ACUTE STRESS, BUT NOT CORTICOSTERONE INJECTIONS, DISRUPTS BOTH SHORT- AND LONG-TERM FORMS OF SYNAPTIC PLASTICITY IN RAT DORSAL SUBICULUM VIA GLUCOCORTICOID RECEPTOR ACTIVATION

A Thesis Submitted to the
College of Graduate Studies and Research
in Partial Fulfillment of the Requirements for the
Degree of Master of Science in the
Department of Physiology at the
University of Saskatchewan

By Matthew J. MacDougall

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ABSTRACT

The subiculum serves as the major output structure of the hippocampus; therefore, exploring synaptic plasticity within this region is of great importance for understanding the dynamics of hippocampal circuitry and hippocampal-cortical interactions. Exposure to acute stress dramatically alters synaptic plasticity within the hippocampal formation. Using in vivo electrophysiological recordings in urethane-anesthetized adult male Sprague-Dawley rats, we tested the effects of either acute restraint stress (30 min) or corticosterone (CORT) injections (3 mg/kg; s.c.) on short- and long-term forms of synaptic plasticity in the CA1-subiculum pathway. Paired-pulse facilitation and two forms of long-term plasticity (long-term potentiation and late-developing potentiation) were significantly reduced after exposure to acute stress but not acute CORT treatment. Measurements of plasma CORT confirmed statistically similar levels of circulating hormone in animals exposed to either acute stress or acute CORT treatment. The disruptive effects of acute stress on both short- and long-term form of synaptic plasticity are mediated by glucocorticoid receptor (GR) activation as these disruptions were blocked by pre-treatment with the selective GR antagonist RU38486 (10 mg/kg; s.c.). The present results highlight the susceptibility of subicular plasticity to acute stress and provide evidence that GR activation is a necessary but not a sufficient physiological parameter for mediating these alterations.

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<tr>
<td>AMPA</td>
<td>α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic</td>
</tr>
<tr>
<td>AP</td>
<td>Anterior-Posterior</td>
</tr>
<tr>
<td>ACTH</td>
<td>Adrenocorticotropic releasing hormone</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>Divalent calcium</td>
</tr>
<tr>
<td>CA1</td>
<td>Cornu Ammonis 1</td>
</tr>
<tr>
<td>CA3</td>
<td>Cornu Ammonis 3</td>
</tr>
<tr>
<td>CaMKII</td>
<td>calcium/calmodulin-dependent kinase II</td>
</tr>
<tr>
<td>CORT</td>
<td>Corticosterone</td>
</tr>
<tr>
<td>CRH</td>
<td>Corticotropin releasing hormone</td>
</tr>
<tr>
<td>DG</td>
<td>Dentate Gyrus</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>DV</td>
<td>Dorsal-Ventral</td>
</tr>
<tr>
<td>EC</td>
<td>Entorhinal cortex</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme linked immunosorbent assay</td>
</tr>
<tr>
<td>fEPSP</td>
<td>Field excitatory postsynaptic potentials</td>
</tr>
<tr>
<td>GC</td>
<td>Glucocorticoid</td>
</tr>
<tr>
<td>GR</td>
<td>Glucocorticoid receptor</td>
</tr>
<tr>
<td>HFS</td>
<td>High frequency stimulation</td>
</tr>
<tr>
<td>HPA</td>
<td>Hypothalamic-pituitary-adrenal</td>
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<tr>
<td>Abbreviation</td>
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<tr>
<td>--------------</td>
<td>------------------------------------------------</td>
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<tr>
<td>LEC</td>
<td>Lateral entorhinal cortex</td>
</tr>
<tr>
<td>LFS</td>
<td>Low frequency stimulation</td>
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<tr>
<td>LTD</td>
<td>Long-term depression</td>
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<td>LTP</td>
<td>Long-term potentiation</td>
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<td>MEC</td>
<td>Medial entorhinal cortex</td>
</tr>
<tr>
<td>mGluR</td>
<td>Metabotropic glutamate receptor</td>
</tr>
<tr>
<td>ML</td>
<td>Medial-Lateral</td>
</tr>
<tr>
<td>PPF</td>
<td>Paired-pulse facilitation</td>
</tr>
<tr>
<td>PP2B</td>
<td>Protein phosphatase 2B</td>
</tr>
<tr>
<td>SUB</td>
<td>Subiculum</td>
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<tr>
<td>VGCC</td>
<td>Voltage gated calcium channels</td>
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1.0. GENERAL INTRODUCTION

1.1. Adaptation and Survival

The ability of an organism to respond and adapt to environmental and homeostatic challenges is paramount for its continued survival. Early physiological accounts on the adaptive capabilities of living organisms can be traced back to Claude Bernard’s theories on the regulation of the internal environment (milieu intérieur) by physico-chemical processes (Cooper, 2008). Bernard held that prolonged departures from a steady-state or disruptions in the regulatory activities that maintain constancy of the internal environment would lead to disease and that prolonged departures would ultimately result in death. Although Bernard’s idea on the constancy of the internal environment was not recognized at the time of its formulation (Gross, 1998), his ideas would later be recognized as the foundation upon which modern experimental physiology was built.

Walter B. Cannon, a nineteenth-century experimental physiologist, expounded upon the ideas of Bernard and would be instrumental in popularizing his theory on the constancy of the internal environment (Gross, 1998). With Bernard’s work in mind, Cannon coined the term “homeostasis” to refer to an organism’s ability to meet external demands and maintain its internal environment. Unlike Bernard, Cannon ultimately viewed an organism as an open system, being both susceptible and responsive to changes in the outer environment. Cannon thus refined and extended Bernard’s terminology from a fixed or steady-state to a more physiologically relevant narrow limit within which the internal environment of an organism is maintained (Cooper, 2008). Cannon noted that threats to homeostasis such as pain or emotional distress would activate the adrenal medulla as well as the sympathetic nervous system and believed that these two effectors
formed a functional unit, the so-called sympathoadrenal system, which upon activation would help to restore homeostasis (Goldstein and Kopin, 2007).

1.2. The Concept of Stress

From Bernard’s steady-state of the *milieu intérieur* to Cannon’s defining concept of homeostasis and the identification of the sympathoadrenal system as an internal regulator, came the first classification of the stress concept. The first report on the physiological effects of stress came when Hans Selye, a Hungarian-born physiologist, identified a *syndrome produced by various noxious agents* (Neylan, 1998). Selye would later define stress as “the nonspecific response of the body to any demand made upon it” (Selye, 1973) and note that such demands would require adaptation. Selye’s early experiments identified three common reactions to stressors: enlargement of the adrenal glands, thymolymphatic dystrophy, and gastrointestinal ulcers (Selye, 1950). Similar to Cannon’s fight-or-flight response, Selye’s classification of the initial response to stress was labeled the alarm reaction stage. Selye would go on to identify two subsequent stages: the “stage of resistance” and the “stage of exhaustion”. These three stages were collectively known as the general adaptation syndrome (GAS) and were considered universal stages of coping with stressors (Selye, 1950, Goldstein and Kopin, 2007). The tri-phasic nature of the GAS demonstrated that the energy requirements for adaptation were finite and if the demands exerted upon the body ensued for extended periods of time, exhaustion and death would invariably ensue (Selye, 1973).

In order to further catalogue the concept of stress, Selye would go on to dichotomize the term and make a distinction between eustress, or positive stressors, and distress, or stressors with negative implications for an organism (Selye, 1973). More
modern views hold that stress is a state in which an organism senses a threat to homeostasis and involves the perceived ability to cope with the stressor in question (Goldstein and McEwen, 2002). Physical and psychological threats to the well-being or survival of an organism activates a complex multi-system response (Wamsteeker and Bains, 2010).

1.3. The Stress Response

The physiological response to stress involves a complex and highly conserved set of systems that strive to maintain the integrity of physiological states during challenging circumstances (Ulrich-Lai and Herman, 2009). The autonomic nervous system provides an initial and rapid response to stressful circumstances through the coordinated activation of sympathetic and parasympathetic branches. Exposure to stressors results in the activation of preganglionic sympathetic neurons within the intermediolateral cell column of the thoracolumbar segment of the spinal cord (Ulrich-Lai and Herman, 2009). These preganglionic cells project to paraganglionic cells, which in turn project to other end organs and the chromaffin cells of the adrenal medulla (Ulrich-Lai and Herman, 2009). The sympato-adrenomedullary arm can cause increases in heart rate and blood pressure by exciting the cardiovascular system, pupil dilation, peripheral vasoconstriction, along with several other physiological responses and it is this pattern of activation that represents the classical “fight-or-flight” response to stress as originally identified by Walter B. Cannon (Cooper, 2008). Importantly, reflexive activation of the parasympathetic branch quickly follows and ensures a relatively transient stress response (Ulrich-Lai and Herman, 2009).
Prolonged physiological responses to stress are brought about via activation of the hypothalamic-pituitary-adrenal (HPA) axis (Fig. 1.1; Goldstein and Kopin, 2007). The parvocellular neuroendocrine cells (PNCs) of the paraventricular nucleus (PVN) of the hypothalamus control the secretion of pro-opiomelanocortin (POMC) products such as adrenocorticotropic hormone (ACTH) and β-endorphin from the anterior pituitary via the release of corticotropin-releasing hormone (CRH) into the median eminence (Habib et al., 2001). Increases in circulating ACTH activates the secretion of glucocorticoids (GCs), cortisol in humans and corticosterone (CORT) in rats, by the adrenal cortex (Ulrich-Lai and Herman, 2009, Wamsteeker and Bains, 2010). Glucocorticoid secretion is the final step in the HPA axis response to stress and increases in circulating GCs have been shown to exert diverse effects on numerous bodily tissues (Leung and Munck, 1975, Tasker et al., 2006), including the shut down of the HPA axis via negative feedback mechanisms acting on PNCs (Wamsteeker and Bains, 2010). This direct negative feedback has been shown to inhibit both the transcription and translation of CRH as well as the secretion into the median eminence of the portal system, thereby suppressing further release of GCs (Watts, 2005).

There are two distinct types of receptors to which CORT can bind in the rat brain: mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs; Reul and de Kloet, 1985). These two receptors have differential binding affinities and can exert both genomic and non-genomic effects on cellular physiology. CORT enters the cell and binds to a cytosolic receptor complex and the GC receptors then dissociate from the multi-protein complex allowing for homodimerization of the ligand-receptor complex and the subsequent translocation to the nucleus of the cell. Once in the nucleus the dimerized
Figure 1.1. *The hypothalamic-pituitary-adrenocortical (HPA) axis.* Exposure to stress stimulates the release of corticotropin releasing hormone (CRH) from the paraventricular nucleus of the hypothalamus. CRH stimulates the release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary. ACTH then stimulates the release of glucocorticoids from the adrenal glands, which results in a systemic increase in plasma levels of glucocorticoids. Glucocorticoids exert a wide range of effects on tissues of the body and can influence neuronal functioning in the hippocampus, a region of the brain that heavily expresses glucocorticoid receptors.
units can act as transcriptional regulators by binding to glucocorticoid-responsive elements and alter the expression of specific proteins either by transactivation or transrepression (Habib et al., 2001). In addition to genomic actions, CORT can also interact with membrane-associated receptors and rapidly alter cellular physiology through disruption or alterations in protein-protein interactions (Borski, 2000, Tasker et al., 2006).

MRs have a high affinity for CORT and are highly occupied (~90%) during basal secretion rates of GCs (Reul and de Kloet, 1985) while GRs have a ten times lower affinity and are thought to bind GCs when there are higher circulating levels of GCs, such as those found following exposure to acute stress (Ulrich-Lai and Herman, 2009). Both receptor types are abundantly expressed throughout the brain with a particularly high density of mRNA expression being found within the hippocampal formation (Reul and de Kloet, 1985, Ulrich-Lai and Herman, 2009).

The hippocampal formation along with the amygdala and prefrontal cortical regions mediate the awareness of a perceived threat, help gauge the severity of a stressor, and controls the adaptability of fear-related behaviours (Habib et al., 2001). Importantly, the hippocampal formation is comprised of numerous structures with dense MR and GR expression profiles (Han et al., 2005). Given the high expression profiles of MRs and GRs within the hippocampal formation (Herman et al., 1989), hippocampal circuitry is likely to be susceptible to the physiological effects of acute stress exposure.
Figure 1.2. The hippocampal formation. (A) The hippocampal formation consists of the entorhinal cortex (EC; with medial and lateral branches), the dentate gyrus (DG), the hippocampus proper (Cornu Ammonis (CA) subfields, CA3 and CA1), and the subiculum (SUB). Adapted from Cajal (1995). (B) A simplified schematic of the major pathways of the hippocampal formation. Highly integrated sensory information enters the hippocampus from the EC and projects to the DG via the perforant path. Information then gets sent to CA3 via the mossy fibres and from CA3 to CA1 via the Schaffer collaterals. The majority of CA1 projections terminate in the SUB and the SUB then sends the processed information back to the EC.
1.4. The Hippocampal Formation

The organization and function of the mammalian hippocampal formation has been rigorously explored and consists of several anatomically distinct regions, including the entorhinal cortex (EC), the granule and hilar cells of the dentate gyrus (DG), the pyramidal cell fields of the hippocampus proper (Cornu Ammonis (CA) subfields, CA3 and CA1), and the subiculum (SUB) as well as surrounding regions (Amaral and Witter, 1989, Cajal, 1995, O’Mara et al., 2001, Anderson et al., 2006, Witter, 2006). While the intrinsic circuitry of the hippocampal formation is inherently complex and extensive, it is important to recognize the major glutamatergic pathways. Highly processed sensory information from the EC projects to the DG and SUB via the perforant path and is considered the major input pathway to the hippocampus (Amaral and Witter, 1989, Jarrard, 1993, Andersen et al., 2006). The mossy fibers of the DG send projections to the CA3 subfield, which in turn innervate the CA1 subfield via the Schaffer collateral fibers (Fig 1.2). These three synapses have been extensively investigated in relation to forms of learning and memory and make up the traditional tri-synaptic descriptor of the hippocampal formation (Andersen et al., 2006).

Although the hippocampal formation is traditionally viewed as a tri-synaptic circuit, there is one pathway that until recently has been largely neglected. The SUB is often considered the major output structure of the hippocampus and as such, the CA1-SUB pathway can be thought of as the major output pathway and an additional synapse to the traditional tri-synaptic descriptor of the hippocampal formation (Jarrard, 1993, O'Mara, 2005, Andersen et al., 2006). As the SUB serves as the major output structure of
the hippocampal formation, it is thought to play a fundamental role in orchestrating hippocampal-cortical interactions (O'Mara, 2005, Behr et al., 2009).

An overwhelming amount of evidence has implicated the hippocampal formation in cognitive operations such as spatial learning and memory (O'Keefe and Nadel, 1978, Eichenbaum et al., 1996, Milner et al., 1998, Eichenbaum, 1999, Andersen et al., 2006). However, despite innumerable considerations and empirical attempts to describe the involvement of the hippocampal formation in these cognitive processes, the nature of how these neural circuits encode, store, and retrieve information related to learning and memory remains elusive (Jarrard, 1993, Andersen et al., 2006).

1.5. Learning and Memory

Learning and memory are two cognitive processes that are fundamental to living organisms. The ability of an organism to encode, store, and retrieve information from past events is vital to its adaptive and survival capabilities. Learning is generally recognized as the ability to acquire new information or skills through experience while memory is generally thought to reflect the ability to retrieve previously learned information (Wright and Watkins, 1987). Much of our knowledge regarding cognitive processes and their underlying neural mechanisms has come from case studies of amnesic patients and animal models of different forms of learning and memory (Wright and Watkins, 1987, Tulving and Markowitsch, 1994, Eichenbaum et al., 1996, Milner et al., 1998, Eichenbaum, 1999). These studies have revealed a particularly large involvement of the medial temporal lobe for mnemonic processing, with the hippocampus playing a fundamental role.
Hippocampal-dependent memory tasks have been particularly useful in elucidating the mechanisms of memory representations within the mammalian brain (Olton and Samuelson, 1976, Morris, 1981, Jarrard, 1993, Eichenbaum et al., 1996). Selective lesion studies using ibotenic acid have further helped establish the necessity of specific structures within the hippocampal formation during different cognitive tasks (Jarrard, 1989). Several studies have used this approach to selectively damage cells within the dorsal SUB and have demonstrated the necessity of the SUB in the acquisition of spatial memories (Bouffard and Jarrard, 1988, Morris et al., 1990, Jarrard, 1993, Cho and Jaffard, 1995). Interestingly, if the lesions to the entire dorsal hippocampus are made after the acquisition period, memory performances often remain unhampered, suggesting that although spatial memories initially require information encoding by the dorsal hippocampus, storage and retrieval processes seem to be less dependent upon the dorsal hippocampus and may be more reliant upon extra-hippocampal structures (Jarrard, 1993). It is generally believed that the hippocampus processes information related to recently formed memories but that over time these memories become less hippocampal-dependent as they get transferred from the hippocampus to the cortex (Frankland and Bontempi, 2005).

How and where memories are stored within neural tissue remains an outstanding question within the field of neuroscience (Frankland and Bontempi, 2005). Although the localization of specific mental faculties can be traced back to the 19th century phrenology movement espoused by Franz Joseph Gall (Fancher, 1996, Milner et al., 1998), more physiological explanations of mental events would begin to emerge in the early part of the 20th century. An early account of the neural mechanisms governing memory...
representations in the brain came with the development of a simple yet elegant
neurophysiological postulate that would later gain experimental acceptance and change
the field of neuroscience for decades after its original formulation (Cooper, 2005).

1.6. The Hebbian Synapse and Synaptic Plasticity

Donald O. Hebb, a Canadian-born psychologist, postulated that if two neurons
repeatedly become activated together that some change would be brought about such that
the communication efficacy between the two cells would be enhanced (Cooper, 2005). In
The Organization of Behavior, Hebb writes: “let us assume then that the persistence or
repetition of a reverberatory activity (or ‘trace’) tends to induce lasting cellular changes
that add to its stability” (Hebb, 1949, p.62) and that “[W]hen an axon of cell A is near
enough to excite cell B and repeatedly or persistently takes part in firing it, some growth
process or metabolic change takes place in one or both cells such that A’s efficiency, as
one of the cells firing B, is increased” (Hebb, 1949, 62). This celebrated and oft-cited
passage has become known as the Hebbian learning rule and forms the basis of modern
views on the neurophysiology of learning and memory.

It is remarkable that Hebb’s postulate remains so modern after several decades
from its original date of formulation (Morris, 1999). His prophetic postulate may be
deconstructed into three main sections: (i) his description of reverberatory activity (or a
memory trace) within cell assemblies, (ii) his prediction for structural changes in one or
both cells (i.e., pre- versus postsynaptic mechanisms), and (iii) his identification of
increases in synaptic efficacy (Cooper, 2005). The ability of cells to undergo changes in
synaptic transmission as a result of activation is known as synaptic plasticity and
synapses that conform to Hebb’s general rule have thus been labeled Hebbian synapses (Brown and Milner, 2003).

Synaptic plasticity is now recognized to be a fundamental property of functional nervous systems and a considerable amount of evidence suggests that learning and memory are made possible through the mechanisms governing synaptic plasticity (Roman et al., 1987, Eichenbaum et al., 1996, Eichenbaum, 1999, Martin et al., 2000, Whitlock, 2006, Collingridge et al., 2010). Both short- and long-term forms of synaptic plasticity exist within the hippocampal formation and are thought to involve distinct yet complementary mechanisms of expression.

Patterns of short-term synaptic enhancement, in the form of paired-pulse facilitation (PPF), are experimentally obtained by delivering two pulses of electrical stimulation in rapid succession to afferent fibers and recording the resulting postsynaptic response (Fig 1.3). PPF is commonly accredited to residual presynaptic Ca\(^{2+}\) levels from the first stimulating pulse summing with the Ca\(^{2+}\) influx from that of the second pulse, which in turn results in an increase in quantal release probability and an increase in the excitability of postsynaptic cells (Fig. 1.3; Wu and Saggau, 1994, Zucker, 1999, Zucker and Regehr, 2002). Other reports have demonstrated that internal Ca\(^{2+}\) stores (Emptage et al., 2001), facilitation of presynaptic Ca\(^{2+}\) currents (Borst and Sakmann, 1998), prolongation of presynaptic action potentials (Sabatini and Regehr, 1997), and phosphorylation of synaptic proteins (Llinas et al., 1991) play significant roles in the facilitation of transmitter release and contribute to PPF.

High levels of PPF are generally suggestive of low initial release probabilities while low levels of PPF are suggestive of high initial release probabilities (Commins et
al., 1998a, Zucker and Regehr, 2002). It has also been argued that changes in PPF following the induction of long-term forms of synaptic potentiation suggest the involvement of a presynaptic locus to the expression of the synaptic plasticity in question (Schulz et al., 1994). Regardless of the specific mechanisms mediating PPF, this synaptic alteration is transient in nature while other synaptic alterations have been demonstrated within the hippocampal formation that have longer lasting profiles.

Bliss and Lomo (1973) were the first to demonstrate long-term potentiation (LTP) using an in vivo electrophysiological preparation with anaesthetized rabbits and were therefore the first to experimentally verify Hebb’s rule of learning (Bliss and Lomo, 1973). By delivering trains of high frequency stimulation (HFS) to the perforant path, they demonstrated long-lasting amplifications in field excitatory postsynaptic potentials (fEPSPs). Ito and colleagues (1982a, 1982b) later demonstrated the complement to LTP, namely long-term depression (LTD), by delivering trains of low frequency stimulation (LFS) to cerebellar Purkinje fibres, which produced a marked long-lasting decrease in the
Figure 1.3. Paired-pulse facilitation (PPF) and the residual calcium hypothesis. During the first stimulation pulse (left) there is a rapid depolarization of the presynaptic neurons, thereby triggering an influx of calcium (Ca\(^{2+}\)) into the terminals via voltage gated calcium channels (VGCC). The influx of calcium results in alterations of the molecular machinery within presynaptic active zones that facilitate the exocytosis of presynaptic vesicles and the subsequent release of glutamate into the synaptic cleft. The glutamate then binds to postsynaptic glutamate receptors (e.g., NMDA or AMPA receptors) to produce postsynaptic excitability. If, after a short interval (inter-pulse interval), the second stimulation pulse (right) is delivered, the Ca\(^{2+}\) influx sums to the residual Ca\(^{2+}\) that had entered the cell after the first stimulation pulse. This leads to an increase in presynaptic calcium levels and the number of exocytosed vesicles. Due to the enhanced release probability and greater transmitter release there is an increase in the overall postsynaptic excitability.
amplitude of fEPSPs (Ito and Kano, 1982, Ito et al., 1982). The strengthening and weakening of synapses following distinct patterns of afferent simulation provided the first empirical support for Hebbian-like synapses.

Classical forms of LTP are dependent upon glutamatergic N-methyl-D-aspartate (NMDA) receptor activation (Nicoll, 2003, Malenka and Bear, 2004). The activation of NMDA receptors following HFS causes an elevation in intracellular Ca$^{2+}$ within postsynaptic neurons and in turn triggers the activation of numerous proteins, most notably calcium/calmodulin-depentant kinsae-II (CaMKII; Nicoll, 2003, Malenka and Bear, 2004), which then phosphorylates α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate acid (AMPA) receptors leading to the insertion of AMPA receptors into the plasma membrane (Fig. 1.4.; Luscher et al., 1999, Malenka, 2003, Malenka and Bear, 2004). The insertion of AMPA receptors causes a rapid increase in synaptic efficacy between cells. Interestingly, classical LTD is also dependant upon NMDA receptor activation (Collingridge et al., 2010). Here, activation of NMDA receptors following LFS results in the activation of several phosphatases such as protein phosphatase 2B, which leads to the dephosphorylation and subsequent endocytosis of AMPA receptors from the plasma membrane. Removal of AMPA receptors from the plasma membrane weakens the synaptic efficacy between cells (Fig. 1.4.; Malenka, 2003, Collingridge et al., 2004, Malenka and Bear, 2004, Collingridge et al., 2010).

While both classical LTP and LTD are reliant upon NMDA receptor activation, several lines of evidence suggest that these NMDA-dependent forms of synaptic plasticity are subunit specific. Importantly, classical LTP involves NMDA receptors expressing NR2A subunits, which are localized within the synapse. Conversely, classical
Figure 1.4. Classical forms of long-term synaptic plasticity. Long-term potentiation (LTP; left) involves the release of glutamate from presynaptic terminals following high frequency stimulation and the subsequent activation of postsynaptic NMDA receptors. Calcium then flows into the postsynaptic neuron through the NMDA receptors and activates intracellular signaling cascades that result in the phosphorylation of AMPA receptors. Long-term depression (LTD; right) involves the release of glutamate from presynaptic terminals following low frequency stimulation and the subsequent activation of postsynaptic NMDA receptors. Calcium then flows into the postsynaptic neuron through the NMDA receptors and activates an intracellular signal cascade that results in the dephosphorylation of AMPA receptors. +P indicates phosphorylation and –P indicates dephosphorylation.
LTD involves NMDA receptors with NR2B subunit compositions (Wong et al., 2007, Collingridge et al., 2010). In addition, it is important to note that the spatiotemporal profile of Ca^{2+} influx into the postsynaptic cell may differentially regulate intracellular cascades by interacting with Ca^{2+}-dependent kinases (e.g., CaMKII) and phosphatases (e.g., PP2B) thereby providing a molecular switch for bidirectional glutamatergic synapses (Lisman, 2001). Lastly, although classical forms of long-term plasticity are NMDA- and Ca^{2+}-dependent, other forms of long-term plasticity exist that are mediated via metabotropic glutamate receptors (mGluRs) and are independent of NMDA receptor activation (Anwyl, 1999, Mukherjee and Manahan-Vaughan, 2012).

Patterns of synaptic plasticity within the hippocampal formation consistent with LTP and LTD are necessary for cognitive operations such as learning and memory (Martin et al., 2000, Malenka and Bear, 2004, Whitlock, 2006, Massey and Bashir, 2007, Collingridge et al., 2010). While research implicating hippocampal synaptic plasticity in learning and memory has focused primarily on the EC, DG, and hippocampus proper, the contributions of the SUB have been largely neglected (O'Mara, 2005, Behr et al., 2009). The relative paucity of research on subicular synaptic plasticity is surprising given the important role of the SUB, along with the CA1 subfield, in providing the majority of output to cortical and subcortical regions and contributions to learning and memory (Morris et al., 1990, Deadwyler and Hampson, 2004, O'Mara, 2006, Sharp, 2006, Behr et al., 2009, O'Mara et al., 2009, Potvin et al., 2010). Distinct roles for the CA1 subfield and SUB in learning and memory have been proposed (Deadwyler and Hampson, 2004, Witter, 2006, van Strien et al., 2009); therefore, a more detailed understanding of the potentially divergent mechanisms governing synaptic plasticity within these regions will
contribute to theories of medial temporal lobe function.

Pyramidal cells in the dorsal CA1 subfield send dense, topographically organized projections to the dorsal SUB (O'Mara et al., 2001, Andersen et al., 2006, van Strien et al., 2009). Studies using electrophysiological recordings have revealed distinct firing patterns of SUB cells (bursting and regular firing neurons; Taube, 1993) and several forms of synaptic plasticity within the CA1-SUB pathway (Behr et al., 2009). Short-term synaptic plasticity, in the form of PPF, has been previously reported in the SUB (Commins et al., 1998a). Long-term alterations including classical LTP are induced in the SUB following trains of HFS delivered to CA1 either in vitro or in vivo (Boeijinga and Boddeke, 1996, Commins et al., 1998b). Interestingly, there appear to be two fundamentally different forms of LTP expression depending on the firing characteristics of SUB neurons (Wozny et al., 2008b). Both types of pyramidal cells express NMDA-dependent LTP but postsynaptic Ca$^{2+}$ elevations do not play a significant role in LTP expression within burst firing cells of the SUB, whereas these elevations are necessary for LTP expression within regular firing neurons (Wozny et al., 2008b). Instead, the LTP within bursting neurons involves the activation of protein kinase A (PKA) by way of elevated cyclic adenosine monophosphate (cAMP) signaling (Wozny et al., 2008a). The HFS-induced LTP can also be suppressed with the activation of 5-HT$_{1B}$ receptors, which produces a decrease in glutamate release from CA1 terminals (Boeijinga and Boddeke, 1996). While divergent mechanisms play a role in LTP expression in the CA1-SUB pathway, a novel form of synaptic potentiation has also been found within these hippocampal output synapses (Anderson et al., 2000).
The idea that HFS produces synaptic potentiation while LFS produces synaptic depression has been challenged by observations of LFS-induced late-developing potentiation within the glutamatergic CA1-SUB pathway of the hippocampal formation (Anderson et al., 2000, Huang and Kandel, 2005, Behr et al., 2009). The LFS-induced late-developing potentiation has been reported within the CA1-SUB pathway *in vitro* as well as *in vivo* (Anderson et al., 2000, Huang and Kandel, 2005, Fidzinski et al., 2008). As with the HFS-induced LTP, the LFS-induced potentiation has differential expression profiles depending upon the firing properties of the neurons in question. Regular firing neurons show a late-developing potentiation following LFS, whereas bursting neurons display classical NMDA-dependent LTD (Fidzinski et al., 2008). Interestingly, when MPCG, a metabotropic glutamate receptor (mGluR) antagonist, is applied to bursting cells the late-developing potentiation is blocked and NMDA-dependent LTD is expressed. Similarly, when APV, an NMDA receptor antagonist, is applied to regular firing neurons the LTD is blocked and the late-developing potentiation is unmasked following LFS (Fidzinski et al., 2008). This LFS-induced synaptic potentiation has also been shown to be modulated via the β-adrenergic system (Huang and Kandel, 2005). Taken together, these data strongly suggest that different forms of synaptic plasticity coexist within regular and burst firing cells of the SUB and are differentially expressed depending on the firing characteristics of subicular neurons. The data also demonstrate that these forms of plasticity are dependent upon modulatory inputs from different brain regions.

The expression mechanisms of HFS and LFS induced synaptic potentiation have been explored *in vitro* and the expression profiles appear to involve distinct physiological
mechanisms and modulatory inputs (Fidzinski et al., 2008, Wozny et al., 2008a, Behr et al., 2009). Despite early attempts to classify the expression mechanisms, the regulation of these three distinct forms of subicular plasticity during challenging circumstances, such as exposure to acute stress, remains largely unexplored (Commins and O'Mara, 2000, Commins et al., 2001).

1.7. Acute Stress and Synaptic Plasticity

Given the evidence implicating synaptic plasticity as a neurobiological substrate of learning and memory (Martin et al., 2000, Malenka and Bear, 2004, Collingridge et al., 2010), it is important to understand how different forms of plasticity are regulated during challenging circumstances. Since the identification of the uptake and retention of glucocorticoids by the hippocampus (McEwen et al., 1968), reports have confirmed the existence of both MRs and GRs within the hippocampal formation (Reul and de Kloet, 1985, Herman et al., 1989). Exposure to acute stress alters patterns of synaptic plasticity within the traditional tri-synaptic glutamatergic pathways of the hippocampal formation (Sandi, 2011), and these alterations have been proposed as the basis for the observed deficits associated with acute stress and spatial learning and memory (Diamond and Rose, 1994, Kim and Diamond, 2002, Howland and Wang, 2008). Interestingly, other behavioural assays capable of raising circulating glucocorticoids, such as exposure to a sexually receptive female or exercise, have been shown to be ineffective means of disrupting memory performance, suggesting that acute stress exerts fundamentally different physiological effects than mere CORT elevations alone (Woodson et al., 2003).
Glucocorticoid hormones exert a wide range of effects on cellular physiology within the mammalian nervous system (Joëls, 2001, Tasker et al., 2006, Howland and Wang, 2008, Lupien et al., 2009, Sandi, 2011). While the effects on cellular physiology are varied, a great deal of research has focused on GR activation and forms of synaptic plasticity within the tri-synaptic circuitry of the hippocampal formation with GR-dependent modifications reported in the DG, and the CA3 and CA1 subfields (Shors and Dryver, 1994, Pavlides et al., 1995, Xu et al., 1998, Cazakoff and Howland, 2010). The DG receives dense projections from EC via the perforant path (PP) and is considered the major input structure to the hippocampus (Andersen et al., 2006). Exposure to acute stress blocks the induction of LTP within this region (Shors and Dryver, 1994). Similarly, the mossy fiber synapses between DG and CA3 also undergo GR-dependent modifications. In addition, acute stress disrupts PPF in CA1 (Cazakoff and Howland, 2010) and disrupts classical LTP within this region while facilitating the induction of LTD (Foy et al., 1987, Diamond and Rose, 1994, Kim et al., 1996, Xu et al., 1997, Wong et al., 2007, Cazakoff and Howland, 2010), effects also reportedly mediated by glucocorticoid receptor (GR) activation (Xu et al., 1998, Cazakoff and Howland, 2010). Interestingly, ablation of the amygdala prior to acute stress exposure prevents the deleterious effects on synaptic plasticity within the CA3-CA1 pathway regardless of the fact that CORT levels remain unchanged, suggesting that the disruptive effects of acute stress on hippocampal plasticity are not solely dependent upon glucocorticoid receptor activation within the hippocampal formation but are also dependent upon amygdalar activation during the stressful circumstance (Kim et al., 2001, Kim et al., 2005).
The role of CORT on hippocampal synaptic plasticity has also been examined (Diamond et al., 1992, Pavlides et al., 1993). An inverted U-shaped function between synaptic plasticity in the CA1 region and peripheral CORT levels has been characterized (Diamond et al., 1992, Lupien and McEwen, 1997). That is to say, both high and low levels of circulating CORT have been shown to exert disruptive effects on patterns of synaptic plasticity within the hippocampus, whereas an intermediate range allows for optimal potentiation to occur (McEwen, 1994). Moreover, glucocorticoids seem to have a biphasic response depending on the specific receptor-ligand interaction. Activation of MRs seems to promote excitability via disinhibition of serotonergic input whereas GR activation has been shown to produce inhibition via suppression of noradrenergic input (Joels and de Kloet, 1992, McEwen, 1994). Thus, it appears that glucocorticoids, namely CORT, exert a sizeable regulatory role on patterns of neuronal excitability as well as synaptic plasticity and therefore heavily contribute in regulating cognitive processes such as learning and memory (Joels and de Kloet, 1992).

Although it was once believed that CORT and glucocorticoid receptor complexes act solely as transcriptional regulators, it is now evident that the effects of CORT, through interactions with both MRs and GRs, can act through non-genomic mechanisms as well (Karst, 2005, Tasker et al., 2006, Sandi, 2011). Given the established susceptibility of the hippocampal formation to elevations in circulating CORT brought about by acute stress, the aim of the present experiments is to explore the effects of acute restraint stress and acute CORT administration on three well-characterized forms of subicular plasticity (Commins et al., 1998a, Commins et al., 1998b, Anderson et al., 2000), all of which have received sparse experimental attention in the context of acute stress. In a second series of
experiments, the necessity of GR activation in the deleterious effects of acute stress on these forms of subicular plasticity is examined using the selective GR antagonist RU38486 (Xu et al., 1998, Cazakoff and Howland, 2010).
2. MATERIALS AND METHODS

2.1. Subjects

Adult male Sprague Dawley rats (>300 g; Charles River Laboratories, Quebec, Canada) were pair housed in plastic cages with ad libitum access to food and water. Rats were housed under a 12:12 hour light/dark cycle (lights on at 07:00) in a temperature and humidity controlled vivarium. Experimentation was conducted during the light phase. After arrival at the facility, rats were given at least 5 days to acclimatize before experiments were initiated. All experiments were conducted in accordance with the Canadian Council on Animal Care and were approved by the University of Saskatchewan Animal Research Ethics Board.

2.2. In vivo electrophysiology

Rats were anaesthetized using urethane (1.5-2.0 mg/kg, i.p.) and placed in a stereotaxic frame (David Kopf, CA). A grounded homeothermic temperature control unit (Harvard Instruments, MA) was used to maintain the rectal temperature of the rats at 37°C ± 1°C during the experimental sessions. A monopolar recording electrode (insulated platinum iridium wire, 125 µm, AM Systems, WA) was lowered into the dorsal SUB through a bored hole in the skull (AP = -6.8 mm, ML = 4.00 mm, DV = -2.5 mm). A concentric-ring bipolar stimulating electrode (NE-100X, Rhodes Medical Instruments, CA; tip separation: 0.5 mm) was lowered into the dorsal CA1 region through a more anterior hole in the skull (AP = -4.5 mm, ML = 2.5 mm, DV = -2.5 mm; Fig. 4.1A). A reference wire for the recording electrode was secured to the skull anterior to bregma with a jeweller’s screw. SUB field excitatory postsynaptic potentials (fEPSPs) were
initially evoked by stimulation of CA1 stratum radiatum (pulse width = 0.12 ms, 200 µA, 0.2 Hz) and were recorded at varying depths. SUB fEPSPs resemble the same characteristics as those found in CA1 with only minor differences in amplitude. Final electrode placement was determined by maximal field response and the electrical current was adjusted in all experiments to elicit fEPSPs of 50-60% of the maximal response (Fig. 2.1D). Recordings were initiated 15-20 min following optimization of electrode placements.

At the start of each recording session, paired-pulse facilitation (PPF) was measured by delivering five pairs of pulses to CA1 at inter-pulse intervals (IPIs) of 25, 50, 100, and 200 ms. Immediately following PPF, baseline fEPSPs were obtained by administering stimulation (0.07 Hz) until a stable baseline was achieved for 20 min. Potentiation was induced by two tetanus protocols: the HFS protocol consisted of 10 bursts of 20 pulses at 200 Hz with an interburst interval of 2 s (Commins et al. 1998b) while the LFS protocol consisted of 900 pulses delivered at 1 Hz for 15 min (Anderson et al. 2000). In all experiments, the baseline stimulation frequency was resumed following the tetanus and responses were recorded for 60 min after which PPF was re-examined as described above.

2.3. Acute stress protocol

Acute stress was accomplished by immobilizing rats in a Plexiglas restraint tube (544-RR, Fisher Scientific) in a brightly lit novel room for 30 min. Rats exposed to acute stress consistently displayed high levels of urination, defecation, and piloerection. All rats were anaesthetized immediately following acute stress and mounted on a stereotaxic frame in preparation for electrophysiological recordings (Fig. 2.1B).
Figure 2.0: (A) A schematic of the experimental design with stimulating electrode placed in dorsal CA1 and recording electrode placed in dorsal subiculum (SUB). (B) A schematic of the experimental conditions. Dotted line represents time spent in home cage and bold line represents time in restraint. All rats were anaesthetized at time zero. (C) Representative electrode placements as indicated by black dots. (D) Input/output curve showing the amount of stimulating current (µA) and the evoked fEPSP responses (mV) for the experimental conditions.
2.4. Pharmacology

CORT (Sigma-Aldrich) was suspended in vegetable oil (Crisco) and injected (3 mg/kg; s.c.; de Quervain et al., 1998) 30 min prior to anaesthesia. Rats were returned to their home cages for 30 min before being anaesthetized (Fig. 2.1B). To examine the role of GR activation, some rats received an injection of vehicle (50:50 DMSO:95% ethanol; 2 ml/kg; s.c.) or an injection of the selective GR antagonist RU38486 (10 mg/kg; s.c.; Xu et al. 1998; Cazakoff and Howland 2010) 60 min prior to anaesthesia. Vehicle and RU38486 treated rats were then randomly assigned to either the stress or a non-stress condition. Rats in the stress group received the acute stress protocol 30 min post-injection while the rats in the non-stress condition remained in their home cages for 60 min before being anaesthetized (Fig 2.1B).

2.5. Corticosterone Assay

Plasma CORT levels were determined using tail blood samples collected from control, stress, and CORT groups at two distinct time points: immediately after anaesthesia and before tetanization. Blood samples were then left to sit for 15-20 min at room temperature. Blood fractionation was achieved by spinning the blood samples at 2000 RPM for 20 min using a centrifuge (Fisher Scientific, accuSpin Micro 17). The supernatant was then collected and stored in plastic tubes at -80°C until subsequent analysis. All samples were analyzed using the commercially available enzyme-linked immunosorbent assay (ELISA) CORT kit (ADI-900-097; Enzo Life Sciences) according to the manufacturer’s specifications.
2.6. Histology

Following the recordings, electrolytic lesions were created by administering direct current (0.2 mA, 20 sec) through each of the electrodes. Rats were then transcardially perfused with 30 ml of physiological saline and their brains removed and stored in a 10% formalin-10% sucrose solution. Brains were sectioned using a sliding microtome and electrode placements were verified (Fig. 2.1C) with the aid of a rat brain atlas (Paxinos and Watson 1997) and a compound light microscope (Fisher Scientific).

2.7. Statistical Analysis

Statistical tests were conducted using SPSS Version 18 for Windows and Graphpad Prism 5.0. All descriptive values are reported as mean ± SEM. P values of less than or equal to 0.05 were considered statistically significant. PPF is expressed as percent change in the second evoked fEPSP slope relative to the first fEPSP slope. For comparisons of pre- and post-tetanus PPF values, difference scores were calculated as (post-tetanus PPF minus pre-tetanus PPF). One sample t-tests revealed that significant PPF was only elicited reliably for trials with 25 and 50 ms IPIs ($P < 0.05$). Thus, we only report the 25 and 50 ms IPIs. Omnibus repeated measures ANOVA revealed no significant effect of Tetanus [$F_{1,52} = 0.523, P = 0.473$]; therefore, PPF data were combined for HFS and LFS groups in all subsequent analyses. The magnitude of long-term plasticity was normalized and expressed as the percent change in fEPSP slope from baseline. For each group, comparisons between the average fEPSP slope for the last 5 min of baseline and the last 5 min of the one-hour decay period were made using paired sample t-tests ($P < 0.05$ for all groups except the stress alone groups; statistics not
shown). ANOVAs were used to determine differences between experimental conditions followed by post hoc comparisons using Tukey’s LSD where appropriate.
3.0. RESULTS

3.1. Acute stress and CORT injections elevate circulating CORT levels

Plasma CORT levels were determined using tail blood samples collected at two time points (following anaesthesia and prior to tetanization) from a subset of animals. Control rats displayed low levels of circulating hormone (4.17 ± 1.84 µg/dL; n = 4) relative to rats exposed to acute stress (17.56 ± 2.56 µg/dL; n = 5) and acute CORT injections (20.79 ± 2.70 µg/dL; n = 10) immediately following anaesthesia (Fig. 3.1A). Rats exposed to acute stress and CORT injections displayed statistically equivalent levels of CORT (P > 0.05). Surprisingly, control rats displayed an increase in basal levels of circulating hormone (33.36 ± 7.75 µg/dL) prior to tetanization, as did rats exposed to acute stress (30.46 ± 9.45 µg/dL) and acute CORT injections (31.37 ± 2.10 µg/dL; Fig. 3.1B). These results were confirmed by a significant main effect of Time [F1,16 = 25.41, P = 0.005] while the Group by Time interaction approached significance [F1,16 = 3.35, P = 0.059]. When data from the two time points were analyzed separately, a significant effect of group was found for the initial plasma levels [F2,19 = 7.16, P = 0.006] but not subsequent measurements taken prior to tetanization [F2,19 = 0.117, P = 0.89]. Post hoc comparisons revealed that the control group differed significantly from both stress and CORT groups (P < 0.05) immediately following anaesthesia.

3.2. Exposure to acute stress, but not CORT, disrupts PPF within the CA1-SUB pathway

In the present experiments, the effects of acute stress and CORT were examined on short-term plasticity using initial pre-tetanus PPF values (averaged 25 and 50 ms IPIs). Significant PPF was observed for the control (30.44 ± 4.69%; n = 17) and CORT (41.61
± 5.98%; n = 10) groups while the stress group (11.76 ± 5.78%; n = 11) did not display significant PPF (Fig. 3.2A, B; one sample t-tests not shown). An ANOVA revealed a significant effect of Group for the pre-tetanus PPF \([F_{2,37} = 6.24, P = 0.005]\). Post hoc comparisons revealed that control and CORT groups displayed significantly higher levels of PPF than the stress group \((P < 0.05)\). These results demonstrate that exposure to acute stress, but not CORT injections, disrupts PPF in the CA1-SUB pathway.

Next, we used the selective GR antagonist RU38486 to elucidate the role of GR activation in the effects of acute stress on PPF. Robust PPF was observed in rats treated with RU38486 (32.52 ± 8.84%; n = 11) as well as rats treated with RU38486 and exposed to acute stress (41.30 ± 7.98%; n = 11; Fig. 3.2A). A 2X2 ANOVA with Stress and RU38486 revealed a significant main effect of RU38486 \([F_{1,46} = 5.81, P = 0.020]\) as well a significant Stress by RU38486 interaction \([F_{1,46} = 4.39, P = 0.042]\). Post hoc comparisons revealed that the stress group displayed significantly lower PPF than the other groups \((P < 0.05)\).
Figure 3.1: Corticosterone (CORT) Assay. (A) Plasma CORT levels taken immediately after anaesthesia for control, acute stress, and CORT groups. (B) Plasma CORT levels taken prior to tetanization for control, acute stress, and CORT groups. * denotes $P < 0.05$ relative to all other groups.
Figure 3.2: Initial paired-pulse facilitation (PPF). (A) Acute stress, but not corticosterone injections, disrupts pre-tetanus PPF values for the averaged 25 and 50 ms IPIs, an effect reversed by pre-treatment with RU38486 (10 mg/kg). * denotes $P < 0.05$ relative to all other groups. (B) Representative fEPSP traces for control and stress conditions.
3.3. HFS of CA1 induces LTP in SUB that is disrupted following exposure to acute stress, but not CORT injections, in a GR-dependent manner

We observed that HFS of the CA1-SUB pathway induces reliable LTP with a magnitude of 18.31 ± 4.46% (n = 7; Fig. 3.3A). Exposure to 30 min of restraint stress completely abolished this synaptic potentiation (-4.33 ± 2.47%; n = 5) while rats treated with acute CORT injections had similar patterns of plasticity as controls (22.55 ± 8.51; n = 5; Fig. 3.3A). These impressions were confirmed by a significant effect of Group \([F_{2,16} = 6.26, P = 0.011]\). Post-hoc analyses revealed that the acute stress alone group displayed significantly lower potentiation than the other groups \((P < 0.05)\). Next, we examined the role of GR activation in the deleterious effects of acute stress using RU38486. Rats injected with RU38486 displayed significant LTP (18.81 ± 8.06%; n = 5), as did rats that received both RU38486 treatment and exposure to acute stress (25.28% ± 8.91; n = 6; Fig. 3.3B). A 2X2 ANOVA with Stress and RU38486 as factors revealed a significant main effect of RU38486 \([F_{1,19} = 5.55, P = 0.029]\) and a significant Stress by RU38486 interaction \([F_{1,19} = 5.18, P = 0.035]\). Post hoc comparisons revealed that the stress group displayed significantly lower LTP than the other groups \((P < 0.05)\). These results demonstrate that HFS-induced potentiation is significantly disrupted following exposure to acute stress, but not CORT injections, and that this disruptive effect is dependent upon GR activation (Fig. 3.3C).
Figure 3.3: Long-term plasticity. (A) High frequency stimulation-induced LTP is present in control (open circle) and CORT (black triangles) rats but not acutely stressed rats (back circles). (B) High frequency stimulation-induced LTP following RU38486 treatment (light grey circles) and RU38486 treated rats exposed to acute stress (dark grey circles). (C) Summary of the effects of acute stress, CORT, and RU38486 treatment on HFS-induced long-term plasticity. (D) Low frequency stimulation-induced late-developing potentiation is present in control (open circles) and CORT (black triangles) but not acutely stressed rats (black circles). (E) Low frequency stimulation-induced late-developing potentiation following RU38486 treatment (light grey circles) and RU38486 treated rats exposed to acute stress (dark grey circles). (F) Summary of the effects of acute stress, CORT, and RU38486 treatment on LFS-induced long-term plasticity. * denotes $P < 0.05$ relative to all other groups.
3.4. LFS of CA1 induces late-developing potentiation in SUB that is disrupted following exposure to acute stress, but not CORT, in a GR-dependent manner

Low frequency stimulation of the CA1-SUB pathway induced late-developing potentiation (21.30 ± 4.97%; n = 8) in SUB that was disrupted following exposure to 30 min of restraint stress (-4.68 ± 7.30%; n = 6). Interestingly, rats that received acute CORT injections demonstrated similar patterns of potentiation as control animals (28.25 ± 14.81%; n = 5; Fig. 3.4D). An ANOVA revealed a significant effect of Group \([F_{2,19} = 3.82, P = 0.044]\). Post-hoc analyses revealed that the acute stress alone group displayed significantly lower potentiation than the other groups \((P < 0.05)\). Again, we examined the role of GR activation in the deleterious effects of acute stress using RU38486. Rats injected with RU38486 displayed significant potentiation (20.17 ± 4.70%; n = 6), as did rats that received both RU38486 treatment and exposure to acute stress (22.04 ± 4.29%; n = 5; Fig. 3.4E). A 2X2 ANOVA with Stress and RU38486 as factors revealed significant main effects of Stress \([F_{1,21} = 4.85, P = 0.039]\), RU38486 \([F_{1,21} = 5.46, P = 0.029]\), and a significant Stress by RU38486 interaction \([F_{1,21} = 6.47, P = 0.019]\). Post hoc comparisons revealed that the stress group displayed significantly lower PPF than the other groups \((P < 0.05)\). In summary, similar to the HFS-induced LTP within the CA1-SUB pathway, the LFS-induced late-developing potentiation is significantly disrupted following exposure to acute stress, but not CORT injections, and this disruptive effect is also mediated via GR activation (Fig. 3.4F).

3.5. Reduced PPF in the CA1-SUB pathway following long-term synaptic potentiation is abolished by acute stress
The magnitude of PPF (averaged 25 and 50 ms IPIs) changed following the induction of HFS- and LFS-induced potentiation. A repeated measures ANOVA on the pre-tetanus and post-tetanus PPF values revealed a significant main effect of Time \( [F_{1,46} = 8.51, P = 0.005] \), a Time by Stress interaction \( [F_{1,46} = 12.95, P = 0.001] \), a Time by RU38486 interaction \( [F_{1,46} = 12.19, P = 0.001] \), as well as a Time by Stress by RU38486 interaction \( [F_{1,46} = 11.37, P = 0.002] \). Difference scores revealed that PPF was decreased in control (-16.82 ± 4.16%), CORT (-17.44 ± 0.76%), RU38486 (-17.45 ± 6.84%), and RU+Stress (-16.26 ± 3.32%) groups while it was significantly increased in the stress alone group (19.84 ± 7.16%; Fig 3.4A). Post hoc comparisons on the PPF difference scores revealed that the change in PPF following tetanization was significantly different in the stress alone group compared to all other groups \( (P < 0.05) \). These results demonstrate that the reduced PPF following the induction of potentiation is abolished by exposure to acute stress, but not CORT injections, and that antagonism of GRs eliminates this effect.

Changes in PPF exhibited an inverse relationship with initial pre-tetanus PPF values for control (Fig. 3.5B; \( r^2 = 0.48, P = 0.002, y = -0.59x + 1.30 \)), RU38486 (Fig. 3.4E; \( r^2 = 0.45, P = 0.024, y = -0.52x - 0.60 \)), and RU+Stress (Fig. 3.4F; \( r^2 = 0.64, P = 0.003, y = -0.90x + 17.53 \)) groups but not stress (Fig. 3.4C; \( r^2 = 0.32, P = 0.069, y = 0.67x + 11.95 \)), or CORT (Fig. 3.4D; \( r^2 = 0.24, P = 0.15, y = -0.35x - 1.50 \)) groups. These data indicate that larger initial PPF is associated with decreases in PPF while smaller initial PPF is associated with little to no changes in PPF following the induction of potentiation in the CA1-SUB pathway.
Figure 3.4: Changes in PPF following tetanic stimulation. (A) Initial pre-tetanus PPF values (left bars; averaged 25 and 50 ms IPIs) compared to post-tetanus PPF values (chequered bars; averaged 25 and 50 ms IPIs). * denotes $P < 0.05$ relative to pre-tetanus value. (B-F) Correlation and regression plots showing changes in PPF following the induction of potentiation across different experimental conditions. The initial pre-tetanus PPF is plotted on the x-axis and the PPF difference scores are plotted on the y-axis.
4.0. GENERAL DISCUSSION

The present experiments provide novel results regarding the effects of acute stress on patterns of both short- and long-term synaptic plasticity within the CA1-SUB pathway. Exposure to 30 min of restraint stress, but not acute treatment with CORT, disrupted PPF (Fig. 3.2) and two forms of long-term synaptic plasticity (Fig. 3.3). Critically, both the stress and CORT manipulations resulted in statistically similar plasma levels of CORT before the surgical procedure was initiated (Fig. 3.1A). Acute stress also reversed the typically observed changes in PPF at this synapse following the induction of long-term synaptic potentiation (Fig. 3.4). The current data provide strong evidence that the disruptive effects of acute stress are mediated in part by GR activation, as these disruptions can be fully blocked by pre-treatment with the selective GR antagonist RU38486. Taken together, these results demonstrate for the first time the necessity, but not sufficiency, of GR activation for the alterations in subicular plasticity caused by acute stress.

4.1. Acute stress and CORT injections elevate circulating CORT levels

Plasma CORT measurements were taken from animals in all three groups near the same time of day (first 4 h of the light cycle); therefore, it is likely that basal levels of CORT were similar between the groups and near the levels of the controls reported in Fig. 2.1 (~ 4 µg/dL). Plasma levels of circulating CORT are well known to increase in response to acute stress (McEwen, 1994, Habib et al., 2001, Ulrich-Lai and Herman, 2009). In the current study, exposure to acute stress significantly enhanced circulating CORT relative to control levels as measured immediately after anaesthesia (Fig. 3.1A). In order to test the effects of elevated CORT alone on subicular synaptic plasticity, we
injected CORT systemically and found that a dose of 3 mg/kg produced comparable levels of circulating hormone to acute stress shortly following anaesthesia (Fig. 3.1A), as has been shown previously in rats (de Quervain et al., 1998). Unexpectedly, CORT measurements made after surgery and prior to tetanization revealed an increase in circulating levels within the control group (Fig. 3.1B), as well as sustained elevations in the CORT and acute stress groups. Previous studies have noted post-operative elevations in circulating CORT in rats (Goldkuhl et al., 2010, Nyuyki et al., 2012), and it is therefore likely that the surgery itself is responsible for the observed CORT elevations. Further experimentation is required to gain better temporal control of these hormonal changes.

4.2. Effects of acute stress on PPF in dorsal subiculum

In the current study, acute stress-induced disruptions of PPF in the CA1-SUB pathway before tetanic stimulation were evident and could be prevented by pre-treatment with RU38486. The magnitude of PPF of the control group at 25 and 50 ms IPIs is similar to that reported in other studies (~ 30-50%; Commins et al., 1998a, Commins and O'Mara, 2000, Commins et al., 2001), although it should be noted that significant PPF was no longer observed at longer intervals (100 and 200 ms). Previous studies are contradictory regarding the effects of acute stress on initial subicular PPF before tetanus. One study reported no change in PPF following exploration of a novel box for 30 min (Commins and O'Mara, 2000), an experience used previously as a stressor in studies of CA1 synaptic plasticity (Xu et al., 1997). Another study demonstrated significantly disrupted PPF (50 ms IPI) 4 hours following lipopolysaccharide treatment (Commins et al., 2001). It is difficult to specify why the study of Commins and O’Mara (2000) failed
to demonstrate a significant effect, although differences between the stressors may be involved. Glucocorticoid receptor-dependent disruptions of PPF have also been reported in the CA3-CA1 pathway in vivo following acute stress induced by exposure to an elevated platform (Cazakoff and Howland, 2010). The current results also indicate that increases in circulating CORT by exogenous means is not a sufficient physiological parameter to alter PPF. Therefore, other factors not specifically related to the endogenous elevation of CORT brought about by acute stress such as modulatory influences of innervating regions or heightened emotionality and arousal may be crucial factors in producing the observed disruptions of synaptic plasticity.

High levels of PPF are generally thought to be more likely in the presence of low initial release probabilities at glutamatergic synapses while low levels of PPF are commonly accredited to high initial release probabilities (Commins et al., 1998a, Zucker and Regehr, 2002). As the current data demonstrate lower PPF values following exposure to acute stress, it may be the case that acute stress causes an increase in glutamatergic release probability within CA1-SUB synapses. However, the exact mechanism and additional factors by which GR activation brings about these alterations in vesicular release probability remains an open question and warrants further investigation.

4.3. Acute stress and long-term plasticity in dorsal subiculum

Two forms of long-term synaptic plasticity have been shown to exist within the CA1-SUB pathway in vivo following either HFS (Commins et al., 1998b) or LFS (Anderson et al., 2000) protocols. Interestingly, these forms of synaptic plasticity are thought to be reliant upon distinct induction mechanisms (Fidzinski et al., 2008, Wozny
et al., 2008b) as demonstrated using patch-clamp electrophysiology. The current data demonstrate that acute stress disrupts both forms of synaptic plasticity and that these disruptions can also be prevented by pre-treatment with RU38486. Specifically, HFS-induced LTP is disrupted following exposure to acute restraint stress, which is dependent upon GR activation (Fig. 3.3C), similar to findings from CA1 (Xu et al., 1998, Cazakoff and Howland, 2010). In the SUB, LFS-induced late-developing potentiation also undergoes GR-dependent disruption following exposure to acute stress (Fig. 3.3F). This is in dramatic contrast to the typically observed enabling of LTD following LFS in the CA1 subfield (Xu et al., 1997, Wong et al., 2007). Interestingly, neither the HFS-induced LTP nor the LFS-induced late-developing potentiation suffered disruptions following acute CORT administration. Taken together, the data again provide novel and compelling evidence that GR activation is a necessary, but not a sufficient, physiological trigger for the observed stress-induced disruptions on patterns of long-term plasticity within the CA1-SUB pathway.

Although a limited number of studies have examined long lasting changes in synaptic strength within the CA1-SUB pathway in the context of acute stress, disruptions in HFS-induced LTP within this pathway have been previously reported in rats exposed to the bacterial endotoxin lipopolysaccharide, which is thought to activate similar neural and endocrine responses as those activated by acute stress exposure (Commins et al., 2001). Moreover, exposure to a novel open field environment has been shown to facilitate the induction of LTD within this pathway following a 10 Hz stimulation protocol (Commins and O'Mara, 2000). However, the current experiments are the first to examine and provide evidence for a putative mechanism by which acute stress disrupts HFS-
induced LTP and LFS-induced late-developing potentiation within this major output pathway of the hippocampal formation.

4.4. A presynaptic locus for potentiation in the dorsal subiculum

Whether the mechanisms governing synaptic plasticity are expressed pre- or postsynaptically is a topic of vigorous debate among researchers (Krueger and Fitzsimonds, 2006, Lisman, 2009). The debate itself dates back to the Hebb’s original postulate that speculated that some change is brought about in one or both cells that results in an increase in synaptic efficacy (Hebb, 1949). It has been argued that changes in PPF, a presynaptic phenomenon, following the induction of potentiation are indicative of presynaptic expression mechanisms for the synaptic plasticity in question (Schulz et al., 1994). Commins and colleagues (1998a) have previously demonstrated reductions in PPF following LTP over a small range of IPIs (30-100 ms) in the CA1-SUB pathway. The present results demonstrate similar changes in PPF for 25 and 50 ms IPIs following LTP and extend these findings to include the late-developing potentiation (Fig. 3.4A). These results suggest that a presynaptic locus may exist for both forms of potentiation within the CA1-SUB pathway. Specifically, we show significant decreases in PPF values following the induction of potentiation (Fig. 3.4A), which may indicate increases in transmitter release following the first stimulation (Zucker and Regehr, 2002).

Interestingly, rats exposed to acute restraint stress displayed significantly lower levels of pre-tetanus PPF, while an increase in PPF following long-term synaptic potentiation was observed (Fig. 5A). Importantly, blocking GR activation abolished these effects of acute stress on PPF before and after the tetanus. The increase in PPF following
tetanization in the acute stress group may be attributed to either the tetanic stimulation itself or the transient nature of the stress effects on synaptic release probability. Thus, the current data provide compelling evidence that acute stress may actually trigger an initial positive shift in the release probability at hippocampal output synapses (Karst et al., 2005).

4.5. Acute stress, the amygdala, and subicular plasticity

It is well established that exposure to acute stress disrupts forms of synaptic plasticity within the hippocampus (Shors and Thompson, 1992, Diamond et al., 1994, Kim et al., 1996, Xu et al., 1997, Kim and Diamond, 2002, Yang et al., 2004, 2005, Cazakoff and Howland, 2010, Chen et al., 2010) as well as memory performance on a variety of hippocampal-dependent tasks (Diamond and Rose, 1994, Kim and Diamond, 2002, Wong et al., 2007, Howland and Wang, 2008, Park et al., 2008, Cazakoff et al., 2010). While GR activation is necessary for the acute stress-induced disruptions in plasticity (the present data; Xu et al., 1998, Cazakoff and Howland, 2010) several lines of evidence suggest that elevated circulating CORT is not a sufficient physiological parameter for disrupting hippocampal processing (Kim et al., 2001, Kim and Diamond, 2002, Woodson et al., 2003, Kim et al., 2005). For example, inactivation of the amygdala during acute stress exposure prevents stress-induced disruption in hippocampal LTP in vitro and prevents the disruptive effects on spatial learning (Kim et al., 2005), despite the fact that circulating plasma levels of CORT have been reported to be statistically similar to those found in rats with intact amygdalae (Kim et al., 2001). In addition, Woodson and colleagues (2003) demonstrated that acute predatory stress, but not other behavioural
approaches capable of elevating CORT, such as exposure to a sexually receptive female or exercise, disrupts memory performance on a hippocampal dependent task (de Quervain et al., 1998, Woodson et al., 2003).

Based on the previous empirical work examining the dissociation between elevated CORT and fear-provoking stressors, it has been suggested that heightened emotionality or fear-evoked arousal, involving the amygdala, plays an integral role in the stress-induced disruptions of hippocampal processing (Kim and Diamond, 2002, McGaugh, 2004, Park et al., 2006). This is evident from studies showing that inactivation of the amygdala prior to acute stress exposure prevents the disruptive effects on patterns of synaptic plasticity within the hippocampus (Kim et al., 2001, Kim et al., 2005). Importantly, amygdalo-hippocampal bundles project directly to CA1 and SUB while indirect projections via the EC to numerous regions of the hippocampal formation have also been demonstrated (Pikkarainen et al., 1999). Thus, there are numerous routes through which the amygdala influences hippocampal information processing following stressful circumstances (Kim and Diamond, 2002). Our data appear to fit well with these general observations and it may be the case that stress-induced emotionality and heightened arousal, thereby activating the amygdala, is a necessary and concurrent component to the observed stress-induced disruptions of subicular plasticity as is the case with CA1 plasticity (Kim et al., 2001, Kim et al., 2005). Further experimentation is required to address this possibility.

4.6 Functional Implications
As SUB undergoes potentiation following both high- and low-frequency afferent input, it has been implicated in mediating hippocampal-cortical interactions via signal amplification (O'Mara et al., 2000, O'Mara, 2005, Behr et al., 2009). The fact that the majority of pyramidal cells within SUB are burst firing cells (Taube, 1993, O'Mara et al., 2001) and the fact that bursting has been shown to strengthen signal transmission by increasing release probability at target synapses (Lisman, 1997) lends credence to these views. While the functional significance of LFS-induced late-developing potentiation remains unknown, it is interesting to note that slow rhythmic activity and synchronous firing in the range of 0.5-2 Hz have been reported in dorsal hippocampal circuits during periods of slow-wave sleep (Sirota, 2003, Isomura et al., 2006). The LFS-induced potentiation in SUB may thus have a functional role in the reorganization and transfer of information from hippocampal circuits to the cortex during critical periods of sleep (Frankland and Bontempi, 2005, Habib and Dringenberg, 2010). Therefore, aberrations in normal SUB functioning brought about by exposure to challenging circumstances could lead to significant disruptions in cognitive processes related to hippocampal-cortical interactions.

Spatial information processing is heavily reliant upon regional connectivity and plastic mechanisms within the traditional tri-synaptic circuitry of the dorsal hippocampus (O'Keefe and Nadel, 1978, Barnes et al., 1994, Moser et al., 1995, Wong et al., 2007). Until recently, the contribution of SUB to spatial cognition has received little consideration (Morris et al., 1990, Cho and Jaffard, 1995, O'Mara, 2005, 2006, O'Mara et al., 2009, Potvin et al., 2010). Based on previous work and the current observations, it is reasonable to suggest that a GR-dependent global remodeling of synaptic transmission
within the entire circuitry of the hippocampal formation may be responsible for the deficits observed in spatial cognition following exposure to acute stress (Diamond and Rose, 1994, Wong et al., 2007, Howland and Wang, 2008, Park et al., 2008, Cazakoff and Howland, 2010). Therefore, future work is required to examine regional-specific dissociations in distinct structures within the hippocampal formation (Passecker et al., 2011) so to provide greater insights into the deleterious effects of acute stress on cellular communication and the consequential behavioural impairments that result.

4.6. General Conclusions

The survival capabilities of organisms are largely dependent upon the ability to adapt to challenging circumstances (Selye and Fortier, 1950). The ability to properly regulate internal states and maintain homeostasis during physiological departures that result from the stresses of life is of paramount importance for adaptive purposes (Selye and Fortier, 1950). Exposure to acute stress activates multiple organ systems and produces an abrupt and transient departure from physiological normalcy (Wamsteeker and Bains, 2010). Although this transient response gradually subsides, the nature of the initial effects can have long lasting effects on neuronal communication. Indeed, the current data demonstrate that exposure to acute stress can provide long-lasting changes in the synaptic efficacy of traditionally viewed Hebbian synapses within the major output structure of the hippocampal formation.

In conclusion, the present experiments highlight the susceptibility of subicular plasticity to acute stress and demonstrate the importance of one mechanism by which this hippocampal output structure undergoes synaptic modification during challenging
circumstances. Moreover, the data show for the first time that GR-activation is a necessary, but not a sufficient, physiological parameter for the effects of acute stress on subicular plasticity. The present experiments thereby extend the literature of acute stress disruptions within the tri-synaptic circuitry of the hippocampal formation to include acute stress-induced disruptions within the CA1-SUB pathway. It is likely then that GR activation, following exposure to acute stress, initiates a global remodeling scheme of synaptic transmission such that the likelihood of potentiation, and plasticity in general, is significantly reduced across the numerous glutamatergic synapses within the hippocampal formation. The specific downstream signalling mechanisms, involvement of the amygdala, the sufficiency of GR activation at other hippocampal synapses, and behavioural consequences of synaptic remodelling within the CA1-SUB pathway following exposure to acute stress remain important and open questions.
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