., 2013; Rosa et al., 2016), auditory processing in the neurons of the nucleus mangocelllularis (Fukui and Ohmori, 2004), olfactory processing (Suzuki and Bekkers, 2006) and relaying sensory information via modulation of thalamic reticular nucleus neurons (Chung et al., 2002; Anwar et al., 2017). In the hippocampus, short-term plasticity plays a role in coding spatial information via short-term facilitation of excitatory synapses and depression of inhibitory synapses, specifically through regulating the activity of CA1 place cells. Place cells are often silent; however, when an animal passes through a place field (specific region or environment that activates place cells), they have a high-frequency firing output (Klyachko and Stevens, 2006; Kandaswamy et al., 2010; Anwar et al., 2017). Excitatory facilitation onto pyramidal cells allows for rapid amplification and specificity of the high-frequency place cell firing when in a particular place field (Klyachko and Stevens, 2006). Additionally, short-term depression of inhibitory interneurons in the hippocampus assists the amplification of these signals to allow selectivity of the high-frequency output of place cell activity (Rotman et al., 2011; Jang et al., 2015).

1.2.2. Long-term synaptic plasticity

LTP, a form of synaptic plasticity whereby synaptic connections are strengthened, is critical in the formation of memories (Bliss and Lomo, 1973). Long-term depression (LTD) is the opposite of LTP and is characterized by an activity-dependent decrease in the strength of

synaptic connections (Dudek and Bear, 1992). LTD is crucial for the removal of old memory traces, to facilitate the formation of new memories and to promote behavioral flexibility. LTP and LTD function synergistically, to allow a stable memory mechanism (Bliss and Lomo, 1973; Dudek and Bear, 1992).

When an action potential reaches the presynaptic terminals of glutamatergic neurons, glutamate is released from specialized vesicles via exocytosis into the synaptic cleft. Glutamate diffuses across the synaptic cleft where it can bind to two vital postsynaptic receptors: NMDA and AMPA receptors. When glutamate binds to AMPA receptors, sodium ions enter the postsynaptic cell, resulting in an EPSP. At resting membrane potential, a magnesium ion blocks the pore of NMDA receptors preventing their activation upon glutamate binding. Once AMPA receptors are activated and depolarize the postsynaptic cell, the magnesium ion blocking the pore of NMDA receptors is removed and subsequent glutamate binding, along with a co-agonist (glycine or serine) can activate these receptors. Once activated, calcium influx occurs through the receptor's pore. The direction of synaptic plasticity in the Schaffer collateral synapse is dependent on the influx of calcium through NMDA receptors, and it is thought that the concentration and subcellular localization of the calcium transients determine the resulting effect (Evans and Blackwell, 2016)

In a brain slice, LTP is induced following high-frequency stimulation. Two of the most common induction paradigms are theta-burst stimulation (TBS; 10 bursts at 5Hz with each burst consisting of 4 pulses at 100Hz) and high-frequency stimulation (HFS; 100 pulses at 100Hz) (Volianskis et al., 2013). LTP induction protocols will unblock NMDA receptors to allow calcium to flood into the postsynaptic neuron, leading to activation of calcium-dependent intracellular cascades. In the early phase of LTP induction, the rise in calcium activates

calcium/calmodulin dependent protein kinase II (CaMKII) (Tao et al., 2021). CaMKII results in the activation of protein kinase A to phosphorylate a variety of targets involved in LTP including GluA1 subunits of AMPA receptors on the neuronal membrane surface (Abel et al., 1997; Diering et al., 2014, 2014; Park et al., 2021). The phosphorylation of AMPA receptors increases the conductance of existing receptors on the neuronal membrane and promotes the lateral diffusion of additional AMPA receptors from extrasynaptic sites to the postsynaptic density (Opazo et al., 2012; Huganir and Nicoll, 2013; Baudry et al., 2015). The increase in conductance and number of AMPA receptors allows a greater proportion of sodium to enter the postsynaptic neuron in response to the same presynaptic stimulation to sensitize the postsynaptic neuron **(Figure 3)**. In addition to the enzymatic function of CaMKII (i.e. activation of protein kinase A), CaMKII has a structural role during the induction of LTP via structural interactions with GluN2B-containing NMDARs (Tullis et al., 2023). These interactions allow for stabilization of NMDA receptors to the postsynaptic density. During the later phases of LTP induction, the synthesis of proteins is required to sustain LTP across multiple hours (Vickers et al., 2005). Protein kinase A (PKA) and CaMKIV can activate cyclic adenosine 3`,5`- monophosphate (cAMP) response element binding protein (CREB) to promote long-term changes in synaptic strength such as increased density of dendritic spines (Hayashi, 2022).

Conversely, low-frequency stimulation (900 pulses, 1Hz) paradigms are used to induce LTD (Dudek and Bear, 1992; Gonzalez et al., 2014). The stimulation paradigm only partially unblocks NMDA receptors resulting in a prolonged, modest increase in intracellular calcium levels. The less robust calcium response is selective to intracellular pathways that activate different enzymes, including protein phosphatase which dephosphorylates and internalize AMPA receptors (Casimiro et al., 2011; Xu et al., 2019). More specifically, NMDA receptor dependent

LTD will activate protein phosphatase 1 (PP1) to dephosphorylate AMPA receptors and promote clathrin- mediated endocytosis of AMPA receptors at neuronal membrane via the activation of glycogen synthetase kinase 3 (GSK3). The dephosphorylation and reduction in the number AMPA receptors will desensitize the postsynaptic neuron. Thus, LTP and LTD reflect the bidirectional regulation of synaptic plasticity (Peineau et al., 2007, 2008; Xu et al., 2019)

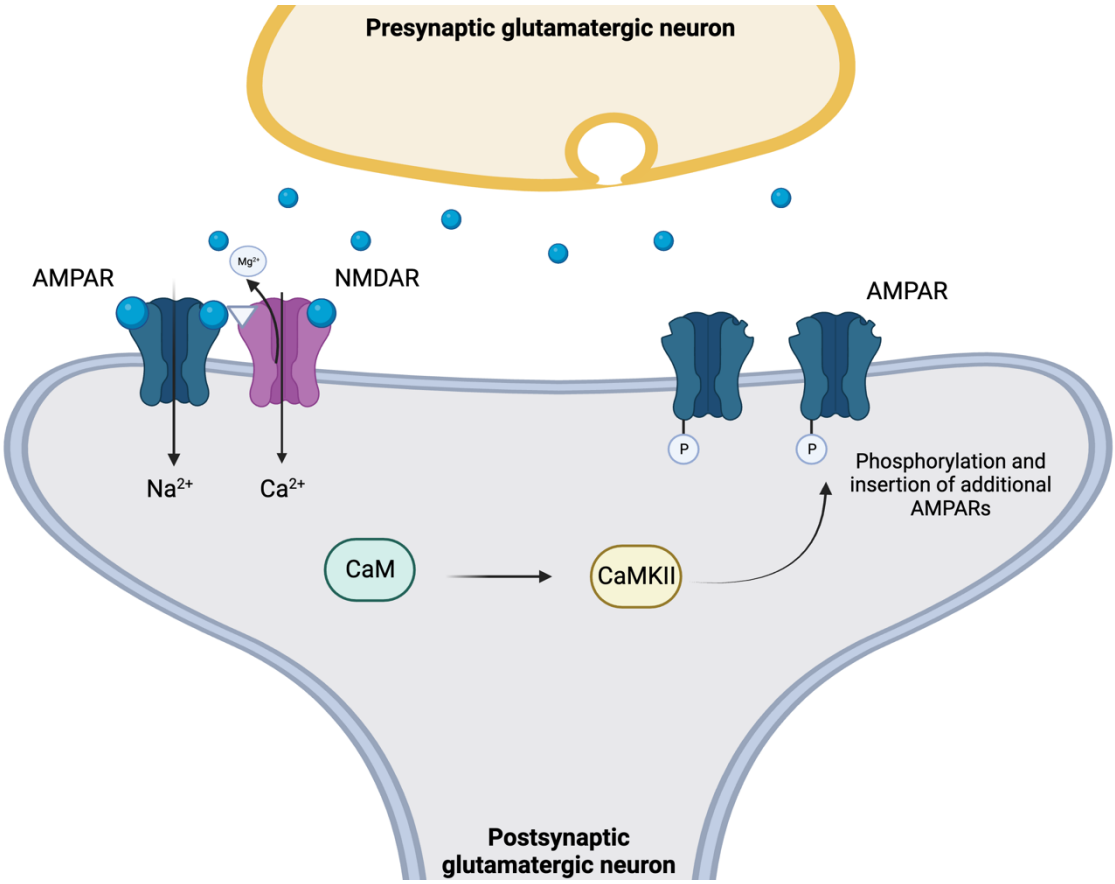


Figure 3: Mechanism of NMDA receptor-dependent LTP induction. Adapted from: https://thebrain.mcgill.ca/flash/a/a_07/a_07_m/a_07_m_tra/a_07_m_tra.html. An illustration of the NMDAR-dependent induction of hippocampal LTP. Calcium influx through NMDA receptors allows the activation of CaMKII to phosphorylate membrane tethered AMPA receptors to increase the ionic conductance and sensitize the postsynaptic neuron via the insertion of

additional AMPA receptors. Abbreviations: CaM – calcium-calmodulin, CaMKII – calcium-calmodulin kinase II

1.2.3. Sex differences in hippocampal LTP

In the hippocampus, sex has region-dependent effects on LTP and can vary across the trisynaptic pathway of the hippocampus (van Eijk et al., 2020). In the DG area, male rats exhibit a greater magnitude of early and late-phase LTP following a HFS protocol compared to female rats (Maren et al., 1994). In the CA3 region, male mice have a significantly higher magnitude of PTP following a HFS stimulation compared to female mice. Interestingly, when comparing LTP, male LTP was weaker compared to females in mossy fiber – CA3 pathways (Harte-Hargrove et al., 2015). Collectively this creates the narrative that in the CA3 region, male mice have higher short-term plasticity compared to females, but the threshold of LTP induction is much lower, and thus, easier to induce in female mice (Harte-Hargrove et al., 2015). In the CA1 region of the hippocampus, the threshold for LTP induction is much higher and more difficult to elicit in female mice, compared to male mice (Maren et al., 1994; Harte-Hargrove et al., 2015; Qi et al., 2016). Interestingly, the sex differences in CA1 LTP have recently been demonstrated to be dependent on the developmental stage of mice (Le et al., 2022). In prepubescent rats (postnatal days 21-28), the threshold of LTP induction is much lower in females versus age-matched male mice. However, in young adult rats (2-3 months old) the reverse occurs and male mice have a lower threshold for LTP induction versus age-matched female rats (Le et al., 2022).

The differences in the magnitude of hippocampal LTP in male and female mice can be related to the dichotomous effects that estrogen and testosterone have on hippocampal synaptic plasticity (Hyer et al., 2018). The hippocampus expresses both estrogen receptors (ERs) and androgen receptors (ARs) allowing it to respond to hormonal fluctuations of estrogens and

testosterone, respectively (Zhang et al., 2002). In female mice, the effects of estrogen fluctuations on hippocampal LTP have been well-studied (Smith and McMahon, 2006; Foy et al., 2010; Kramár et al., 2013; Pettorossi et al., 2013; Tozzi et al., 2019). Namely, 17B-estradiol (E2) has been shown to increase the magnitude of LTP in CA3-CA1 synapses (Kramár et al., 2013; Pettorossi et al., 2013). Accordingly, female mice in the proestrus phase of their estrous cycle (where estradiol levels are at their peak) have heightened LTP compared to the diestrus and estrus phases (Warren et al., 1995). These enhancements of LTP can be related to the interaction of E2 with NMDA receptors in the hippocampus, as E2 treatments have been shown to increase the levels of NR1 (a NMDA subunit expressed in all subtypes of NMDA receptors), and thus, an increase in the overall number of NMDA receptors in the hippocampus. Additionally, antagonizing the NR2B subunit of NMDA receptors prevents the E2-mediated enhancement of LTP (Smith and McMahon, 2006). In male mice, the effects of testosterone on hippocampal LTP are less understood. However, there have been reports that dihydrotestosterone (DHT) reduces the magnitude of hippocampal LTP in Schaffer- collateral synapses (Harley et al., 2000). The lack of literature investigating the effects of male-specific hormones on hippocampal LTP highlights the perceived bias that the neuroscience field has on excluding female mice. A common reason for the exclusion of female mice from neuroscience studies originates from the hyperfixation on female-related hormonal fluctuations and their effects on neuronal function. However, male mice have an equal number of hormonal fluctuations (Bartake et al., 1973), and have been recently shown to be more variable than female mice (Levy et al., 2023). Despite this, male mice continue to dominate as the preferred sex for most studies. Thus, while this section highlights sex differences in hippocampal LTP, with an emphasis on LTP changes in female mice, the inclusion of female mice in neuroscience studies continues to be important.

1.3. The effects of stress on the hippocampus

1.3.1. Hypothalamic-pituitary-adrenal axis

The body's stress response is regulated by a complex neuro-endocrine pathway known as the hypothalamic - pituitary - adrenal (HPA) axis. The HPA axis governs our stress response and maintains general homeostasis in the body. The three major components of the HPA axis: the hypothalamus, the pituitary and the adrenal glands work synergistically resulting in the production of stress hormones known as glucocorticoids (Herman et al., 2016). The HPA axis cascade begins with the activation of corticotropin releasing factor (CRF) neurons in the paraventricular nucleus (PVN) of the hypothalamus. These neurons will release CRF into the hypophyseal portal vessels that connect the PVN to the anterior pituitary gland, which is the major target for PVN-released CRF. The binding of CRF to CRF receptors (CRFRs) on the anterior pituitary gland results in the release of adrenocorticotropic hormone (ACTH) into the peripheral circulation (Herman et al., 2016). ACTH will then bind to melanocortin receptors in the adrenal cortex to stimulate the synthesis and release of glucocorticoids (Taves et al., 2011). In humans the major glucocorticoid is cortisol and in rodents it is corticosterone (CORT). CORT targets various cells in the body to modify our behavioral and physiological responses to under basal conditions and in response to stress.

Hyperactivity of the HPA axis can result in unfavourable conditions of hypercortisolemia; and thus, the activity of the HPA axis must be tightly regulated. In addition to inputs from other regions of the brain, such as the limbic system, the activity of the HPA axis can be largely -self regulated via negative feedback mechanisms (Gjerstad et al., 2018). More specifically, CORT production from the adrenal cortex will feedback to the pituitary gland or hypothalamus to suppress its own activity by binding to their target glucocorticoid receptors

(Gjerstad et al., 2018). Thus, the HPA axis represents one of the fundamental biological examples of a negative feedback loop. The negative feedback has two district temporal mechanisms, both of which are mediated by CORT (Dallman and Yates, 1969; Jones et al., 1974, 1977; Keller-Wood and Dallman, 1984; Osterlund et al., 2016). The fast, non-genomic mechanism of negative feedback will suppress the secretion of CRH and ACTH from the PVN and pituitary gland (Herman et al., 2016). The slow, genomic negative feedback reduces the transcription of CRH in the PVN and of proopiomelanocortin (POMC; the precursor for ACTH) in the pituitary gland (de Kloet et al., 2005). These mechanisms of self-regulation of the HPA axis limits tissue exposure to elevated glucocorticoids to prevent dysfunction of target cells across the body, including cells within the brain.

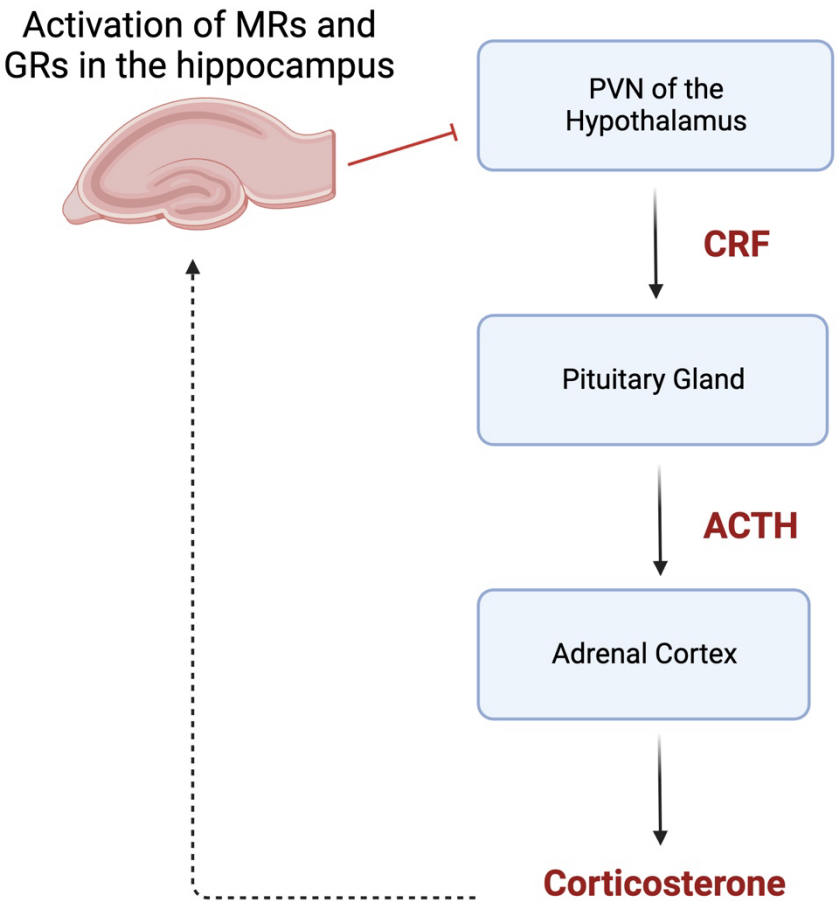


Figure 4: Simplified overview of the hypothalamic - pituitary - adrenal (HPA) axis and the representative inputs from the hippocampus. An illustration of the HPA axis and the hippocampal inputs that regulate the negative feedback loop of its activity. Release of CORT will act on GRs and MRs in the limbic system brain regions to allow these brain regions to input onto the HPA axis to regulate its function. Abbreviations: MRs – mineralocorticoid receptors; GRs – glucocorticoid receptors; CRF – corticotropin releasing factor; ACTH – adrenocorticotropic hormone. Adapted from: Cognitive Impairment in Major Depressive Disorder (Strawbridge and Young, 2016). Created with BioRender.com

1.3.2. Corticosteroid Receptors

The action of CORT in the body is mediated by two types of receptors: low-affinity glucocorticoid receptors (GRs) and high-affinity mineralocorticoid receptors (MRs). MRs and GRs respond to the same ligand; however, the high affinity of MRs allows them to respond to basal levels of CORT and play a predominant role in the circadian regulation of CORT production (Koning et al., 2019). GRs are low-affinity receptors, and thus, respond to major peaks of CORT, such as that released by a stressful situation (Jones et al., 1974; Reul et al., 1987). In addition to their affinity differences, MRs and GRs have differential expression patterns across the brain. GRs are highly expressed in most regions of the brain and most cell types, while MRs are predominantly found in the limbic system of the brain (i.e. amygdala, hippocampus and prefrontal cortex) (Reul and de Kloet, 1985). Accordingly, MR and GR activation can have opposing effects on brain function. For example, in the CA1 region of the hippocampus MR activation increases the excitatory drive of CA1 neurons, but GR activation reduces their excitation (Joëls and de Kloet, 1989; Joels et al., 1991; Kerr et al., 1992). More recently, Chatterjee and Sikdar (2014) elucidated on how MR and GR activation have

differential effects on synaptic transmission in hippocampal cultures. More specifically, activation of MRs with low concentrations of CORT (25nM) increased the frequency and amplitude of miniature EPSCs (mEPSCs) accompanied by an enhancement of depolarization-mediated calcium influx. Similarly, the activation of GRs with higher concentrations of CORT (100nM) increased the frequency and amplitude of mEPSCs; however, 100nM of CORT also increased the decay tau of mEPSCs, which was not present in low concentrations of CORT (Chatterjee and Sikdar, 2014). These changes highlight that the recruitment of MRs vs GRs in the hippocampus can have small, but differential effects on synaptic function.

MR and GRs can also work synergistically to regulate how our body responds to CORT. As an example, during an acute stressor, the activation of MRs plays an important role in the appraisal of our environment and memory retrieval, while GR activation promotes the consolidation of new memories and adaptation of our behavior to the stressor (de Kloet et al., 2005; Koning et al., 2019). Additionally, MR and GRs have both been implicated in the psychopathology that underlies stress-related mental illnesses. The hyperactivation of GRs has been implicated in the progression of mood disorders such as bipolar disorder and major depression (Yehuda et al., 1993). Accordingly, Cushing syndrome patients (a disease characterized by excessive release of cortisol) experience psychiatric symptoms that are mediated by the activity of GRs (Lin et al., 2020b). MRs are also associated with psychopathologies; however, the opposite activity pattern is implicated in psychiatric symptoms. More specifically, reduced activity of MRs has been tied to schizophrenia, bipolar disorder, and depression (Paul et al., 2022). The reduction in MR activity are likely compensated by an increase in the activity of GRs, which highlights the hypothesis that suggests the balance of MRs to GRs dictates the effects on the brain. The MR-GR balance hypothesis suggests that the

imbalance of MR or GR activity can dysregulate the HPA axis and increase the susceptibility to psychopathologies (Harris et al., 2013). Thus, a precise balance of activity of corticosteroid receptors is pivotal for our body's capacity to respond to stressful events.

Corticosteroid receptors have two major mechanisms of action in the brain: non-genomic and genomic actions. The non-genomic effects of GRs are faster and involve a rapid intracellular signaling cascade independent of changes in gene expression (Song and Buttgereit, 2006). These effects are mediated through cytosolic and membrane-bound GRs. Both receptor subtypes are localized within the plasma membrane and thus, exert their actions through calcium-dependent intracellular cascades (Mitre-Aguilar et al., 2015). Cytosolic GRs have been shown to mediate their effects through modulation of the MAPK pathway, a pathway that has been shown to be fundamental for synaptic plasticity in the hippocampus (Kelleher et al., 2004; Ordóñez-Morán and Muñoz, 2009). Membraine-bound GRs exert their effects through the activation of G-protein coupled receptors (GPCRs). Thus, these receptors have the capacity to activate adenylate cyclase to influence the expression of CREB and the phosphorylated isoform of CREB (pCREB) to influence gene transcription (Han et al., 2005; Leite-Dellova et al., 2008; Mitre-Aguilar et al., 2015). Additionally, membrane-bound GRs activate phospholipase C to mobilize internal calcium stores (Falkenstein et al., 2000; Löwenberg et al., 2008). Conversely, the genomic effects of GRs and MRs are the result of directly modulating gene expression, and these effects are often much slower than that of the non-genomic effects of corticosteroid receptors (Koning et al., 2019). The binding of CORT to cytosolic GRs or MRs will result in a confirmation change of the receptor complex to allow it to translocate to the nucleus. Once inside the nucleus, cytosolic GRs and MRs can interact with glucocorticoid response elements (GREs) and mineralocorticoid response elements (MREs), respectively (Koning et al., 2019). The binding of the CORT-

receptor complexes to their respective response elements will directly alter transcription. In the context of neuronal function, GREs have been found to be located on target genes that influence processes such as catecholamine synthesis (dopamine decarboxylase and tyrosine hydroxylase), synaptic transmission (potassium channel subunit beta-1 (Kcnab1)), and neuronal morphogenesis (Prx1 and Prx2) (Polman et al., 2012). Thus, GREs being localizaed to genes that can influence neuronal function and synaptic transmission highlights the impact that stress-induced activation of GRs can have on brain function.

1.3.3. Sex differences in the physiological response to stress

In rodents, there are pronounced differences in HPA axis activity between males and females. At baseline, it has been reported that circulating CORT is higher in female mice compared to male mice (Oyola and Handa, 2017; Heck and Handa, 2019). Additionally, in response to stress, females often have a heightened CORT and ACTH response in comparison to male mice (Handa et al., 1994; MacLusky et al., 1996; Viau et al., 2005; Iwasaki-Sekino et al., 2009; Babb et al., 2013). These higher CORT values in females have been reported in response stressors such as: early-life maternal stress (Fuentes et al., 2014), chronic restraint stress (Babb et al., 2013), and an acute swim stress (Vecchiarelli et al., 2022). The exaggerated CORT response in female mice is also accompanied by changes in neuronal activity and genes related to HPA axis function. More specifically, PVN mRNA expression of stress-related genes such as CRH and POMC are elevated in female mice compared to male mice (Viau et al., 2005; Iwasaki-Sekino et al., 2009). Additionally, following an acute restraint stress (30 mins), female mice have a greater number of c-fos positive cells (a marker for neuronal activation) in the PVN compared to males (Babb et al., 2013). Thus, the innate differences in HPA axis function between males and females are likely due to differences in neuronal communication within the PVN and the

communication of the PVN with other stress-associated brain regions. In addition to innate differences in HPA axis function in female mice, there is also literature supporting sex-differences in glucocorticoid receptor expression levels between male and female mice (Turner and Weaver, 1985; Turner, 1990; Bourke et al., 2013; Palumbo et al., 2020). The differences in GR and MR expression between male and female mice could be a potential mechanism for the differences in basal and stress induced HPA axis function. Female mice have lower expression levels of MRs and GRs in the pituitary gland compared to male mice (Turner, 1990). Additionally, in response to an acute swim stress (20 minutes) and chronic swim stress (20 mins, 14 days) stress-induced increases of MR and GR mRNA were selective to male mice, and unaffected in female mice (Karandrea et al., 2002). Thus, the lower level of MRs and GRs would reduce the ability of CORT to bind to its target receptors, and thus, reduce the ability to exert a negative feedback function (Heck and Handa, 2019).

In addition to lower GR and MR expression levels, Weister et al. (2009) elaborated on another potential mechanism of why female mice have differences in HPA axis negative feedback. The authors demonstrated that estradiol could reduce the magnitude of HPA axis negative feedback in female rodents (Weiser and Handa, 2009). Ovariectomized rodents that were treated with exogenous sources of estradiol had increased basal CORT and heightened the CORT response following a chronic restraint stress. Furthermore, the authors infused estradiol and estrogen receptor agonists into the PVN of the ovariectomized rodents and completed a dexamethasone (DEX) test to investigate the interaction between female hormones and HPA axis negative feedback (Weiser and Handa, 2009). DEX is a synthetic corticosteroid with a high affinity for GRs and is intended to suppress HPA axis activity (Noreen et al., 2021). As an example, in patients with Cushing's syndrome, DEX has no effect and the lack of effect of DEX

suggests dysregulated HPA axis function and cortisol production (Dogra and Vijayashankar, 2023). Interestingly, estradiol and estrogen receptor agonists implanted into the PVN of female mice impaired the ability of DEX to block CORT production. The inability of DEX to suppress CORT with estradiol implantation suggests that estradiol disinhibits the negative feedback of the HPA axis (Weiser and Handa, 2009).

1.4. The effects of stress on the hippocampus

The hippocampus is a brain region sensitive to the effects of stress and plays a direct role in regulating the activity of the HPA axis (Jankord and Herman, 2008). Hippocampal pyramidal cells project excitatory terminals to CRH neurons in the PVN to reduce the secretion of CRF by activating GABAergic neurons in the bed nucleus of stria terminalis (BNST) (Cullinan et al., 1993; Herman et al., 2003). In addition to functional connections with the HPA axis, GRs and MRs are copiously expressed in the hippocampus, making it sensitive to circulating CORT in the brain (Reul and de Kloet, 1986; Herman et al., 1989). Accordingly, lesions to the hippocampus elevate basal levels of CORT (Fendler et al., 1961; Knigge, 1961; Herman and Mueller, 2006) and increase the CORT response to acute restraint stress (Sapolsky et al., 1989). The inhibitory role of the hippocampus on the HPA axis has been demonstrated to be dependent upon the activation of GRs. More specifically, hippocampal infusion of a GR antagonist prevents the capacity of DEX to reduce circulating CORT (Boyle et al., 2005). The inability of DEX to reduce circulating CORT, suggests that GR activation in the hippocampus is vital for the hippocampus-HPA axis negative feedback. Due to the interactions of the hippocampus with the HPA axis, and the large number of glucocorticoid receptors, the hippocampus is inherently sensitive to the effects of stress, and can be affected on a behavioural, cellular, and synaptic scale.

1.4.1. The effects of stress on hippocampal-dependent behaviors

Stress-induced impairments of cognitive function have been well established in humans and rodents. These impairments of cognitive function can be linked back to abnormal functioning of the hippocampus (Kim et al., 2015). More specifically, in healthy subjects, both stress and exogenous cortisol administration have been shown to specifically inhibit the retrieval of long-term memories (Newcomer et al., 1994; de Quervain et al., 1998; Bremner et al., 2000). Interestingly, patients that have been diagnosed with Cushing's syndrome (a disease characterized by excessive production of glucocorticoids due to an adrenal cortex tumor) have marked atrophy of the hippocampus that is accompanied by deficits in hippocampal-dependent memory tasks (Starkman et al., 1992; Kim et al., 2015). The stress-induced impairment of hippocampal-dependent behaviours have also been characterized in rodents (Francis et al., 1995; Diamond et al., 1999; Kim and Diamond, 2002; Kim et al., 2015). As a proxy for hippocampal-dependent memory task, the Morris water maze test can be used in rodents to assess spatial memory. Briefly, mice are trained to locate a hidden platform submerged in opaque water to without any visual cues to the location of the platform, and thus, the rodent relies exclusively on spatial memory (Vorhees and Williams, 2006). Mice that have been exposed to foot shock stress have marked deficits in their performance on the Morris water maze test indicating spatial memory retrieval deficits (Francis et al., 1995). Similarly, mice that are exposed to a predator-based psychosocial stress (i.e. in close proximity to a cat) have reduced performance on the Morris water maze test (Park et al., 2008). Moreover, transgenic mice overexpressing CRF (i.e. excess CORT production) have reduced performance on the water Morris maze test and perform significantly worse than their non-transgenic littermates (Heinrichs et al., 1996). However, the effects of stress are not always uniform on hippocampal-dependent behaviors. Rats that have

been exposed to either an acute (2 hours) or chronic restraint stress (21 days of restraint) have enhanced performance on a contextual fear-conditioning paradigm (hippocampal-dependent associative memory task between an aversive stimulus and an environment (context)) (Cordero et al., 2003). Similarly, in neonatal rats (postnatal day 15) that have been exposed to experimental handling as a model of stress have heightened performance during a contextual-fear conditioning paradigm compared to non-handled littermates (Beane et al., 2002). Thus, stress has bidirectional effects on hippocampal-dependent learning by enhancing the consolidation of fear-related behaviors and impeding spatial memory. The marked behavioural deficits associated with stress can be related to the fact that stress has negative effects on the cellular composition of the hippocampus, as well as effects on hippocampal synaptic transmission and plasticity.

1.4.2. The effects of stress on hippocampal cellular dysfunction

Chronic elevations of CORT (i.e. prolonged or intense stressors) have been shown to alter the cellular composition across the hippocampus via dendritic atrophy (reduced volume of dendritic spines), changes in the morphology of dendritic spines and the arrest of neurogenesis (Kim et al., 2015). Atrophy of dendritic spines in response to a chronic stressor have been reported in pyramidal neurons of the CA3, CA1 regions, as well as granule cells of the DG (McEwen, 2000). In additional to pyramidal and granular neuron dysfunction, literature has also shown that stress has the capacity to affect GABAergic interneurons in the hippocampus (Czéh et al., 2015; Rossetti et al., 2018; Kraus et al., 2022). More specifically, chronic mild unpredictable stress (9 weeks) reduced the number of parvalbumin interneurons in the CA3 and CA1 region of the hippocampus (Czéh et al., 2015). Thus, stress has the capacity to hinder cellular function across the entire hippocampus. In addition to stress-induced dysfunction of neurons within the hippocampus, increasing literature supports the role of glial cells in the

psychopathology associated with stress (Murphy-Royal et al., 2019). Astrocytes, the brain's most abundant glial cell (Sofroniew and Vinters, 2010), play a critical role in healthy neuronal function, such as: neurovascular coupling (Haydon and Carmignoto, 2006), neurotransmitter clearance (Bergles and Jahr, 1997), supplying L-lactate as an energy source for neurons (Giaume et al., 2010), and potassium buffering (Wallraff et al., 2006). Interestingly, mRNA expression profiles from the mouse brain, including the hippocampus, show that the expression levels of GRs in astrocytes is seven-fold higher than that of neurons (Zhang et al., 2014). As such, chronic and acute stress have been shown to reduce expression of glial fibrillary acidic protein (GFAP; an intermediate filament protein expressed abundantly in astrocytes) (Nichols et al., 1990; O'Callaghan et al., 1991; Banasr et al., 2010; Adedipe et al., 2022), reduce the expression of GLT-1 (glutamate transporter expressed predominately on astrocytes) (Reagan et al., 2004; Wood et al., 2004; Yu et al., 2019) and reduce the expression of connexin 30 (Murphy-Royal et al., 2020) and connexin 43 (Sun et al., 2012; Adedipe et al., 2022), two astrocyte-specific gap junction channel proteins responsible for the shuttling of metabolic substrates between these cells (Giaume et al., 2010). In addition to stress-induced structural changes in astrocytes, stress has been shown to impair hippocampal synaptic function by modulating the activity of astrocytes. More specifically, an acute swim stress constrains the shuttling of astrocyte-derived L-lactate to neurons. These defects in astrocyte-neuron lactate shuttling are accompanied by impairments of hippocampal LTP (Murphy-Royal et al., 2020).

1.4.3. The effects of stress on hippocampal synaptic plasticity

1.4.3.1. The effects of stress on basal synaptic transmission

The precise balance of excitatory and inhibitory neurotransmission in the hippocampus ensures optimal function. Dysregulation of the excitatory/inhibitory balance of individual cells in

the hippocampus, can result in global hippocampal dysfunction, and thus, behavioural deficits. In general, it seems that stress increases excitatory drive of CA1 neurons, and reduces inhibitory drive, creating a hyperexcitable synaptic phenotype (Al-Chami et al., 2020; Mei et al., 2020). In response to a chronic social defeat stress (CSDS; experimental mice are exposed to an aggressive CD-1 mouse to induce stress) stressed mice had an increase in the frequency of sEPSCs in the hippocampus (Mei et al., 2020). Similarly, maternal separation early life stress has shown to increase sEPSC frequency (Al-Chami et al., 2020). Interestingly, both stress paradigms were shown to decrease the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) (Al-Chami et al., 2020; Mei et al., 2020). The increases in sEPSCs frequency and decrease in sIPSC frequency, shifts the balance of excitation/inhibition to favour excitation. Consistent with these results, stress had the capacity to influence the basal intrinsic excitability of hippocampal CA1 neurons (Weiss et al., 2005; MacKenzie and Maguire, 2015). More specifically, chronic restraint stress, but not acute stress depolarizes the reversal potential for GABA. These changes in the GABA reversal potential were accompanied by increases in the frequency of AP firing in response to current, both indicating hyperexcitability of CA1 neurons (MacKenzie and Maguire, 2015). Furthermore, acute restraint stress in combination with foot shocks also resulted in an increase in action potential firing frequency in response to current injections (Weiss et al., 2005). Thus, stress has an impact on the activity of individual hippocampal neurons, which could influence the induction of long-term plasticity.

1.4.2.2. The effects of stress on long-term plasticity

The first report of stress-induced impairments of hippocampal LTP was noted in 1987 in the Schaffer-collateral pathway. Following an inescapable foot shock paradigm, rats had a lower magnitude of CA1 LTP in comparison to their naïve littermates (Foy et al., 1987). Since this

initial description of stress-induced impairments of hippocampal LTP, multiple reports have followed to establish that stress has the capacity to impair NMDA receptor-dependent hippocampal LTP in the Schaffer-collaterals (Diamond et al., 1990; Aleisa et al., 2006; Lesuis et al., 2019). For example, reduced hippocampal LTP was noted following an early-life maternal separation stressor (Lesuis et al., 2019), a chronic psychosocial defeat stress (Aleisa et al., 2006), and exposure to a novel environment (Diamond et al., 1990). Furthermore, impairments of hippocampal LTP are accompanied by an enhancement of CA1 LTD (Xu et al., 1997), suggesting that stress can have a dichotomous effect on synaptic plasticity in the hippocampus. Impairments of LTP are also noted in other subregions of the hippocampus such as the DG and CA3 regions (Shors and Dryver, 1994; Pavlides et al., 2002). Mice subjected to a chronic restraint stress (21 days, 6 hours per day) had marked impairments of LTP induction in the performant pathways (entorhinal cortex to DG) and mossy fiber inputs to the CA3 region 24 (Shors and Dryver, 1994; Pavlides et al., 2002).

Despite literature supporting stress-induced impairments of hippocampal LTP, there have been inconsistent reports of stress and hippocampal LTP impairments. These discrepancies in the literature have highlighted that the stress paradigm and the LTP stimulation paradigm used can influence whether stress impairs hippocampal LTP. For example, one study showed mice that underwent an acute restraint stress (1 session, 6 hours) had an impairment of LTP (Jin et al., 2015). However, an acute exposure to a two-way active avoidance conditioning paradigm (acute foot shocks) resulted in an enhancement of hippocampal LTP (Bartsch et al., 2021). While both paradigms are acute stressors, they have different effects on hippocampal LTP. Thus, the intensity and duration of the stressors may influence the effect stress has on hippocampal LTP. Additionally, the effects of stress on hippocampal LTP can also vary based on the LTP

stimulation paradigm being used (Blank et al., 2002; Kim and Diamond, 2002). Generally, stress has been shown to enhance TBS-induced LTP, but impair HFS-induced simulation (Blank et al., 2002; Kim et al., 2006). Overall, there is no concrete conclusion on whether LTP goes up or down or remains unaltered following stress.

Despite discrepancies in the literature, there has been an association between CORT and the resulting alterations of hippocampal LTP. More specifically, In-vivo (via CORT pellets) and in-vitro (exogenous application of CORT to brain slices) CORT applications have been shown to impair hippocampal LTP, when administered at high doses (100nM) (Diamond et al., 1992; Pavlides et al., 1996; Alfarez et al., 2002). These concentrations of CORT are similar to those that may be produced during an intense and prolonged stressor. Conversely, low to moderate amounts of CORT (0.5-10 nM) have been shown to facilitate and increase the magnitude of hippocampal LTP (Diamond et al., 1992). These levels of CORT are similar to those that may be produced by a less intense stressor. Therefore, stressors that result in chronic and high CORT production may impair LTP and stressors that result in low to moderate CORT production can facilitate the induction of LTP (Diamond et al., 1992). The dose-dependent effects of CORT on hippocampal LTP can be further highlighted by manipulating the activity of MRs and GRs. Since MRs respond to basal or low amounts of CORT, artificially activating these receptors could mimic a less intense stressor, and thus enhance LTP. Since GRs respond to high levels of CORT, their activity could reflect a strong and robust stressor, and thus, impair LTP (Diamond et al., 1992; Mitre-Aguilar et al., 2015). Avital et al. (2006) highlighted this concept by specifically antagonizing MRs (with spironolactone) or GRs (with mifepristone) prior to an acute swim stress and recorded hippocampal LTP experiments. When blocking GRs, acute stress produced a large increase in LTP. Since MRs are the activated receptor in this scenario, this suggests MRs

enhance LTP when bound with CORT. However, blocking MRs prior to stress resulted in an impairment of LTP. Since GRs are the activated receptor in this scenario, this suggests that GR activation impairs LTP (Avital et al., 2006). Thus, there are varying factors that contribute to outcomes that stress has on hippocampal LTP including: the length of the stressor, the amount of CORT produced by the stressor and the LTP induction paradigm. Further studies are needed to understand how these different variables can precisely modulate the impact of stress on hippocampal LTP.

1.5. Sex differences in the effects of stress on the hippocampus

Sex differences in hippocampal function and plasticity are also revealed following exposure to varying stress paradigms. On a behavioural scale, hippocampal-dependent behaviours show a great deal of sex differences following stress (Wood and Shors, 1998; Bowman et al., 2001; Yagi and Galea, 2019). In response to a chronic restraint stress (21 days, 6 hours a day) male mice had an impairment in the radial arm maze task (hippocampal-dependent measure of spatial memory). However, female mice had an enhancement of performance on the same task (Wood and Shors, 1998; Bowman et al., 2001; Yagi and Galea, 2019). More recently, a direct comparison was made between male and female mice following a chronic unpredictable intermittent restraint stress (30- or 60-minute restraints with variations in time of day, and time between restraints). Interestingly, performance on the radial arm maze task was only reduced in male mice following the stressor. Female mice were unaffected by the chronic stressor (Peay et al., 2020). Thus, this suggests there are consistencies in male mice having poor spatial memory following stress, and female mice have either an improvement or resilience following stress.

On a cellular scale, stress has dichotomous effects on spine morphology and neurogenesis within the hippocampus in male and female mice (Galea et al., 1997; Shors et al., 2001; Yagi and

Galea, 2019). The sex dependent effects of stress are noted across all subregions of the hippocampus but can vary depending on the region being studied. For example, acute stress increases the spine density of CA1 apical dendrites in male mice; however, decreases spine density in female mice (Shors et al., 2001; Yagi and Galea, 2019). In the CA3 region of the hippocampus, chronic stress decreases the complexity and density of dendrites in both male and female rats. However, the chronic stress paradigm has different effects on different subpopulations of dendrites in the hippocampus (i.e. apical dendrites in male rats, basal dendrites in female rates) (Galea et al., 1997; Yagi and Galea, 2019). In the DG, male mice exposed to a social isolation have an increase in the complexity of granular neuron morphology, which does not translate to female mice who remain unaffected (Juraska et al., 1985; Yagi and Galea, 2019). Thus, the sex differences on hippocampal cellular dysfunction can change depending on the subregion of the hippocampus being studied.

In addition to sex differences in hippocampal morphology following stress, hippocampal neurogenesis is affected in a sex-dependent manner (Hillerer et al., 2013; Yagi and Galea, 2019). In the CA1 region of the hippocampus, chronic restraint stress increases the rates of neurogenesis in male rats, but reduces the rate in female rats (Barha et al., 2011). Interestingly, the sex differences in neurogenesis have developmental implications, as the age at which the stress takes place influences the outcome. Mice that went through an early life maternal separation stress (postnatal day 2 to day 9) showed marked deficits in neurogenesis in adulthood. However, these deficits were only noted in adult male mice, with female mice being unaffected (Naninck et al., 2015). Interestingly, when postpartum rats (days 2 to 29; preweaning) were given CORT to mimic postpartum depression, there was a decrease in neurogenesis in adult female offspring, however, males were not affected (Gobinath et al., 2016). Thus, there are sex-differences in

neurogenesis following stress; however, they depend heavily on the age mice are exposed to the stress as well as the length of the stressor (7 days vs. 27 days).

1.5. Hypothesis and Aims

Stress can have detrimental effects on brain function and can impair hippocampal-dependent cognitive functions. Historically, females have been excluded from neuroscience studies, including the literature that investigates the effects of stress on the brain. Only recently have female rodents become increasingly represented in neuroscience research, and even then, literature is lacking making a direct comparison between male and female mice in response to stress. Additionally, the precise mechanisms of stress and GR activation on synaptic function and plasticity in male and female mice remains unclear. Thus, we wanted to compare the differences in hippocampal synaptic function and plasticity between male and female mice in the context of an acute swim stress.

The two major aims of my master's thesis are:

Aim 1: To characterize the neuroendocrine response to acute stress in male and female mice

Aim 2: To investigate the effects of acute stress on basal measures of hippocampal synaptic function and short- and long-term plasticity in male and female mice.

We hypothesize that female mice will exhibit a pronounced neuroendocrine response to an acute swim stress in comparison to their age-match male counterparts. The heightened neuroendocrine response will lead in hyperexcitability of CA1 neurons and impairments of hippocampal LTP.

2.0. Methods

2.1. Animals

Both male and female C57BL/6J mice (6-10 weeks) were used in the present study with ad libitum access to food and water. Mice were housed on a 12h:12h light: dark cycle (lights on at 6:30 am). Mice were group housed until the stress protocol. The acute stress protocol consisted of 20 minutes of swimming in a beaker of water (32 ℃) before placing the mouse in a single-housed cage for 90 minutes. Following the 90-minute recovery period, mice were sacrificed and used for experimental procedures. All experiments were performed in accordance with the guidelines for the maintenance and care of the Canadian Council on Animal Care (CCAC) and approved by the Institutional Committee for the Protection of Animals (CIPA) at the Centre Hospitalier de l'Université de Montréal.

2.2. Acute brain slice preparation

Adolescent male and female C57 mice (6-8 weeks old) were deeply anesthetized with isoflurane and transcardially perfused with ice-cold N-methyl-D-glucamine (NMDG) slicing solution saturated with 95% O2 and 5% CO2 containing the following (in mM): 119.9 NMDG, 2.5 KCL, 25 $NaHCO_3$, 1.0 $CaCl_2$-2 H_20, 6.9 $MgCl_2$ – 6 H_20, 1.4 NaH_2PO_4-H_20 and 20 glucose. The brain was rapidly removed and placed in the ice-cold NMDG solution. Acute brain slices (300μm) were cut in NMDG slicing solution on a Lecia VT1200 vibratome. Slices were transferred to an oxygenated NMDG slicing solution at 34 ℃ for 12 minutes for optimal recovery of slice health (Ting et al., 2018). Following the brief recovery, slices were maintained in oxygenated artificial cerebrospinal fluid (aCSF) containing (in mM): 130 NaCl, 2.5 KCL, 1.25 NaH_2PO_4, 1.3 $MgCl_2$, 2 $CaCl_2$, 10 Glucose and 26 $NaHCO_3$ at room temperature for at least 1 hour before experiments.

2.3. Electrophysiology

Acute brain slices were placed in the recording chamber and the hippocampus was visualized with a Scientifica SciCam Pro camera using a 4x objective. Oxygenated aCSF was continuously perfused over the slice at a flow rate of 1.5-2 ml/min. For recordings of LTP, glass pipettes (Precision Instruments; 1B100F) were pulled using Sutter Instruments P-97 pipette puller with a resistance of 1-3 mΩ and filled with aCSF. The Schaffer collateral pathway was stimulated by controlling an Iso-flex stimulator (AMPI) with digital outputs of a Digidata 1440A digitizer (Molecular devices). Field excitatory postsynaptic potential (fEPSPs) were evoked using a bipolar electrode at a frequency of 0.33Hz and the stimulus intensity was set to 30% of the maximal fEPSP response. For LTP experiments, stable fEPSP responses were recorded for a minimum of 10 minutes. To induce LTP, a high-frequency stimulation protocol (two trains of 100 pulses at 100Hz, 1s intertrain interval) was delivered. Following HFS, fEPSPs were evoked at a frequency of 0.33Hz for 40 minutes. The % potentiation was expressed as an increase in slope from baseline at the 0-3 minutes (PTP) and 35–40-minute (LTP) time points.

For whole-cell patch clamp experiments, glass recording pipettes with a resistance of 3-6 mΩ were filled with a potassium gluconate internal solution containing the following (in mM): 105 K Gluconate, 30 KCl, 10 Phosphocreatine, 10 HEPES, 4 ATP-Mg, 0.3 GTP-Tris, 0.3 EGTA. Neurons were visualized with a Scientifica SciCam Pro with a 40x objective. Recordings were made with a MultiClamp 700B amplifier and pClamp10 software (Molecular Devices). Data were low pass filtered at 1kHz and digitized at 10kHz with a digitizer (Digidata 1440; Molecular Devices). Access resistance was monitored and did not exceed 25 mΩ and cells that displayed a change of more than 30% throughout the recording were excluded. To measure sEPSCs, voltage clamp recordings were performed at a holding potential of -70mV. To measure

spontaneous action potentials and resting membrane potential, current clamp recordings were performed, and cells were recorded without any current input. Analysis of all parameters were performed offline using Clampfit (Molecular devices).

2.4 Immunohistochemistry

For the quantification of GR signaling in astrocytes and neurons, mice were transcardially perfused with 4% paraformaldehyde (PFA) and brains were immediately extracted and placed in PFA overnight at 4C and then cryoprotected with 30% sucrose in 0.01M phosphate buffer saline (PBS). Brains were flash frozen, embedded in optimal cutting temperature (OCT) compound and stored at -80 C. Brains were cryosectioned (-20 C) using a Lecia CM3050S cryostat to 30 μm. Free floating brain slices were washed three times in 0.01M PBS for 15 minutes and permeabilized in a block-perm solution (3% Bovine serum albumin (BSA), 0.5 % triton X-100 in 0.01M PBS) for one hour. After blocking slices were incubated with the following primary antibodies: rabbit anti-NeuN (1:250, NovusBio NBP1-77686) or rabbit anti-S100B (1:1000, Abcam Ab73593) co-stained with mouse anti-glucocorticoid receptor (1:500, ThermoFisher MA1-510) overnight at 4C. The following day, slices were again washed in 0.01M PBS. In the absence of light, slices were then incubated in the following secondary antibodies diluted in 0.01M PBS with 1:1000 DAPI: goat anti-rabbit Alexafluor 488 (1:1000, Jackson Immuno Research, 111-545-144) and goat anti-mouse Alexafluor 647 (1:1000, ThermoFisher A32728) for 1 hour. After secondary antibody incubation slices were washed with 0.01M PBS. Stained slices were mounted onto Fisherbrand microscope slides using ProLong glass antifade mountant (P36982).

2.5. Spinning disc confocal microscopy

Imaging of the CA1 region of slice-mounted hippocampal resections was performed using an ZEISS AxioObserver inverted spinning disc confocal microscope with an oil immersion 40x/1.3 NA objective. 16-bit images of 170 x 170 μm were acquired (frame size (x,y): 512x512). 20-30 μm z-stacks were acquired with a progressive z-step of 0.5 μm. For hippocampal CA1 analysis of neuronal and astrocyte GR, max intensity z-projections of were analyzed in Image-J. For measures of astrocyte and neuronal GR, astrocytes (S100B +) and neurons (NeuN) DAPI nuclei were thresholded and used as an ROI for nuclear measures of GR fluorescence (S100B+ DAPI + or NeuN+DAPI+). The integrated density of GR fluorescence was measured in each ROI to quantify nuclear astrocyte GR signalling and nuclear neuronal GR signalling using Fiji ImageJ.

2.6. Blood collection and corticosterone ELISA

Blood samples were collected through trunk blood collection. An additional cohort of mice were anesthetized with isoflurane, decapitated and trunk blood was collected into capillary blood collection tubes (BD 365963) and placed on ice. To isolate serum, blood samples were centrifuged at 4 ℃ for 5 minutes at 5000 rpm. Serum was aliquoted and stored at -80 ℃. Corticosterone measurements were obtained using an ENZO ELISA kit (ADI-900-097).

2.7. Statistics

Results are presented as mean ± SEM. The statical tests include: one-way ANOVA with post-hoc Dunnett's multiple comparisons test and unpaired t-tests. The statistical tests used in each experiment is stated within the results section. Graphic significant levels were *, $p<0.05$, **, $p< 0.01$ and *** $p<0.001$. GraphPad prism 9.3.1. was used to perform all statistical analyses (Version 9, GraphPad, USA). For all experiments, the goal number of mice per each condition was alteast 3 mice per condition; however, due to experimental error, certain parameters have

less mice per condition. The reported N-values represents the number of mice, and n-values represent the number of cells (patch clamp experiments), slices (field recordings) or fixed brain slices (immunohistochemistry)

3.0. Results

3.1. The neuroendocrine response to acute stress in male and female mice

To characterize the CORT (ng/mL) response in male and female mice exposed to acute stress, we collected trunk blood from mice after a 20-minute forced swim stress paradigm. Blood was collected from group-housed naïve mice (N = 4) at 0 minutes (N = 5) 45 minutes (N= 4), and 90 minutes (N = 5) after the stress (Figure 5A). One-way ANOVA analysis of CORT levels at different time points in male mice demonstrated a significant effect of time after stress ($F = 71.08$, $p<0.0001$, Figure 5B). Post-hoc analysis further demonstrated a significant difference between naïve (21.17 ng/ml) vs. 0 min post-swim (0 min: 231.8 ng/ml, $p<0.0001$, Figure 5B) and naïve vs. 45 min post-swim (45 min: 126.0 ng/ml, $p = 0.001$, Figure 5B). At the 90 min post-swim time point, CORT was not significantly different from naïve levels (90 min: 39.37, $p = 0.5674$, Figure 5B). These results highlight the time-sensitive effects of CORT elevations following the acute swim stress paradigm. In female mice, One-way ANOVA analysis of CORT levels at different timepoints after the swim stress showed no significant effect of time ($F=0.8681$, $p= 0.4866$). Post-hoc analysis showed no significant difference between naïve vs 0 min (Naïve: 50.75 ng/ml, N=4; 0 min: 101.5 ng/ml, N= 3; $p=0.4015$, Figure 5C), vs. 45 min (45 min: 68.61ng/ml, N=4, $p = 0.9120$, Figure 5C), and vs. 90 min (90 min: 92.80 ng/ml, N=4; $p = 0.4844$, Figure 5C). There were no differences between naïve male and female serum CORT levels (Supplemental Figure 1A). These results suggest a sexual dimorphism of the CORT response to acute stress. Male mice have a pronounced, and immediate CORT response that reverts to naïve values following the 90-min recovery period. However, in female mice, there is a lack of increase in CORT following the stress paradigm.

Next, we investigated whether changes in blood CORT also result in changes in GR expression across different cell-types at the 90 minute timepoint following swim stress. We conducted immunostaining with an anti-GR antibody co-stained with NeuN or S100B to quantify neuronal and astrocyte GR expression levels, respectively (Figure 5D). More specifically, we looked at the nuclear translocation of GRs defined as co-expression with a DAPI marker. These experiments were conducted in two different cohorts: cohort 1: Male Naïve and Acute Stress, cohort 2: Female Naïve and Stress. Thus, due to potential confounding factors between cohorts such as antibody incubation times and experimental variance, fluorescence data were not compared between male and female mice. In male mice, unpaired t-tests revealed a significant increase in the Integrated Density (IntDen) of S100B+, GR+, DAPI+ in stressed mice compared to their naïve counterparts (Naïve: 7122 AU, n = 13; Stress: 10062, n= 12; p = 0.0336, Figure 5E). The IntDen of NeuN+, GR+, DAPI+ cells were not significantly different between male naïve and stressed mice (Naïve: 3154 AU, n = 12; Stress: 3693 AU, n = 12; p = 0.2700, Figure 5G). In female mice, there were no significant changes in the IntDen of S100B+, GR+, DAPI+ expression 90 minutes after the swim stress (Naïve: 8521 AU, n = 12; Stress: 6604 AU, n= 12; p = 0.1200, Figure 5F). Similarly, there were no significant differences in the IntDen of NeuN+, GR+, DAPI+ expression (Naïve: 6601 AU, n =12; Stress: 8174 AU, n= 12; p = 0.0706, Figure 5H). Thus, GR signalling seems to be specifically increased in male mice following our acute stress paradigm. More specifically, astrocytes have a specific increase in the nuclear translocation of GRs in male mice. In female mice, the lower CORT response did not increase GR signalling in astrocytes or neurons.

3.2. The effects of acute stress on basal hippocampal synaptic function in male and female mice

Since the activation of GRs has been shown to impair hippocampal function and cognitive behaviours (Kim and Diamond, 2002; Kim et al., 2006), we sought to investigated how the stress-induced increase of CORT and GR signalling could have a impact on hippocampal synaptic function. Since the function of individual CA1 neurons can influence the hippocampus at the circuit level, we conducted whole-cell current clamp recordings of spontaneous action potentials (sAPs) and resting membrane potential (RMP) were recorded from male (Figure 6A) and female (Figure 6D) mice following acute stress to assess intrinsic excitability of CA1 neurons. Unpaired t-test results showed that stressed male mice have a significant depolarization of CA1 RMP (Naïve: -53.59 mV, n =8; Stress: -48.37mV, n = 9; p = 0.0264, Figure 6B). The changes in RMP were accompanied by a significant increase in the frequency of sAPs (Naïve: 0.4754 Hz, n = 6; Stress: 3.292 Hz, n = 8; p = 0.0067, Figure 6C). In female mice, stress did not significantly alter the RMP of CA1 neurons (Naïve: -58.84 mV, n = 7; Stress: -55.69 mV, n = 7; p = 0.4618, Figure 6E). However, stress increased the frequency of sAPs in female CA1 neurons following stress (Naïve: 0.3219 Hz, n = 6; Stress: 1.538 Hz, n = 6; p = 0.0217, Figure 6F). There were no significant differences in RMP and sAP frequency between naïve male and female mice (Supplemental Figure 1B-C). The depolarization of the RMP in male mice following stress is likely the underlying mechanism that contributes to increased sAP firing of CA1 neurons. However, in females, since stress did not alter the RMP of CA1 neurons but increased the sAP firing frequency; differences in waveform properties of action potential firing may contribute to increased sAP firing frequency. Thus, using time-derivative analysis, we analyzed the afterhyperpolarization (AHP, mV) and threshold (mV) of sAPs to determine whether increased

sAP firing could be correlated to changes in these parameters (Figure 6G). There were no significant differences in AHP or threshold values between naïve male and female mice (Supplemental Figure 1D-E). In male mice, unpaired t-tests showed no significant effect of stress on the AHP (Naïve: -7.980 mV, n = 7; Stress: -6.500 mV, n = 5; p = 0.1855, Figure 6I) or sAP threshold (Naïve: 3.818 mV, n = 7; Stress: 3.144 mV, n = 5; p = 0.5126, Figure 6H). Similarly, in female mice, stress had no significant effect on sAP threshold (Naïve: 3.363 mV, n = 4; Stress: 2.499 mV, n = 6; p = 0.1301, Figure 6K) and sAP AHP (Naïve: -6.913 mV, n = 4; Stress; -6.000 mV, n = 4; p = 0.5242, Figure 6J). Overall, these results suggest that acute stress increases the intrinsic excitability of CA1 neurons by increasing the frequency of sAPs in male and female mice.

Since increased excitatory inputs onto CA1 pyramidal neurons could be a potential mechanism for the increased sAP firing after stress in male and female mice, we next wanted to determine whether excitatory inputs onto individual CA1 neurons can be altered by stress. Here, I conducted whole-cell voltage clamp recordings of CA1 neurons at -70mV to measure sEPSCs (Figure 7A, E). The peak amplitude (pA), interevent interval (ms) and, frequency (Hz) of sEPSCs were quantified in male and female mice following stress (Figure 7). There was no significant difference in the peak amplitude of sEPSCs in naïve male vs female mice (Supplemental Figure 1F). However, naïve female mice have a significantly lower interevent interval compared to naïve male mice (Supplemental Figure 1G; p=0.0084). In male mice, unpaired t-tests showed a significant decrease in the interevent interval of sEPSCs in response to stress (Naïve: 644.3 ms, n = 8; Stress: 418.4 ms, n = 11 ; p = 0.0061, Figure 7C). Accordingly, stress also increased the frequency of sEPSCs in male mice (Naïve: 1.711 Hz, n = 8; Stress: 2.701 Hz, n = 11; p = 0.0151, Figure 7D). However, there was no significant difference in the

peak amplitude of sEPSCs (Naïve: -23.22 pA, n = 8; Stress: -25.14 pA, n = 11; p = 0.4284, Figure 7B). In female mice, acute stress has no significant effect on the interevent interval (Naïve: 327.6 ms, n = 4; Stress: 318.9 ms, n = 7; p =0.9253, Figure 7G), frequency (Naïve: 0.9401 Hz, n = 4; Stress: 1.011 Hz, n = 7; p = 0.7728, Figure 7H), or peak amplitude (Naïve: -21.01 pA, n = 4; Stress: -19.74 pA; p = 0.5018, Figure 7F) of sEPSCs. These results suggest that in male mice, stress increases the frequency of excitatory inputs onto pyramidal CA1 neurons, with no effect in female mice.

3.3. The effects of acute stress on hippocampal synaptic plasticity

The coordinated activity of individual neurons in the hippocampus contributes to its ability to undergo activity-dependent synaptic plasticity. Since stress altered the function of individual CA1 neurons, we wanted to see how these individual neuronal changes can influence the induction of short-term and long-term plasticity after stress. Thus, we recorded fEPSPs in the Schaffer collateral pathway of the hippocampus and recorded short-term and long-term plasticity in male and female mice. To measure short-term plasticity, paired fEPSP pulses were recorded at varying time intervals (50ms, 100ms, and 250ms) to measure paired-pulse ratio (PPR) (Figure 8). PPR was quantified by dividing pulse 2 / pulse 1, and the resulting ratio gives PPR. An increase in PPR suggests heightened presynaptic release probability and decreased PPR suggests a reduced release probability. When comparing naïve male mice to naïve female mice, there were no significant differences in PPR at 50ms, 100ms or 250 ms (Supplemental Figure 1H-J). In male mice, unpaired t-tests of PPRs demonstrate a significant decrease in PPR at 50ms (Naïve: 2.150, n = 5; Stress 1.679, n = 7; p = 0.0318, Figure 8C) and 100ms (Naïve: 2.040 , n = 5, Stress: 1.505, n = 7; p = 0.0258, Figure 8D) intervals following acute stress. However, when the interval was extended to 250ms, there was no significant difference in PPR (Naïve: 1.540, n = 5, Stress:

1.384, n = 7; p= 0.2067, Figure 8E). The longer interval (250ms) is expected to not have as pronounced of an effect, as the longer interval between pulses will result in less accumulation of neurotransmitter in the presynaptic terminals during neurotransmission (Regehr, 2012). In female mice, stress did not influence short-term synaptic plasticity (Figure 8F). More specifically, unpaired t-tests showed no significant difference between the 50ms (Naïve: 1.923, n = 7, Stress: 2.202, n = 6; p = 0.1665, (Figure 8H), 100ms (Naïve: 1.829, n = 7, Stress: 1.995, n = 6; p = 0.4112, Figure 8I) and 250 ms (Naïve: 1.394, n = 7, Stress: 1.645, n = 6; p = 0.2297, Figure 8J) time internals. These findings suggest that release probability is reduced in male mice and remains unaltered in female mice following acute stress.

To determine whether long-term plasticity is affected by stress in male and female mice, LTP was recorded in the Schaffer collateral pathway of the hippocampus (Figure 9). PTP was quantified at the 0–3-minute timepoint, and LTP was quantified at the 35–40-minute timepoint following HFS (Figure 9C, H). In male mice, unpaired t-tests revealed a signification reduction in the % potentiation during the PTP timepoint (Naïve: 239.7 %, n = 5; Stress: 82.23 %, n = 5; p = 0.040) (Figure 9D). Similarly, there was a significant reduction in %potentiation at the LTP timepoint (Naïve: 73.12 %, n = 5; Stress: 32.69 %, n = 5; p = 0.0324) (Figure 9E). In female mice, stress did not have any significant effect on the % potentiation at the PTP timepoint (Naïve: 221.1 %, n = 7; Stress: 198.4 %, n = 7; p = 0.7168) (Figure 9I) or the LTP timepoint (Naïve: 57.0 %, n = 7; Stress: 73.90 %, n = 7; p = 0.3759) (Figure 9J). There was no difference in the magnitude of PTP or LTP between male and female mice (Supplemental Figure 1K-L). These results suggest that male mice have pronounced deficits in PTP and LTP following stress, and female mice remain unaffected. Thus, the whole-cell recordings of individual CA1 neurons

that highlighted deficits in male mice translate to dysfunction of Schaffer-collateral plasticity following stress.

4.0. Discussion

4.1. Summary of main results

The goal of the current thesis was to investigate the effects of acute stress on hippocampal synaptic function in male and female mice. We hypothesized that an acute swim stress would have a greater effect on hippocampal synaptic plasticity in female mice, compared to male mice. Surprisingly, we found that male mice had a robust increase in CORT in response to the acute swim stress, while female mice had no major changes in CORT following the stress. Accordingly, male mice had an increase in the nuclear translocation of astrocyte GRs, with no change in neuronal GR signalling. In female mice, both neuronal and astrocyte GR signalling were not changed by the acute stress paradigm. We also found that both male and female mice have an increase in the intrinsic excitability of CA1 neurons via an increase in the frequency of CA1 sAPs with acute stress. However, only male mice had an increase in the excitatory inputs onto CA1 neurons after acute stress. Finally, only male mice had a significant impairment of PPR, PTP and LTP following acute swim stress. Based on these findings, we suggest that female mice are much less sensitive to the stress-induced impairments of hippocampal function in response to an acute swim stress, while male mice have pronounced hippocampal synaptic dysfunction.

4.1. Acute stress has an effect on CORT production in male mice, but not female mice

Acute stress has been shown to promote hyperactivation of the HPA axis resulting in a rapid, but transient increase in CORT. Additionally, there are innate differences in HPA axis function between male and female mice at basal levels, with female mice having a heightened HPA axis activity compared to male mice (Handa et al., 1994; MacLusky et al., 1996; Weiser and Handa, 2009; Babb et al., 2013). This forms the hypothesis that female mice will have a stronger CORT response to an acute swim stress compared to male mice. Thus, we set to

determine the effects of our acute swim stress paradigm on the CORT profiles in male and female mice. We showed that a 20-minute swim stress drastically and immediately increases CORT production in male mice which gradually returns to the CORT values of their naïve littermates, 90 minutes after the stress. The time course of CORT release (i.e. high initially, and low at 90 mins) suggests that CORT has been removed from the periphery and exerted its effects on the brain. However, in female mice, the swim stress paradigm did not increase CORT levels from their naive counterparts at any point following the swim stress. Thus, the lack of CORT response in female mic suggests that they are unaffected, or even resilient to the effects of an acute swim stress protocol. These are exiciting preliminary results; however, the lower sample size in the female cohorts could underpower our statistical analysis, and thus, increasing the number of mice in these groups would further validate these findings. Nevertheless, these results were not as we expected, as previous literature suggests that female mice generally have higher CORT values in response to acute and chronic stress compared to male mice (Handa et al., 1994; Viau et al., 2005; Larkin et al., 2010). A potential explanation for these results could be the effect of the acute swim stress on female reproductive hormones, which could be interacting with the HPA axis at varying levels (Larkin et al., 2010). Thus, future experiments would be to correlate rises of estradiol with the serum CORT levels in female mice and the levels of androgens in female mice to elaborate on the hormonal influence during the response to an acute swim stress.

4.2. Acute stress increases GR translocation in male mice, but not female mice

The effects of stress on the brain are mediated largely by CORT binding to low-affinity GRs. More specifically, GR signalling in neurons has been well established to contribute to the stress response and activation of these receptors can result in neuronal dysfunction (Diamond et

al., 1992; Pavlides et al., 1995). However, the contribution of non-neuronal cell type GR signalling in mediating the effects of stress on the hippocampus remains underdeveloped. Here, we wanted to investigate whether the CORT changes following acute swim stress in male and female mice resulted in changes in neuronal or astrocyte GR signalling. Surprisingly, in male and female mice, there was no change in neuronal nuclear GR expression in response to an acute swim stress. Additionally, there was no increase in astrocyte nuclear GR expression in female mice in response to stress, but male mice had a pronounced increase in nuclear GR expression in astrocytes. These results suggest two things: 1) The lack of CORT response in female mice is consistent with a lack of nuclear GR signalling in astrocytes and neurons, 2) astrocytes serve as a putative target for the effects of stress on the hippocampus in male mice. The expression levels of the mRNA for GRs in astrocytes is 7-fold that of neurons, making them a central target for peripherally released CORT (Zhang et al., 2014). Thus, acute stress increasing only astrocyte GR expression levels could be due to their vulnerability to the effects of CORT. Additionally, recent literature has implicated astrocyte GR signalling in mediating the effects of stress on the medial prefrontal cortex (mPFC) and the lateral amygdala (Adedipe et al., 2022; Lu et al., 2022). In the mPFC, genetic ablation of the *Nr3c1* gene (gene for GR) in astrocytes increases the susceptibility to a social defeat stress. Astrocyte GR knock-out increased depressive-like behaviours (i.e. increased immobility in response to a forced swim test) and increased social avoidance following a social defeat stress (Lu et al., 2022). In the lateral amygdala, targeting astrocyte GRs has a neuroprotective effect in response to an early-life stress (ELS). In response to ELS, mice had reduced performance on an auditory fear discrimination memory task; however, ablation of astrocyte GRs rescues the ELS-induced impairments of memory (Adedipe et al., 2022). In addition to mediating the stress response, astrocyte GRs have also been implicating in regulating

astrocyte proliferation (Crossin et al., 1997; Unemura et al., 2012), neurodegeneration (Maatouk et al., 2019) and opioid reward processing (Skupio et al., 2020) making these receptors vital for proper astrocyte function. Thus, our results suggest that astrocyte GR signalling could be a putative target for the stress-induced impairments of hippocampal function in male mice.

4.3. Sexually dimorphic effects of acute stress on hippocampal synaptic function

Acute stress results in a rapid amnesia that results in cognitive dysfunction, including deficits in hippocampal-dependent memories (Diamond et al., 1999; Bowman et al., 2001; Kim et al., 2015). The aberrant functioning of the hippocampus in response to acute stress can be related to the fact that acute stress also results in hyperexcitability of individual hippocampal pyramidal neurons (Weiss et al., 2005; MacKenzie and Maguire, 2015) or LTP (Shors and Dryver, 1994; Pavlides et al., 2002; Aleisa et al., 2006; Murphy-Royal et al., 2020). Despite a clear sex-differences in basal hippocampal synaptic plasticity, few studies have directly compared the effects of acute stress on hippocampal synaptic function and plasticity in male and female mice. To determine the sex-differences in basal hippocampal synaptic function, we focused on measuring the sAP firing in hippocampal CA1 neurons and sEPSC inputs to CA1 neurons. Acute stress increased the frequency of sAPs in male and female mice; but only depolarized the RMP of CA1 neurons in male mice. These changes indicate hyperactivity of CA1 neurons after stress in both male and female mice, which could lead to global hippocampal dysfunction. In male mice, the increase in sAP firing frequency could be related to the increase in sEPSC frequency, as an increase in excitatory inputs to a cell with a depolarized RMP will increase the firing rate of CA1 neurons. In female mice, the lack of changes in RMP and action potential properties suggests an alternative mechanism for increased sAP firing. While difficult to elucidate a precise mechanism, potential target points could be: voltage-gated sodium channel currents (Nav1.6, the major channel located in CA1 neurons) (Zybura et al., 2020), voltage-gated

potassium channel currents (transient K+ channels) (Akemann and Knöpfel, 2006) or sodium-potassium pump activity (Pivovarov et al., 2019). The activity of these currents may be modulated by stress, independently of the RMP. In addition to sAP firing, we looked at the excitatory inputs to CA1 neurons by recording sEPSCs in male and female mice exposed to acute stress. Our results show that only male mice have an increase in the frequency of excitatory inputs to CA1 neurons, while female mice are unaffected by stress. The increase in sEPSC frequency suggests more frequent release of glutamate from presynaptic terminals onto CA1 neurons; however, recording miniature EPSCs would give precise pre- vs post-synaptic mechanisms (Malkin et al., 2014). These results highlight another potential mechanism for why male mice have an increase in sAP firing patterns with stress. Since their RMP is depolarized (and more likely to fire), the increase in spontaneous excitatory inputs can increase the number of postsynaptic sAPs. The lack of an effect in female mice could be related to the fact that they do not have an exaggerated CORT response to the acute swim stress, as their serum CORT levels did not significantly change across anytime point, nor did the expression of neuronal or astrocyte GR levels. CORT (via the activation of MRs and GRs) has been shown to elevate the frequency and amplitude of EPSC in cultured neurons (Chatterjee and Sikdar, 2014)and thus, the neuroendocrine response following the acute swim stress is likely the mechanism of action for the alterations in synaptic properties of the hippocampus.

After we quantified basal synaptic parameters, we next wanted to compare the effects of stress on activity-dependent synaptic plasticity in male and female mice. Here, we found that an acute swim stress impairs short-term plasticity (PPR and PTP) and LTP in male mice. This suggests that male mice have an impairment on presynaptic release probability (i.e reduced PPR) and have marked deficits in the ability to potentiate the postsynaptic response after HFS (i.e

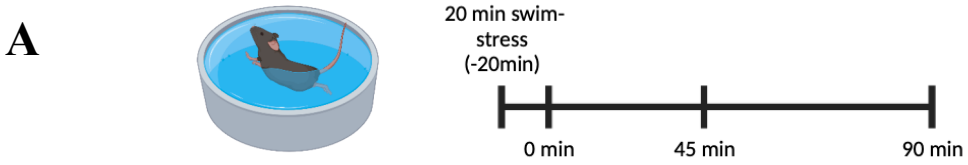
reduced PTP and LTP). However, both parameters remain unaffected in female mice. A potential hypothesis for the sex-differences of stress on hippocampal synaptic plasticity may be due to sex-specific alterations of the astrocyte-neuron lactate shuttle (ANLS). The ANLS provides a framework where astrocytes supply metabolic substrates (i.e., L-lactate) to neurons to sustain their high energy demands (Mason, 2017). The delivery of lactate from the ANLS has been shown to play a role in potentiating PPR in CA3 pyramidal neurons (Herrera-López et al., 2020) as well as being essential for the induction of LTP in the hippocampus (Suzuki et al., 2011; Descalzi et al., 2019). Additionally, the same 20-minute swim stress protocol has been shown to impair hippocampal LTP by reducing the metabolic shutting of L-lactate from astrocytes to neurons in male mice. Furthermore, when supplementing exogenous L-lactate, stress-induced impairments of hippocampal LTP were rescued (Murphy-Royal et al., 2020). However, most of our knowledge of stress-induced impairments of the ANLS is derived from studies conducted in male mice. In female mice, a recent study showed that knock-out of lactate dehydrogenase A (catalyzes the formation of L-lactate) in astrocytes promotes in the mPFC promotes a depressive-like and anxiety-like phenotype (Yao et al., 2023). However, the relationship between astrocyte lactate dynamics and stress-induced synaptic dysfunction remains unexplored in female mice. Thus, since the acute swim-stress: 1) increases astrocyte GR signalling only in male mice, and not female mice and 2) impairs the shutting of L-lactate from astrocytes to impair hippocampal LTP, it may be possible that acute stress does not alter the metabolic shutting of astrocyte L-lactate to neurons in female mice, and thus, does not impair PPR or LTP. Exogenous applications of CORT have been shown to alter the metabolic shuttling of substrates between astrocytes (Murphy-Royal et al., 2020), and thus, the lack of CORT response in females would allow astrocytes to function properly and supply lactate to neurons.

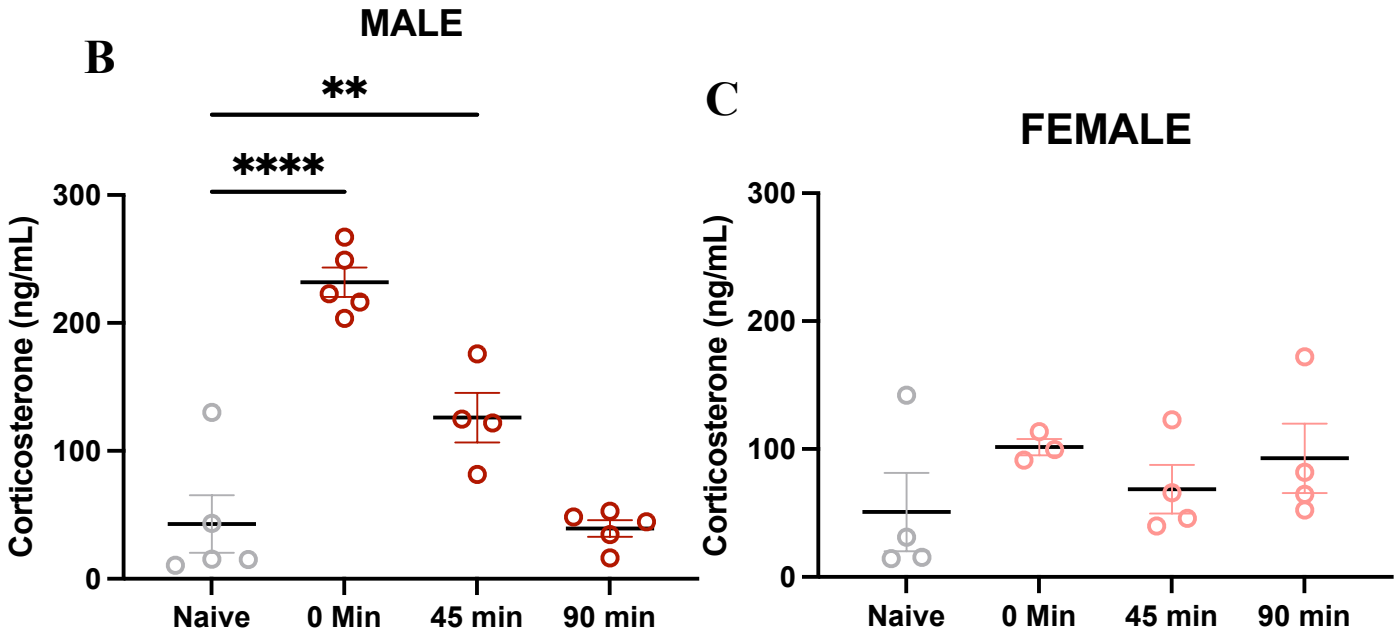
4.4. Limitations and future studies

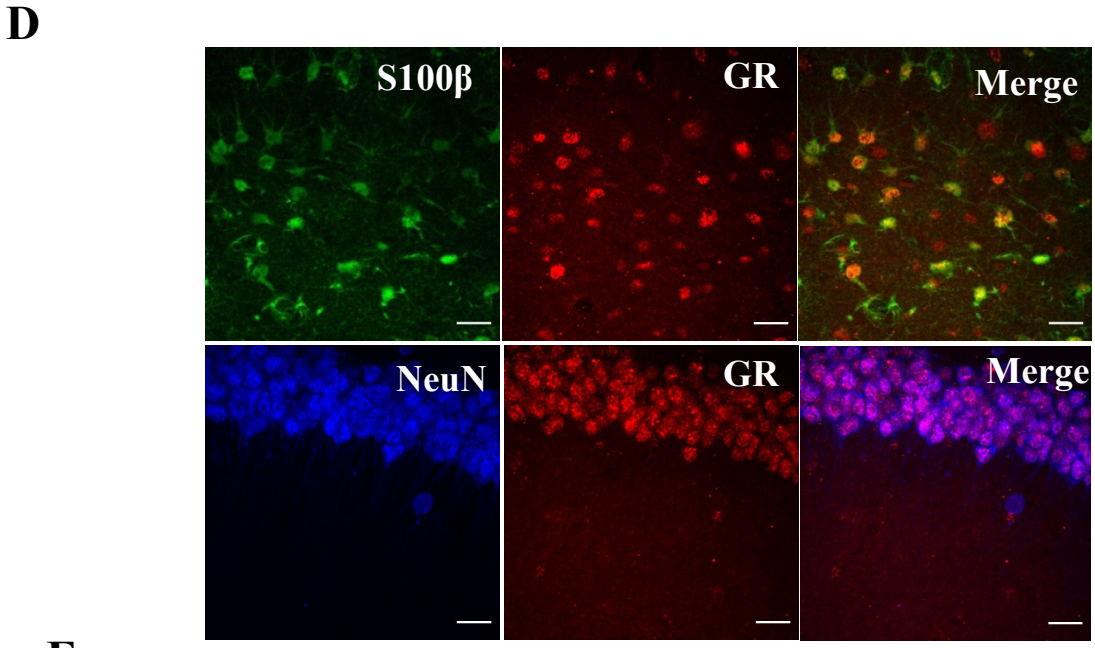
The limitations of the present study include a low sample size in the female cohorts of naïve and stressed mice. The low sample size will not provide adequate power for the analysis to reach statistical significance. Thus, the lack of an effect of the acute stress on female mice could be related to the low sample size rather than a sex-dependent effect of stress on the hippocampus. Secondly, a limitation of the present study was neglecting GR signalling in other non-neuronal cell types such as microglia and oligodendrocytes. Recent literature supported a role for microglia GR signalling in mediating the effects of chronic unpredictable mild stress paradigms on the hippocampus (Woodburn et al., 2023). Thus, investigating whether other non-neuronal cell types are involved in the acute swim stress response could give further insight to the precise molecular mechanisms contributing to stress-induced impairments of hippocampal function.

Future directions could focus on whether the acute swim stress paradigm results in a sex-dependent effect on hippocampal-dependent behaviours. Since LTP contributes heavily to the consolidation of long-term memory (Ishihara et al., 1997) and has been shown to be induced in the hippocampus during a radial arm maze (a measure of spatial memory), impairments of LTP should correlate to reduced performance on hippocampal-dependent behaviours. Thus, correlating the LTP impairments in the hippocampus with behavioural deficits in male mice would be an interesting future experiment to conduct. Additionally, whether the sexual dimorphisms continue to exist on hippocampal-dependent behaviours would be an interesting avenue to explore. Secondly, whether targeting the neuroendocrine response could prevent stress-induced hippocampal dysfunction in male mice could provide an interesting avenue for future experiments. In the PVN, administration of metyrapone (a CORT synthesis inhibitor) was successful at blocking the effects of social isolation stress in males and female, where there was

a pronounced CORT response (Senst et al., 2016). Thus, whether metyrapone prevents stress-induced synaptic dysfunction in male mice, and would be ineffective in female mice (due to their lack of CORT response) could further validate our findings. Finally, a future direction could be targeting astrocyte GR signalling in male mice to rescue the stress-induced impairments on hippocampal synaptic dysfunction and plasticity. These manipulations have been shown to be effective in a chronic, ELS paradigm in the lateral amygdala (Adedipe et al., 2022); however, whether these results translate to the hippocampus, and to an acute stress paradigm could provide fascinating insight to the role of astrocytes in mediating the effects of stress.


A
20 min swim-stress (-20min)
0 min
45 min
90 min


B
MALE
**
****
Corticosterone (ng/mL)
300
200
100
0
Naive
0 Min
45 min
90 min
C
FEMALE
Corticosterone (ng/mL)
300
200
100
0
Naive
0 Min
45 min
90 min


D
S100β
GR
Merge
NeuN
GR
Merge

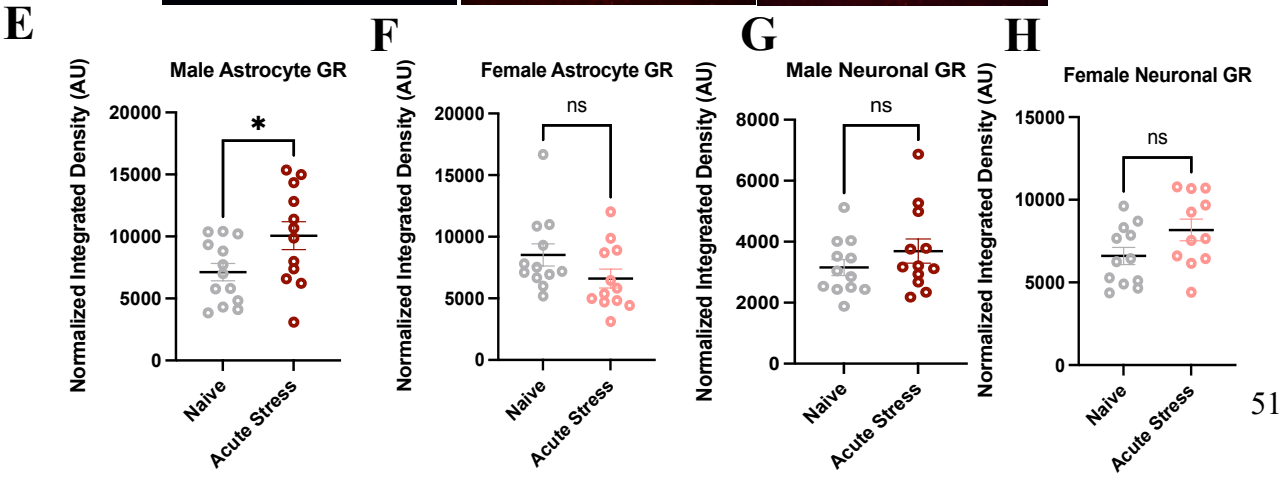

E
Male Astrocyte GR
Normalized Integrated Density (AU)
20000
15000
10000
5000
0
*
Naive
Acute Stress
F
Female Astrocyte GR
Normalized Integrated Density (AU)
20000
15000
10000
5000
0
ns
Naive
Acute Stress
G
Male Neuronal GR
Normalized Integreated Density (AU)
8000
6000
4000
2000
0
ns
Naive
Acute Stress
H
Female Neuronal GR
Normalized Integrated Density (AU)
15000
10000
5000
0
ns
Naive
Acute Stress

Figure 5: The neuroendocrine response to an acute swim stress in male and female mice. **A.,** Representative experimental timeline of a 20-minute forced swim stress followed by CORT collection at 0 min, 45 min, and 90 min after the swim. **B-C.,** Aligned dot plot (mean ± SEM) showing the serum corticosterone (ng/ml) response in male and female mice 0, 45 and 90 min after a 20-minute swim stress. **D.,** Representative spinning disc confocal microscopy images at 60x magnification of CA1 S100B (Green), NeuN (blue) merged with GR (red). Scale bar = 20 μm. **E-F.,** Aligned dot plot (mean ± SEM) showing neuronal and astrocyte GR expression from male naïve and stressed mice. **G-H.,** Aligned dot plot (mean ± SEM) showing neuronal and astrocyte GR expression from female naïve and stressed mice. Unpaired t-test results are indicated by *$p<0.05$, **$p<0.01$, ***$p<0.001$

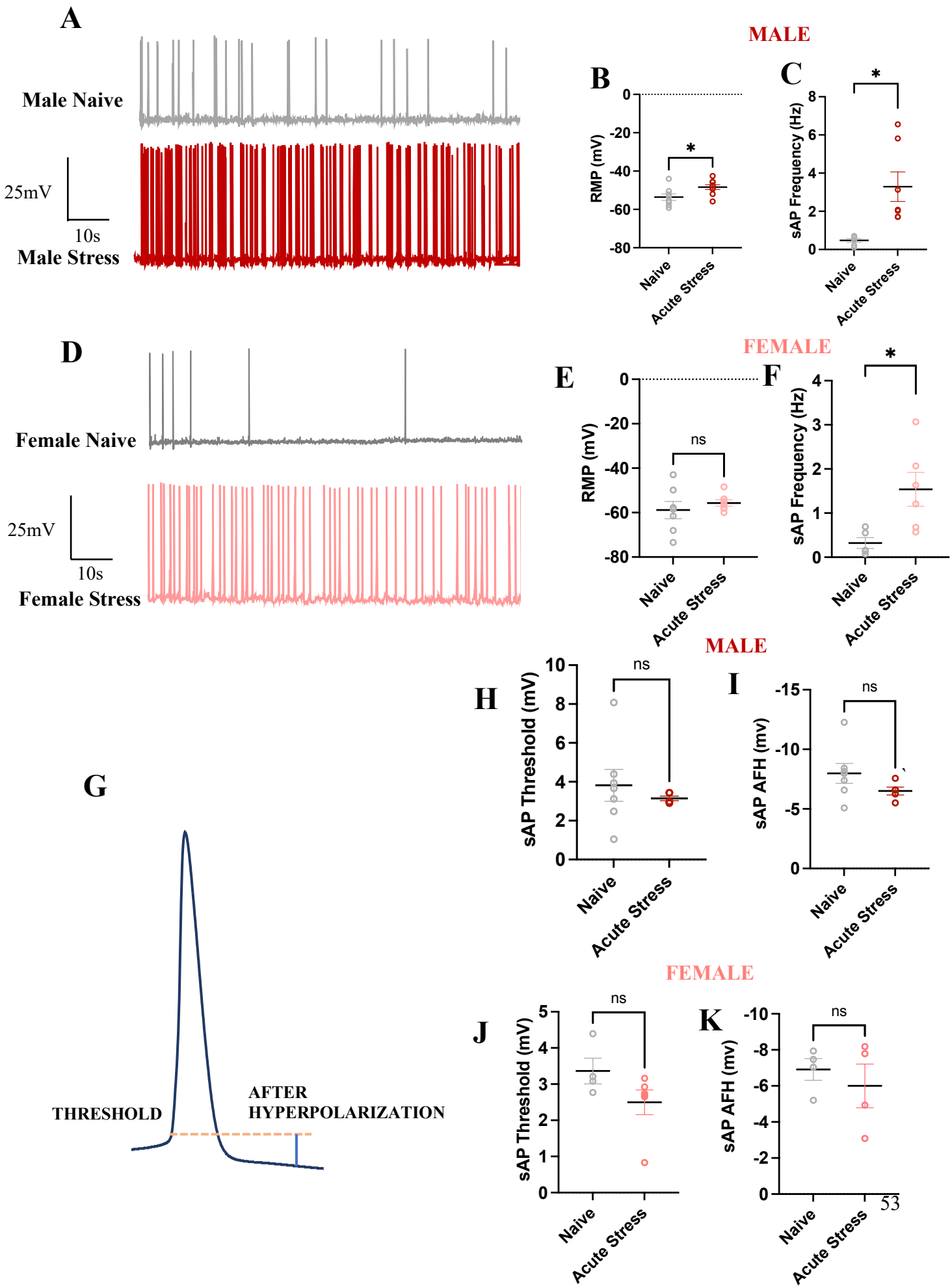

A
Male Naive
25mV
10s
Male Stress
MALE
B
RMP (mV)
0
-20
-40
-60
-80
*
Naive
Acute Stress
C
sAP Frequency (Hz)
8
6
4
2
0
*
Naive
Acute Stress
D
Female Naive
25mV
10s
Female Stress
FEMALE
E
RMP (mV)
0
-20
-40
-60
-80
ns
Naive
Acute Stress
F
sAP Frequency (Hz)
4
3
2
1
0
*
Naive
Acute Stress
MALE
H
sAP Threshold (mV)
10
8
6
4
2
0
ns
Naive
Acute Stress
I
sAP AFH (mv)
-15
-10
-5
0
ns
Naive
Acute Stress
G
THRESHOLD
AFTER
HYPERPOLARIZATION
FEMALE
J
sAP Threshold (mV)
5
4
3
2
1
0
ns
Naive
Acute Stress
K
sAP AFH (mv)
-10
-8
-6
-4
-2
0
ns
Naive
Acute Stress

Figure 6: The effects of acute stress on spontaneous action potential firing and waveform. **A.,** Representative current clamp recordings spontaneous action potentials in naïve and stress male mice. **B-C.,** Aligned dot plot (mean ± SEM) showing resting membrane potential (mV) and sAP frequency (Hz) in male naïve and stressed mice. **D.,** Representative current clamp recordings spontaneous action potentials in naïve and stress female mice. **E-F.,** Aligned dot plot (mean ± SEM) showing resting membrane potential (mV) and sAP frequency (Hz) in female naïve and stressed mice. **G.,** Sample action potential from a naïve mouse showing the waveform properties analyzed: threshold (mV) and afterhyperpolarization (mV). **H-I.,** Aligned dot plot (mean ± graphs showing the sAP threshold (mV) and sAP afterhyperpolarization (mV) in male naïve and acutely stress mice.). **J-K.,** Aligned dot plot (mean ± graphs showing the sAP threshold and sAP afterhyperpolarization in female naïve and acutely stress mice. Unpaired t-test results are indicated by *$p<0.05$, **$p<0.01$, ***$p<0.001$

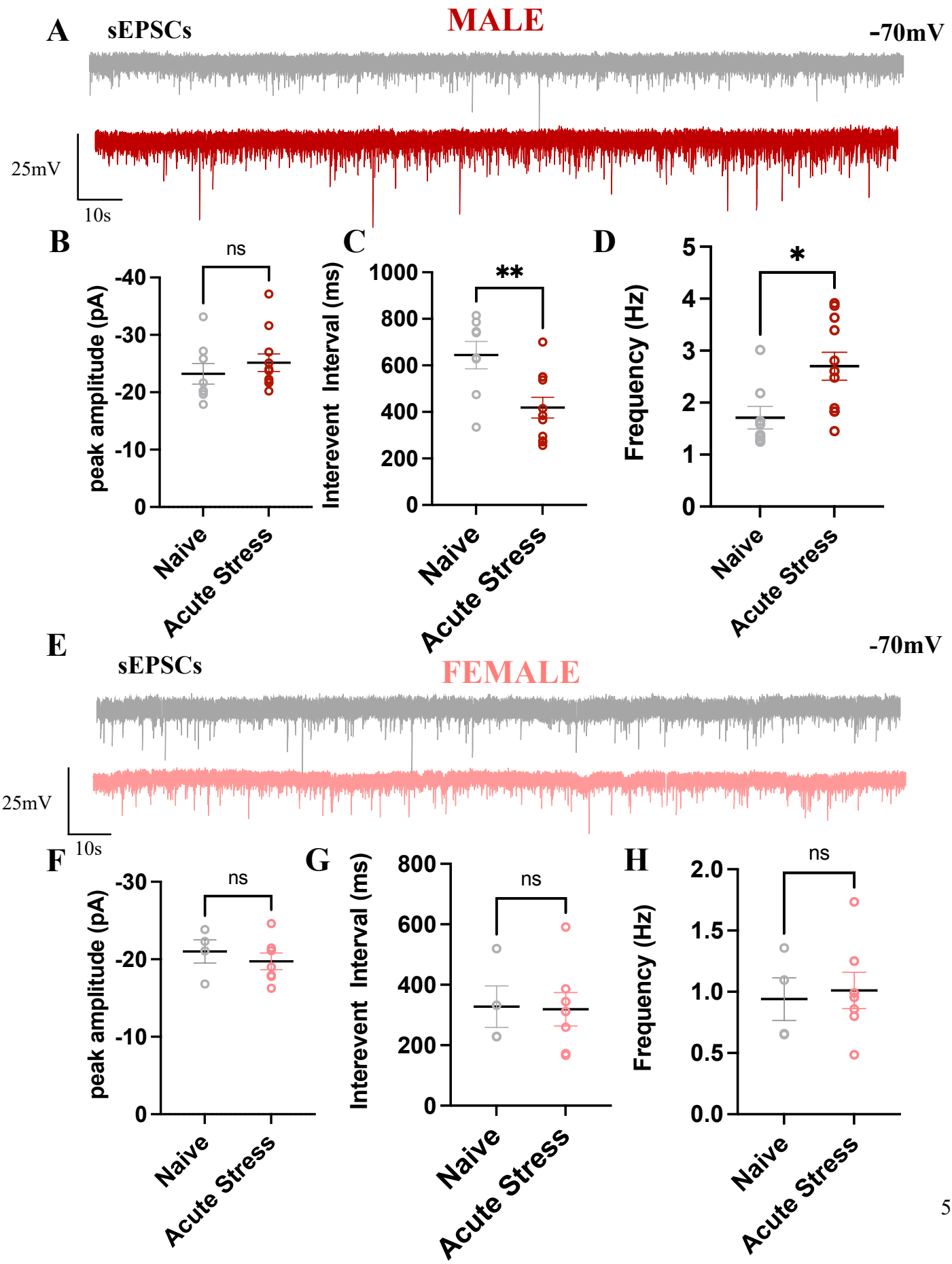

A
sEPSCs
MALE
-70mV
25mV
10s
B
ns
peak amplitude (pA)
-40
-30
-20
-10
0
Naive
Acute Stress
C
**
Interevent Interval (ms)
1000
800
600
400
200
0
Naive
Acute Stress
D
*
Frequency (Hz)
5
4
3
2
1
0
Naive
Acute Stress
E
sEPSCs
FEMALE
-70mV
25mV
10s
F
ns
peak amplitude (pA)
-30
-20
-10
0
Naive
Acute Stress
G
ns
Interevent Interval (ms)
800
600
400
200
0
Naive
Acute Stress
H
ns
Frequency (Hz)
2.0
1.5
1.0
0.5
0.0
Naive
Acute Stress

Figure 7: The effects of acute stress on CA1 excitatory inputs. A., Representative voltage clamp recordings (-70mV) of sEPSCs in male naïve (grey) and stress mice (red). **B-D.,** Aligned dot (mean ± SEM) plots of the peak amplitude (pA) and interevent interval (ms) and frequency (Hz) in male naïve and acute stress mice. **E,** Representative voltage clamp recordings (-70mV) of sEPSCs in female naïve (grey) and stress mice (pink). **F-H.,** Aligned dot (mean ± SEM) plots of the peak amplitude (pA) and interevent internal (ms) and Frequency (Hz) in female and naïve and acute stress mice. Unpaired t-test results are indicated by *$p<0.05$, **$p<0.01$

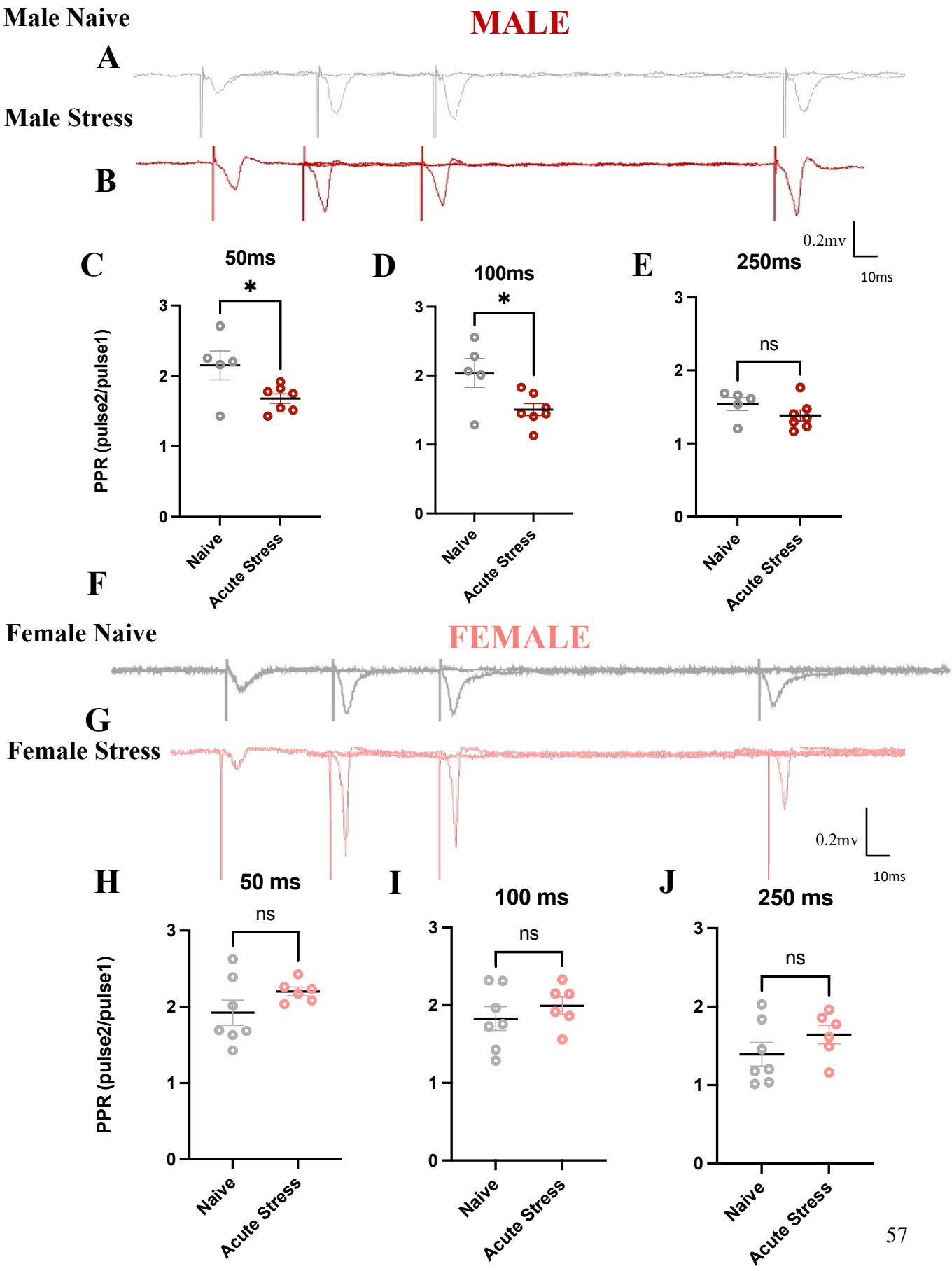

Male Naive
MALE
A
Male Stress
B
0.2mv
10ms
C
50ms
*
D
100ms
*
E
250ms
ns
PPR (pulse2/pulse1)
Naive
Acute Stress
F
Female Naive
FEMALE
G
Female Stress
0.2mv
10ms
H
50 ms
ns
I
100 ms
ns
J
250 ms
ns

Figure 8: The effects of acute stress on hippocampal PPR. **A-B.,** Representative fEPSP paired pulses (50ms) interval from male naïve (grey) and male stressed mice (red) **C-E.**, Aligned dot (mean ± SEM) plots of paired-pulse ratios at 50, 100 and 250 ms intervals in male naïve and acute stress mice. **F-G.,** Representative fEPSP paired pulses (50ms) interval from male naïve (grey) and female stressed mice (pink) **H-J .,** Aligned dot (mean ± SEM) plots of paired-pulse ratios at 50, 100 and 250 ms intervals in female naïve (grey) and acute stress (pink) mice. **H-K.,** Aligned dot (mean ± SEM) plots of paired-pulse ratios at 50, 100 and 250 ms intervals in female naïve (grey) and acute stress (pink) mice. Unpaired t-test results are indicated by *$p<0.05$, **$p<0.01$, ***$p<0.001$

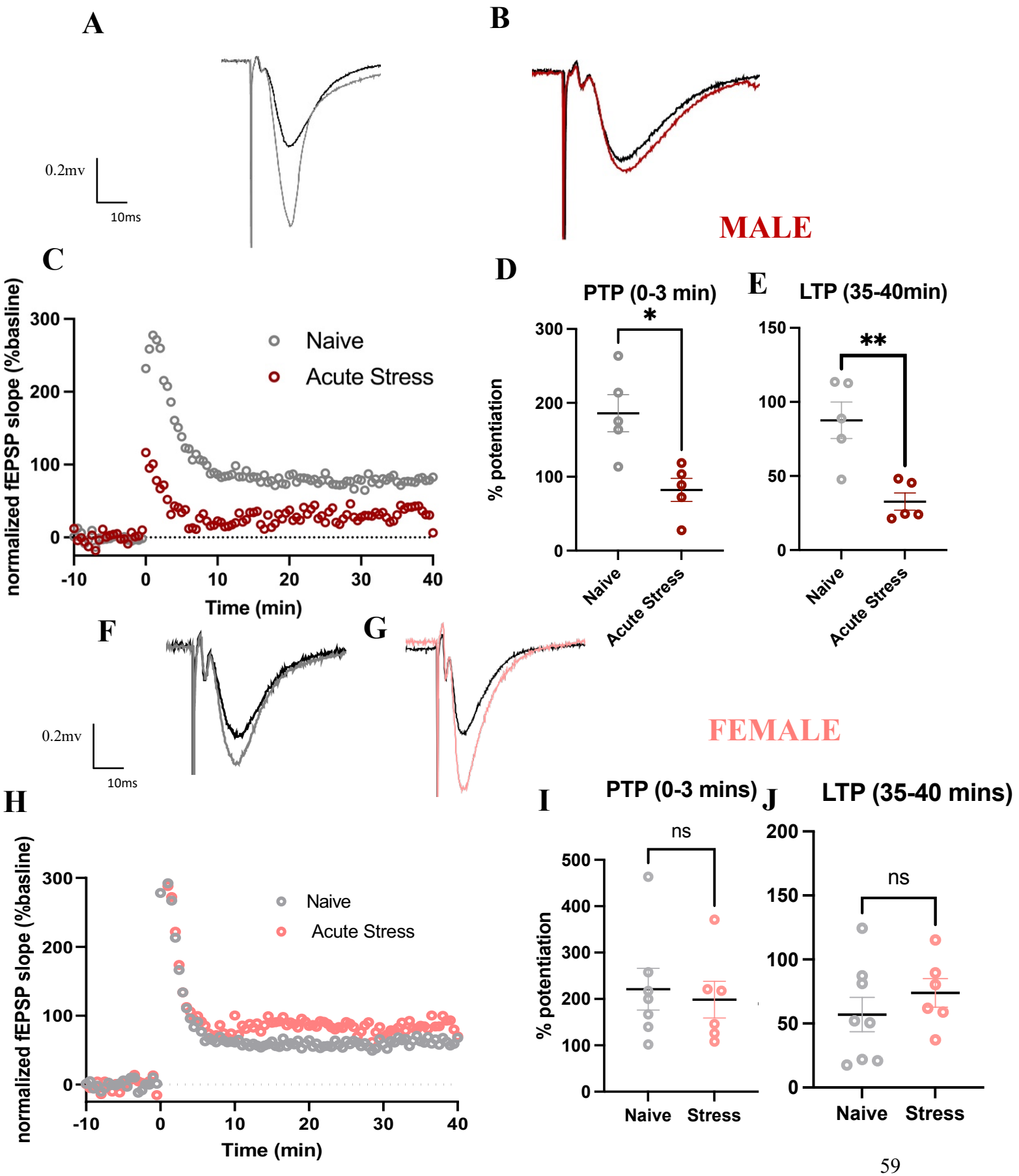
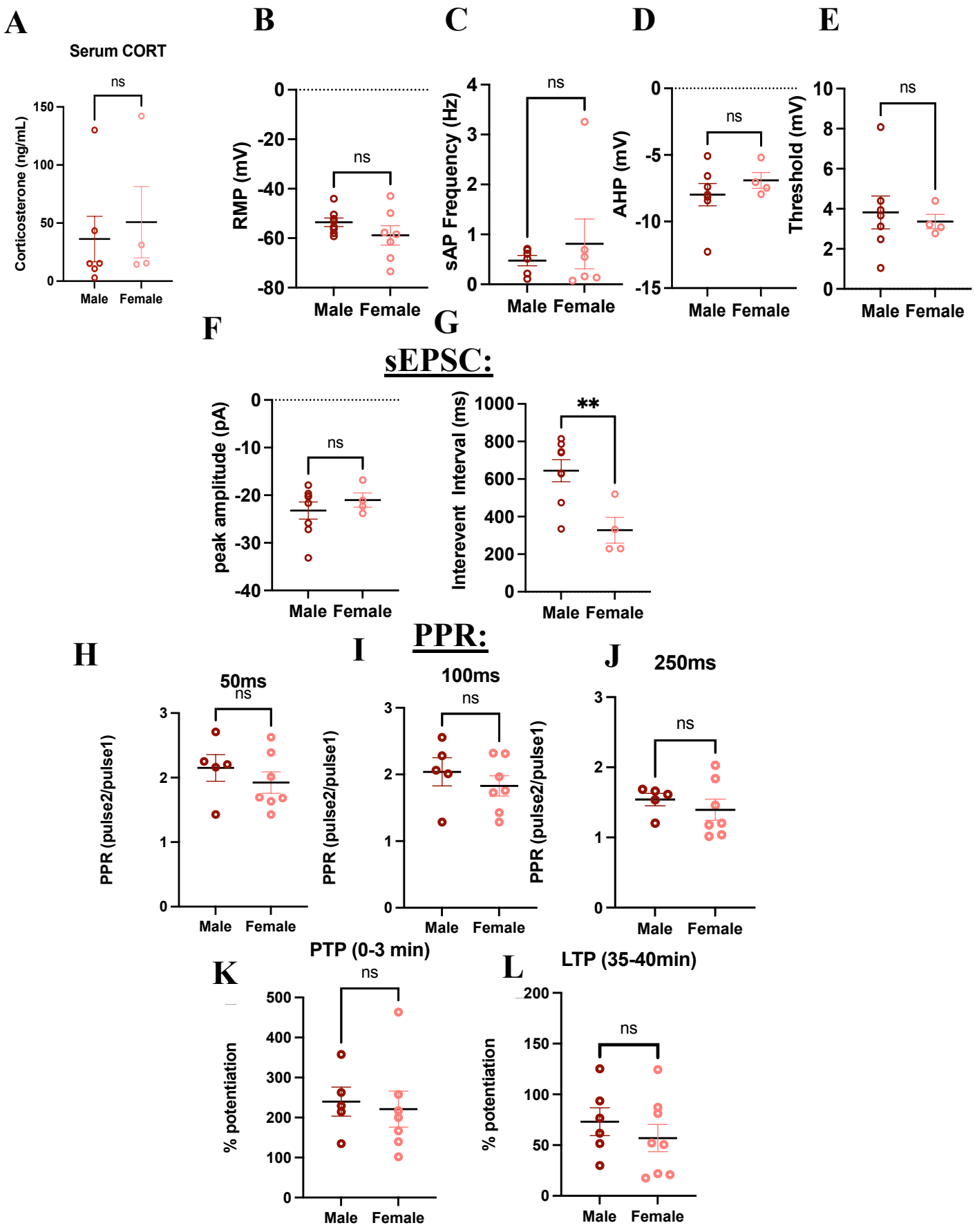

A
B
0.2mv
10ms
MALE
C
D
E
PTP (0-3 min)
LTP (35-40min)
normalized fEPSP slope (%basline)
Naive
Acute Stress
Time (min)
% potentiation
*
**
Naive
Acute Stress
F
G
0.2mv
10ms
FEMALE
H
I
J
PTP (0-3 mins)
LTP (35-40 mins)
ns
Naive
Stress

Figure 9: The effects of acute stress on hippocampal LTP. A., Representative fEPSP traces before and after HFS from naïve (grey), acute stress (red). **C**, Graph of %potentiation 40 minutes following HFS (applied at time = 0 min) in naïve (grey) and acutely stressed (red) male mice. **D-E** Aligned dot graph showing mean (±SEM) %potentiation in naïve and acutely-stressed mice at 0-3 min (PTP) and 35-40 min post-HFS (LTP). **F-G.,** Representative fEPSP traces before and after HFS from naïve (grey), acute stress (pink) female mice. **I-J.,** Aligned dot graph showing mean (±SEM) %potentiation in naïve and acutely-stressed female mice at 0-3 min (PTP) and 35-40 min post-HFS (LTP). Unpaired t-test results are indicated by *$p<0.05$, **$p<0.01$, ***$p<0.001$.


A
Serum CORT
Corticosterone (ng/mL)
150
100
50
0
ns
Male
Female
B
RMP (mV)
0
-20
-40
-60
-80
ns
Male Female
C
sAP Frequency (Hz)
4
3
2
1
0
ns
Male Female
D
AHP (mV)
0
-5
-10
-15
ns
Male Female
E
Threshold (mV)
10
8
6
4
2
0
ns
Male Female
F
peak amplitude (pA)
0
-10
-20
-30
-40
ns
Male Female
G
sEPSC:
Interevent Interval (ms)
1000
800
600
400
200
0
**
Male Female
PPR:
H
50ms
PPR (pulse2/pulse1)
3
2
1
0
ns
Male
Female
I
100ms
PPR (pulse2/pulse1)
3
2
1
0
ns
Male Female
J
250ms
PPR (pulse2/pulse1)
3
2
1
0
ns
Male Female
PTP (0-3 min)
K
% potentiation
500
400
300
200
100
0
ns
Male
Female
L
LTP (35-40min)
% potentiation
200
150
100
50
0
ns
Male Female

Supplemental Figure 1: Baseline comparison between male and female mice across all parameters. A., dot graph showing mean (±SEM) serum CORT in naïve male (red) and female (pink). **B.,** Aligned dot graph showing mean (±SEM) RMP in naïve male and female mice. **C-D.,** Aligned dot graph showing mean (±SEM) action potential waveform properties (AHP and threshold) in naïve male and female mice. **F-G.,** Aligned dot graph showing mean (±SEM) sEPSC properties (peak amplitude, interevent interval) in naïve male and female mice. **H-J.,** Aligned dot graph showing mean (±SEM) PPR values (50, 100 and 250 ms) in naïve male and female mice. **K-L.,** Aligned dot graph showing mean (±SEM) PTP (0-3 mins; % potentiation) and LTP (35-40 mins; % potentiation) in naïve male and female mice. Unpaired t-test results are indicated by *$p<0.05$, **$p<0.01$, ***$p<0.001$.

5.0. References

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