Antidepressant-like Effects of Peripheral Reelin Administration in a Preclinical Model of Depression

A Thesis Submitted to the College of

Graduate and Postdoctoral Studies

in Partial Fulfillment of the Requirements

for the Degree of Master of Arts

in the Department of Psychology

University of Saskatchewan

Saskatoon

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ABSTRACT

Depression is a serious psychiatric disorder characterized by a range of debilitating symptoms. Long-term exposure to stress is a significant risk factor for the onset and maintenance of depression. Rats exposed to repeated treatment with the stress hormone corticosterone (CORT), a well-established rodent model of depression, begin to display depression-like symptoms. The development of depression-like symptoms with prolonged exposure to CORT is accompanied by reductions in the number and maturation rate of immature dentate granule cells within the hippocampus. Furthermore, these changes are paralleled by gradual decreases in reelin-expressing cells in the dentate subgranular zone of the hippocampus. Reelin is a large extracellular matrix protein that has been implicated in a number of neuropsychiatric disorders, including szchiphrenia, bipolar disorder, autism, as well as major depression. It holds important roles in learning and memory, cell migration and integration, synaptic contact formation, and adult neurogenesis. Mice deficient in reelin are more susceptible to CORT-induced impairments in hippocampal neurogenesis and the development of a depressive phenotype. Previous work has shown that intra-hippocampal infusions of reelin into the hippocampus reverse CORT-induced increases in depression-like behavior in rats, while restoring accompanying impairments in hippocampal neurogenesis.

Reelin is also expressed in peripheral organs and tissues, though its roles here are not well understood. However, reelin-deficient mice show peripheral alterations in the clustering pattern of the serotonin transporter (SERT) in membranes of blood lymphocytes. The serotonin transporter is one of the main targets of antidepressant action, and importantly, this altered pattern of SERT clustering in reelin-deficient mice is mirrored both in patients with depression and in rats exposed to prolonged CORT treatment.

Based on the previous findings, we were motivated to examine whether peripheral injections of reelin could restore CORT-induced increases in depression-like behavior. To investigate possible mechanisms, we examined (i)

the SERT clustering pattern in peripheral lymphocyte membranes, and (ii) the maturation rate of immature hippocampal neurons.

40mg/kg of CORT was administered subcutaneously once per day for 21 consecutive days. In conjunction, we utilized a novel reelin injection paradigm, where reelin was delivered via the lateral tail vein at either 3µg/ml or 5µg/ml every 5 or 10 days during the period of CORT injections. Depression-like behavior was measured using the forced swim test the day following the last CORT injection. The open field test was included as a measure of locomotive and anxiety-like behavior.

Importantly, peripheral reelin at all dosages administered restored CORT-induced increases in depression-like behavior in the forced swim test, normalizing both immobility and swimming behaviors. Neither CORT nor reelin impacted open field behavior. As expected, CORT-treated rats displayed alterations in SERT membrane protein clustering, and importantly this was restored by all doses of reelin administered. Peripheral reelin did not significantly reverse the CORT-induced deficits in immature neuron maturation rate.

These novel findings demonstrate that reelin has antidepressant-like actions when given peripherally and provide evidence for the regulation of serotonin transporter clustering in lymphocyte membranes as a mechanism for the antidepressant action of reelin.

ACKNOWLEDGEMENTS

I would first like to thank my supervisor, Dr. Lisa Kalynchuk, for provding me with the invaluable opportunity to pursue my Master's degree under her supervision, and affording me the chance to learn and grow as a researcher. Without your continual commitment and insight, this would not have been possible.

I would also like to thank Dr. Hector Caruncho for his immense support throughout my program. Your time, patience, and guidance are very much appreciated.

Thank you to the members of my committee, Dr. John Howland and Dr. Steve Prime, for providing me with valuable direction and mentorship in my research.

Next, I would like to thank Dr. Raquel Romay-Tallon for taking extensive time to train and teach me. Thank you for your continued support and patience, and for allowing me to come freely to you with all of my questions. I significantly gained from your example as a researcher.

I would also like to thank my colleagues, Josh Allen, Kyle Brymer, Nikita Nogovitsyn, Dr. Katherina Lebedeva, and Jamie Kim for the academic support you provided, while cultivating a work space I was grateful to be a part of.

Thank you to my dear friends, Raquel and Josh, for making my Master's journey uniquely memorable and enjoyable. I am forever grateful for your lasting and treasured friendships. Nikita, thank you for your motivating presence and the continued encouragement that you provided me. Thank you to Gavin Scott, for sharing your knowledge and friendship, and for being a researcher whom I can admire. Thank you to Payden Laderoute for supporting me and encouraging me to achieve my goals.

Lastly, I would like to thank my mother, Melody Mitchell, for her unwavering love and support throughout my educational career. Thank you for the foundation you have provided me, not only throughout my Master's but throughout my whole life.

Thank you again to all of you, for shaping who I am as a student and as a person.

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5-HT	5-hydroxytryptamine (serotonin)
ACTH	Adrenocorticotrophin Hormone
BBB	Blood Brain Barrier
CA	Cornu Ammonis
CMS	Chronic Mild Stress
CORT	Corticosterone
CRF	Corticotropin-Releasing Factor
DA	Dopamine
DCX	Doublecortin
DG	Dentate Gyrus
DLPFC	Dorsal Lateral Prefrontal Cortex
DSM-V	Diagnostic and Statistical Manual of Mental Disorders, 5th Ed.
DST	Dexamethasone Suppression Test
ECT	Electroconvulsive Therapy
FST	Forced Swim Test
GABA	*Gamma*-Aminobutyric Acid
GCL	Granule Cell Layer
HPA	Hypothalamic-Pituitary-Adrenal
HRM	Heterozygous Reeler Mice
IHC	Immunohistochemistry
LH	Learned Helplessness
LTP	Long-term potentiation
MAOI	Monoamine Oxidase-Inhibitor
MDD	Major Depressive Disorder
MRI	Magnetic Resonance Imagining
MWM	Morris Water Maze
NA	Noradrenaline
NMDA	N-methl-D-asparate
NR2B	NMDA Receptor Subtype 2B
NR2A	NMDA Receptor Subtype 2A
OFT	Open Field Test
OLT	Object Location Test
PFC	Prefrontal Cortex
PVN	Paraventricular Nucleus
RR	Reelin Repeats
SC	Subcutaneous
SEM	Standard Error of the Mean
SERT	Serotonin Transporter
SNRI	Serotonin–Noradrenaline Reuptake Inhibitor
SPSS	Statistical Package for the Social Sciences
SPT	Sucrose Preference Test
SSRI	Selective Serotonin Reuptake Inhibitor
SGZ	Sungranular Zone
SVZ	Subventricular Zone

TCA	Tricyclic Antidepressant

CHAPTER 1

General Introduction

1. Introduction to Depression and Stress as a Predisposing Factor for the Onset and Maintenance of Depression

1.1 Depression

Major Depressive Disorder (MDD) is a debilitating psychiatric disorder with a lifetime prevalence rate of 15-30% (Kessler et al., 2003). It is estimated by the World Health Organization (WHO) that MDD will be the leading cause of disease burden worldwide by 2030 (Willner et al., 2013). MDD presents as a complex and highly heterogeneous disorder with regard to its symptoms (McGlinchey et al., 2006). It is characterized by a range of disabling symptoms, that include low mood, mood variability, anhedonia (loss of pleasure or interest), impairments in emotion regulation, disrupted sleep and appetite, low energy and motivation, suicidal ideation, and altered cognitive attributes such as rumination, impaired attentional control, and dysfunctional attitudes (Fales et al., 2007; Fava & Kendler, 2000; Ramel, Goldin, Carmona, & McQuaid, 2004). Not surprisingly, these symptoms can present as serious challenges and setbacks in an individual's daily living. To fit the diagnostic criteria for MDD, an individual must present with at least 5 of the following symptoms, nearly every day, for at least 2 weeks, of which at least one of the symptoms must be (1) depressed mood or (2) anhedonia.

(1) Depressed or irritable mood most of the day, as indicated through subjective report or observations made by others

(2) Reduced interest/pleasure in most or all activities, most of each day (as made by subjective report or observation)

(3) Significant weight change, or change in appetite

(4) Change in sleep (insomnia or hypersomnia)

(5) Change in activity level (psychomotor agitation or retardation); made

through observation and not merely subjective evaluation

(6) Fatigue or loss of energy

(7) Feelings of worthlessness or excessive/inappropriate guilt

(8) Diminished ability to think or concentrate

(9) Suicidal ideations.

In addition, the depressive episode cannot be attributed to the physiological effects of a substance or another medical condition, or be better explained by the diagnosis of another mood or psychotic disorder. There must be no prior history of a manic or hypomanic episode (American Psychiatric Association, 2013).

Despite both the severity and ubiquity of major depressive disorder, there still remains a lack of understanding about the neurobiological mechanisms that underlie its development and recurrence. Since the initial discovery and development of monoamine oxidase inhibitors (MAOIs) and tricyclic antidepressants (TCAs), the pathophysiology of depression has been largely dominated by the monoamine hypothesis (Hindmarch, 2002). According to the monoamine hypothesis of depression, decreased neural transmission of the cerebral monoamines, which include serotonin, dopamine, and norepinephrine, underlie depressive pathology. However, the monoamine hypothesis alone cannot entirely explain the pathophysiology of depression or the action of common serotonergic and noradrenergic antidepressants, given that there is an appreciable delay period between antidepressant regulation of monoamine availability and therapeutic outcomes (Hindmarch, 2002; Sanacora et al., 2012), suggesting that the antidepressant effects are due to events occurring downstream of monoamine regulation. Other biological theories exist that attempt to explain the pathophysiology of depression, which include theories of: dysregulated hypothalamic-pituitary-adrenal (HPA) axis functioning, reduced hippocampal volume, and compromised immune functioning related to the secretion of pro-inflammatory cytokines (Post, 1992; Sapolsky, 2000; Maes, 2001; Maes et al., 2009; Raison et al., 2006; Miller et al., 2009; Blume et al., 2011; Young et al., 2014).

Despite the relative short-term effectiveness of pharmacological and

psychological treatments for depression, there is a continued need for the development of fast-acting and effective long-term treatments in order to address the current large-scale treatment resistance experienced by many patients (Marchetti, Koster, Sonuga-Barke, & De Raedt, 2012). This need stems from the complicated nature of treatment development in the field of depression due to the complex etiology of the disorder. Heritability of depression based on twin studies is estimated at 40-50% (Levinson, 2006). However, in addition to genetic pre-determinants, there are potent environmental factors, such as early life trauma and recent stressful experiences, which inarguably come into play when determining the influencing factors that contribute to the development of depression (Checkley, 1996; Parker et al., 2003; Kendler et al., 2002, 2004). Additionally, there exists substantial variability in stress susceptibility among individuals, making it more or less likely for some individuals to confer stress resilience than others.

1.2 Structural and Functional Alterations in Depression

Although the monoamine hypothesis dominated the field of depression for many years, more recent research has elucidated prominent changes in the structure, function, and responsiveness of the brain associated with depressive disorder (Berton & Nestler, 2006; Pittenger & Duman, 2008). The pathophysiology of depression is increasingly being studied in relation to the neural circuitry involved in the interactions between emotional and attentional processes, contributing to an understanding of depression as a failure to employ top-down attentional processes to manage limbic activity (Farb, Segal, & Mayberg, 2007; Drevets, 2000; Marchetti et al., 2012). Neuroimaging of patients experiencing a depressive episode has revealed a profile of brain metabolic changes that reflect abnormal activation of various brain structures involved in emotion regulation and stress response (e.g., hippocampus and amgydala). For instance, individuals with major depression show hyperactivity of the amygdala when processing emotionally-laden information (Sheline et al., 2001). In contrast, other brain regions involved in sensory processing and attention, such as the

dorsolateral prefrontal cortex (DLPFC), are aberrantly deactivated (Drevets, 2001). Increased number of depressive episodes has been associated with reduced connectivity between frontal and limbic areas, supporting the idea for a disruption of network circuitry involved in top-down cognitive control over limbic activity in depression (Dannlowski et al., 2009).

In addition to the abnormal activation of various brain regions, neuroimaging and post-mortem analyses in patients with depression have revealed structural alterations in limbic and forebrain regions, including the hippocampus, amygdala, and prefrontal cortex (Drevets, 2000, 2001; Jaracz, 2008). Of these regions, the hippocampus has been the most extensively studied in the context of depression. The hippocampus plays a central role in long-term potentiation (LTP), in the consolidation of short-term memory into long-term memory, and in spatial memory, and consequently, navigation. A simplified description of hippocampal neuroanatomy and neuro-circuitry will be given in the following paragraph in order to provide a brief introduction to the unique characteristics of the hippocampal formation.

The hippocampus is part of the broader hippocampal formation, which is comprised of the: hippocampus proper, dentate gyrus, subiculum, presubiculum, parasubiculum (together making up the subicular cortex), and the entorhinal cortex (EC). The hippocampus proper is made up of the Cornu Ammonis (CA) which consists of three subfields making up the hippocampal circuit: CA1, CA2, CA3. The passage of information through intrahippocampal circuits is largely unidirectional, a unique feature of hippocampal neuroanatomy. First, cells in the superficial levels of the entorhinal cortex have axons that extend primarily to the dentate gyrus, in a unidirectional manner. It is these axonal projections from the entorhinal cortex to the dentate gyrus that form the perforant pathway - a major input pathway into the hippocampus. Next, granule cells, the primary cells of the dentate gyrus, project mossy fiber axons to the pyramidal cells of the CA3 hippocampal field. Pyramidal CA3 cells then project to the CA1 hippocampal field via Schaffer collateral axons. The CA2 field is a narrow zone of cells located between CA3 and CA1 fields. It has large pyramidal cells like CA3, but unlike

CA3 it is not innervated by mossy fibers from the dentate gyrus. Following innervation by CA3 Schaffer collaterals, CA1 pyramidal cells then project to the subiculum, affording the main source of excitatory input to the subiculum. In all cases, these projections create a unidirectional course for information flow. Pyramidal cells of the CA1 hippocampal field also project to the entorhinal cortex. As well, the subiculum projects primarily to the entorhinal cortex. It is through these connections back to the entorhinal cortex, that both the CA1 field and the subiculum close the hippocampal circuitry loop that began in the superficial layers of the entorhinal cortex (Amaral & Lavenex, 2007).

The laminar organization of the hippocampus is similar for all of its major fields. The stratum oriens is the deepest, or most subficial layer of the CA, located superficial to the alveus, which borders the wall of the lateral ventricle. This description is based on conventional superficial-deep nomenclature, which defines regions closer to the pia or hippocampal fissure as superficial and those regions in the opposite direction (closer to the alveus) as deep. The basal dendritic tree of pyramidal cells extends into the stratum oriens. Superficial to the stratum oriens is the stratum pyramidal layer, which is the principal cell layer and is most tightly packed in CA1. Superficial to the stratum pyramidal layer is the stratum radiatum, which contains interneurons that form inhibitory and excitatory contacts, as well as the CA3 to CA1 Schaffer collaterals. Next, is the stratum-lacunosum-moleculare (SLM), the most superficial layer of the hippocampus proper. Like the stratum radiatum, the SLM contains a variety of interneurons. Fibers from the entorhinal cortex and afferent fibers from other regions also terminate in the SLM. The dentate gyrus is a three-layered cortical structure, characterized by its U shape. Its most superficial layer, residing closest to the hippocampal fissure, is the molecular layer, containing relatively few cells. The principal cell layer of the dentate gyrus is the granule cell layer (GCL), which is made up of densely packed excitatory granule cells, whose apical dendrites extend into the superficial portion of the molecular layer. The U shape created by the molecular layer and the GCL surrounds the polymorphic cell layer, which is also referred to as the hilus. The hilus contains inhibitory interneurons as well as

the mossy fiber axons of granule cells that extend to the CA3 field. (Amaral & Lavenex, 2007). Importantly, between the GCL and the hilus is the subgranular zone (SGZ), which is one of the few sites in the adult brain where new neuron birth takes place. This important region will be discussed in more detail later on.

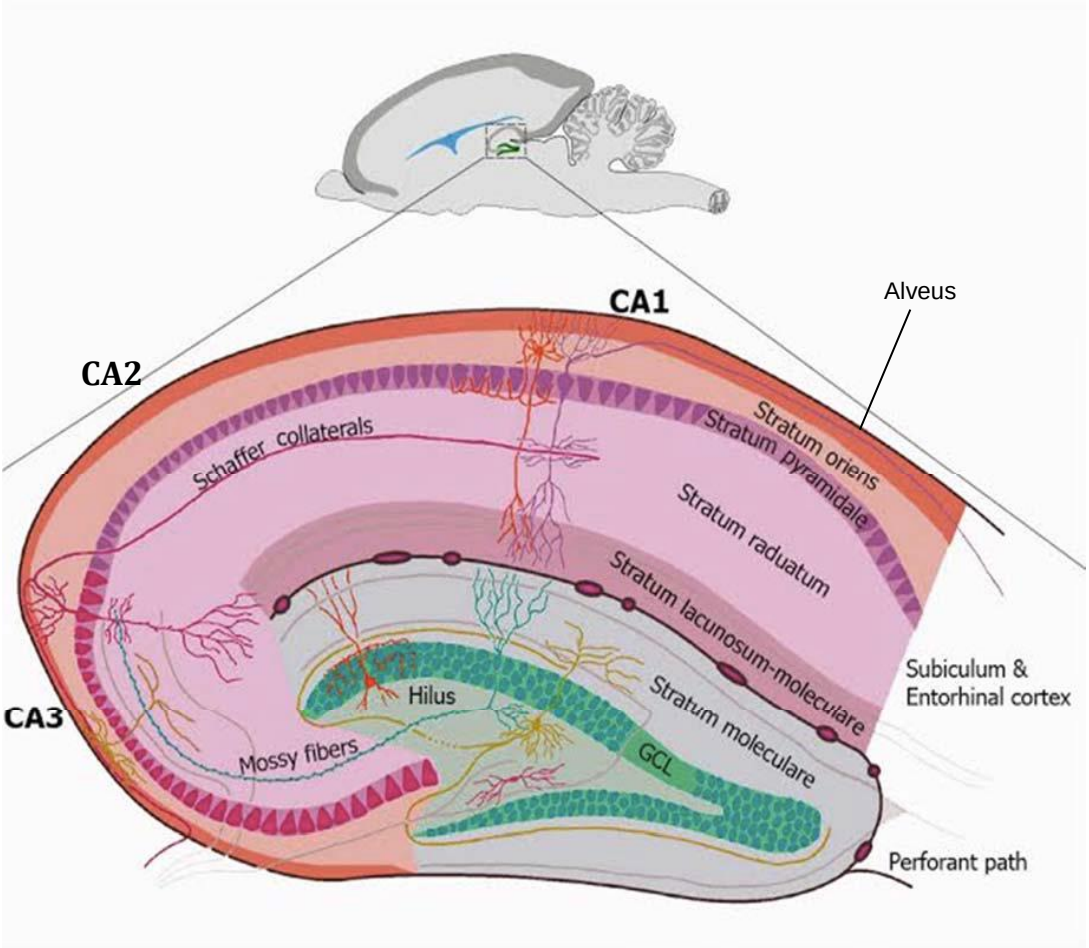

Fig 1. Representation of the laminar organization and connectivity of the rodent hippocampal formation. Adapted from Lebedeva (2017), with permission.

While there exists obvious hippocampal differences between species, for example significantly greater hippocampal volumes and more complex hippocampal organization in primates as compared to rodents, the basic hippocampal architecture and the pattern of connectivity just described are similar in rats, monkeys, and humans. It is these similarities that allow for cross-species inferences to be made when elucidating various structural and functional brain abnormalities associated with a range of psychiatric disorders.

As mentioned, the hippocampus has received extensive focus in depression research. Hippocampal volume reductions are associated with depressive illness, with more pronounced reductions observed with increased severity and duration of the disorder (MacQueen et al., 2003; Caetano et al., 2004; Campbell et al., 2004; Frodl et al., 2004, 2006; Jaracz, 2008; Lorenzetti et al., 2009). Reduced PFC volumes have also been observed in patients with depression (Konarski et al., 2008), whereas amygdalar volume appears to change from an initial increase in size at onset of illness to a reduction in volume with progression of the disorder (Lorenzetti et al., 2009). Notably, these brain regions that show structural alterations associated with depression (i.e., hippocampus, PFC, and amygdala), are part of the limbic-cortico-thalamic circuit, which regulates attentional control of emotional processing, and has been implicated in depressive pathophysiology (Farb et al., 2007; Segal & Mayberg, 2007; Drevets, 2000; Marchetti et al., 2012). Collectively, these brain regions are involved in cognition and emotion regulation, and the shared dysregulation in their structure and function may lead to impairments in emotion, motivation, cognition, and behavior that characterize mood disorders.

1.3 The HPA Axis: Relation to Stress and Depression

The hippocampus is also involved in stress adaptation and resilience, which can pose environmental contributing factors for the onset and development of psychiatric and mood disorders. Stress may be defined as a physiological adaptation to environmental challenges or threats that cause activation of the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis comprises a complex set of interactions among three endocrine glands, the hypothalamus, pituitary gland, and adrenal glands. The HPA system regulates many body processes. It is involved in homeostasis, digestion, immune response, mood and emotions, energy storage and expenditure, and regulation of the stress response. The normal response to a stressful event is the engagement of the HPA axis, resulting in the subsequent release of corticosteroid hormones into systemic circulation (de Kloet et al., 1998). This stress response begins with the release of

corticotrophin releasing hormone (CRF) from the paraventricular nucleus (PVN) of the hypothalamus. CRF then acts on the anterior pituitary gland to release adreno-corticotrophin releasing hormone (ACTH) into the circulatory system. ACTH reaches the adrenal glands where it stimulates the release of glucocorticoids (cortisol in humans and corticosterone in rodents) from the adrenal cortex into the circulatory system. This cumulative elevation in levels of circulating glucocorticoids acts on bodily tissues to mobilize resources to essential functions and limit the activity of non-essential functions, ultimately allowing an organism to adapt to the stressful situation.

In addition to exerting their effects on peripheral organs and tissues, corticosteroid hormones also act on localized regions of the brain containing two main subtypes of receptors. The mineralcorticoid receptor (MR) has a high affinity for both aldosterone and corticosterone; thus, these receptors are activated even at low levels of circulating CORT. The glucocorticoid receptor (GR) holds about ten times less affinity for corticosterone than the MR. For this reason, the GR is less activated than the MR under conditions of low circulating stress hormone. However, at peak circadian rhythm or following stress exposure, the GR will become markedly activated (de Kloet et al., 1998). The GR is more widely distributed throughout the brain than the MR; however, both types are present in the CA1 hippocampal field and in granule cells in the dentate gyrus (DG). With this concentration of GR and MRs in the hippocampus, it is not surprising that the hippocampus is particularly susceptible to the deleterious effects of chronic stress (Herman et al., 1995; Drevets, 2001; McEwen, 2005, 2007), as GRs in the hippocampus play a critical role in regulating stress responses through negative feedback control of the stress system (de Kloet et al., 1998). Additionally, the GR remains concentrated in the PVN of the hypothalamus. GRs in the PVN of the hypothalamus receive complex signals from many regions of the brain (NE+, E+/-, GABA-, 5-HT+/-, Ach+, alpha, beta). The PVN integrates these signals, and when necessary will stimulate the cascade of glucocorticoid events, beginning with the release of CRF.

As mentioned, these interactions of the HPA axis comprise a negative

feedback system in which elevated levels of cortisol/corticosterone act on GR and MR in the pituitary, hypothalamus, hippocampus, and various other regions throughout the brain to inhibit the further release of corticosteroids (Herman & Cullinan, 1997). These regions include the PFC and amygdala, which both express mineralcorticoid and glucocorticoid receptors, and participate in negative feedback control of the HPA axis. The hippocampus and PFC are typically involved in the inhibition of HPA axis activity, while the amygdala enhances HPA axis activity (Feldman et al., 1995; Herman and Cullinan, 1997; Jacobson and Sapolsky, 1991). When glucocorticoids activate hippocampal GR, projections from the ventral hippocampus activate GABAergic interneurons in the stria terminalis, and these then inhibit the production of CRF from the PVN of the hypothalamus (Anaker et al., 2011).

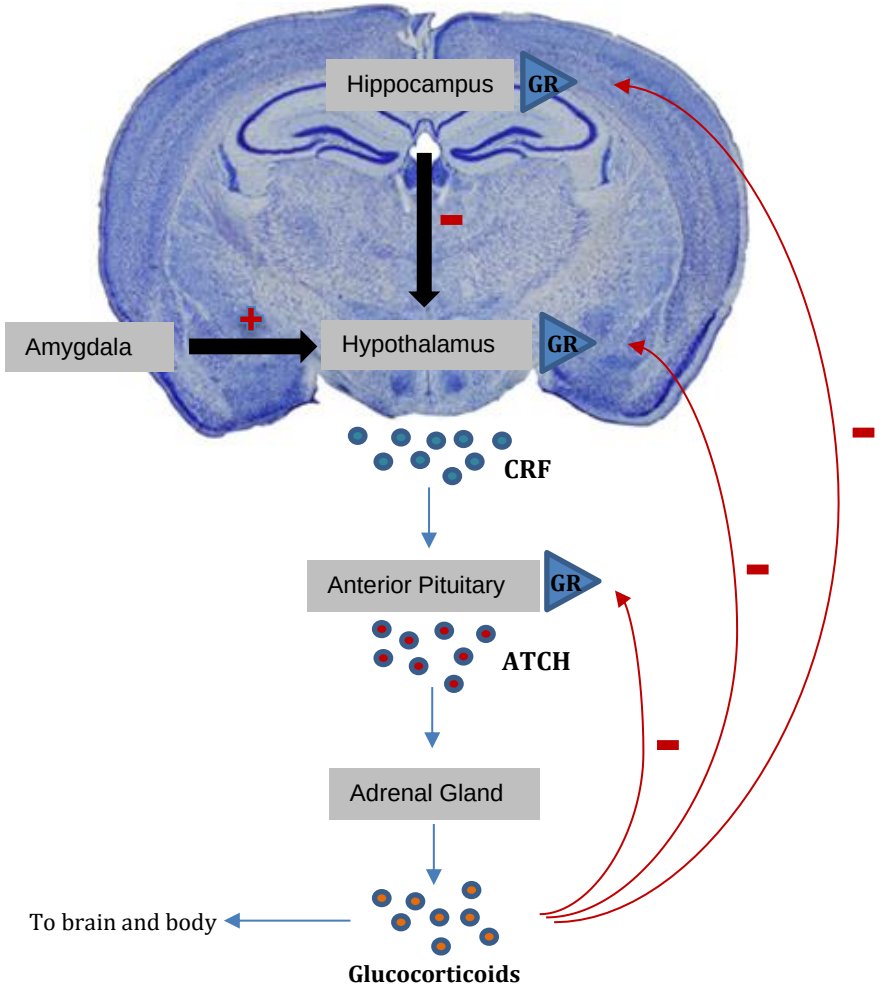

Fig 2. The hypothalamic-pituitary-adrenal axis. A cascade of events resulting in the cumulative release of glucocorticoids (i.e., cortisol/corticosterone) which travel via the bloodstream and act on GRs, as part of a negative feedback loop (shown by red arrows). Black block arrows represent the neural connections via which the hippocampus and amygdala affect the activity of the hypothalamus.

In the case of persistently high levels of glucocorticoids, the integrity of GRs is jeopardized and the regular functioning of the HPA axis can become disrupted. In optimal conditions, when hippocampal GR numbers are high, feedback inhibition is efficient and the HPA axis is tightly regulated. However, when GR numbers in the hippocampus are low, feedback inhibition becomes inefficient, resulting in higher than normal levels of glucocorticoids (de Kloet et al., 1998). This resultant dysregulated glucocorticoid action has been shown to cause hippocampal remodeling and cell death, such as dendritic arbor retraction of CA3 pyramidal neurons, hippocampal volume loss, reduced number of corticosteroid receptors, decreased expression of hippocampal GR mRNA, and reduced hippocampal neurogenesis (Magarinos & McEwen, 1995; Herman et al., 1995; Sapolsky, Krey, & McEwen, 1992; McEwen, 2000a, 2005, 2007; Fuchs, 2007). This hippocampal damage as a result of elevated levels of stress hormones likely only serves to further disrupt HPA axis functioning, which in turn only exacerbates damage (de Kloet et al., 1998). It is important to note that the activation of the HPA axis following exposure to a stressor is an important mechanism that prepares an organism for survival (de Kloet et al., 1998). By contrast, prolonged activation of the HPA axis leads to negative health outcomes, and an important goal for research is to better understand the mechanisms by which it does so and how they can ultimately contribute to the development of depressive symptoms.

Stress exposure has been linked to some of the volume changes in several brain regions seen in patients with depression. Hippocampal remodeling and cell death as a result of elevated levels of glucocorticoids have been suggested to contribute to the volumetric changes and ensuing pathological outcomes seen in depressed patients (Drevets, 2001; McEwen, 2007; Sapolsky, 2000). In line with this, there is evidence to support an association between hypersecretion of

cortisol, known as hypercortisolemia, and hippocampal, PFC, and amygdalar volume in patients with depression (Lorenzetti et al., 2009; Pariante, 2003; Pariante and Miller, 2001; Sheline, 2000). These hippocampal volume reductions are improved with correction of cortisol levels. Animal studies have shown that persistently high levels of glucocorticoids induce dendritic atrophy and sometimes cellular death in hippocampal and PFC regions, and dendritic hypertrophy in amygdalar regions (McEwen, 2007; Mitra & Sapolsky, 2008).

A large body of clinical research has shown that exposure to stress can be an instigating factor in the development of depression. For instance, the onset of depression has been shown to correlate with major stressful life events such as divorce or the death of a loved one (Kessing, Agerbo, & Mortensen, 2003). Furthermore, a significant number of patients with depression (~ 50%) display hypercortisolemia (Gillespie & Nemeroff, 2005; Sachar & Baron, 1979), and this condition has been shown to be reversed with antidepressant treatment (Holsboer, 2001). Similarly, patients with Cushing's syndrome, which is characterized by pathological hypercortisolemia, show significantly high rates of depression (Sonino & Fava, 2002). Moreover, several classes of effective antidepressant treatments function by influencing neuroendocrine control of cortisol secretion (Pariante et al., 2001, 2003; Pariante & Miller, 2001). Some patients with major depression show aberration in HPA axis function as evidenced by the dexamethasone suppression test. In healthy subjects, dexamethasone will bind to glucocorticoid receptors resulting in the inhibition of ACTH synthesis and consequently a suppression of cortisol release. However, some patients with depression do not show the expected stress response to this synthetic glucocorticoid (Southwick et al., 2005), due to a disrupted negative feedback loop that results in a failure to lower cortisol levels in response to dexamethasone. These abnormalities are partially normalized when clinical symptoms have been treated with pharmacotherapy, and some studies show that a failure to normalize is linked to poor symptom outcome and early relapse (Grenden et al., 1983). These correlations provide compelling evidence that elevated levels of stress hormones, or glucocorticoids, may lead to certain

aspects of depression (Sterner & Kalynchuk, 2010).

Given the aforementioned research supporting a role for stress-related endocrine changes and neurobiological alterations of the limbic system in the pathological functioning seen in patients with depression, the question may be asked: does stress precede the onset of depression by altering certain brain structures and functions, and by what mechanisms does it do so?

2. Animals Models to Study Depression

2.1 Usefulness of Animal Models

Animal models hold an essential place in the study of many human psychiatric disorders, including in the examination of the link between stress and depression. Research with human patients is limited by concerns for ethical issues, and a constrained ability to precisely and independently manipulate variables in order to make causal inferences. For instance, with clinical correlational data alone, it is difficult to determine whether elevated cortisol levels seen in some patients with depression are a causal factor leading to development of the disorder, or merely a result of the disorder itself. Preclinical animal studies can address this limitation by allowing for strict variable control, such as control of confounding variables that influence gene-environment interactions, while it is difficult for human studies to do so. However, no animal model exists that can perfectly replicate the range of complex and varying aspects of human psychiatric disorders. Moreover, some depressive symptoms, such as suicidal ideations, can simply not be measured in animals. Yet, despite the complex phenotypes associated with depression and other psychiatric disorders, these disorders are also comprised of intermediate phenotypes that can be reproduced and evaluated in animals. These so-called intermediate phenotypes that can be modeled in rodents include behavioral, endocrinological, physiological and neuroanatomical alterations (Deussing, 2006). For instance, anhedonia, the loss of interest in a normally pleasurable activity, is a core symptom of depression that can be measured in rodents by evaluating

preference for an appetizing reward, such as sucrose. Disrupted functioning of the HPA axis, which is widely present in major depression, can be effectively measured in rodents using the dexamethasone suppression test. Lack of motivation and behavioral despair can also be evaluated in rodents using behavioral tests such as the forced swim test or the tail suspension test. Examination of physiological alterations in appetite, weight gain, and sleep levels that are associated with MDD, can also be measured in rodent models. Finally, neuroimaging research has elucidated the presence of hippocampal volume changes, specifically hippocampal volume loss, that present with major depression. Hippocampal volume changes have also been measured in animal models of depression that employ chronic stress paradigms, wherein rodents have been shown to exhibit similar reductions in hippocampal volume.

However, in the examination of the aforementioned alterations common to both the human state of depression and those seen in animal models of depression, it is necessary to employ strict and established criteria in order to draw valid commonalities. In this vein, rodent models of depression must meet several measures of validity in order to be considered an adequate model. The model should (i) reasonably recapitulate the symptomology and neurobiology of the human disorder, or in other words, hold face validity, (ii) produce behavioral changes that can be measured objectively and systematically, (iii) produce behavioral alterations that can be restored by the same treatments that are effective in human patients, or in other words, hold predictive validity, (iv) be able to be reproduced in different laboratory settings, while holding replicability of results between investigators (Duessing, 2006; McKinney & Bunney, 1969), (v) be developed with reference to a rational theory that can be used to explain and answer questions about the etiology of the human disorder, or in other words, hold construct validity (Willner, 2005).

2.2 Rodent Models of Depression

There are various animal models that have been developed in order to study depressive etiology and symptomology, and the choice of which model to use will

largely depend on what aspects of the disorder one is attempting to study. Many animal models of depression employ a chronic stress paradigm in order to examine the behavioral and physiological consequences of chronic stress and the factors that contribute to the development of depression. Encompassed under animal models to study depression are those that employ either: (i) experimenter-induced stress, or (ii) exogenous CORT treatment. There are various experimenter-induced stress animal models that attempt to examine the influence of stress on the development of depression. Experimenter-induced stress falls under several different paradigms: (1) Chronic restraint stress, which has been widely used to study degeneration of specific brain regions as a result of stress, as well as explore stress-induced cognitive deficits associated with depression (Beck and Luine, 2002; Bowman et al., 2003; Conrad et al., 2003; Luine et al., 1994, 1996; Pham et al., 2003; Vyas et al., 2002, 2003; Watanabe et al., 1992b). However, there are some inconsistencies between studies in the range of behaviors it produces. For example, in regard to depression-like behavior, some studies have reported increased immobility on the forced swim test (FST) and a decreased preference for sucrose (Bravo et al., 2009; Joo et al., 2009; Regenthal et al., 2009; Veena et al., 2009), whereas other studies have reported no effect on the FST (Gregus et al., 2005; Lussier et al., 2009; Perrot-Sinal et al., 2004); (2) Chronic mild unpredictable stress (CMS), which incorporates a combination of psychosocial and physical stressors to reduce the chance of adaptation and to better simulate the variety of stressors encountered in daily life. Like the chronic restraint model, CMS also shows opposing behavioral results, but this may be due in part to difficulties in executing the model. Its ability to produce depression-like symptomology is still more robust than that of chronic restraint stress. CMS has been shown to lead to an increase in depression-like behavior (Willner et al., 1987; Willner, 2005), a decrease in neurogenesis, as well as signs of glucocorticoid overexposure, including increased adrenal weight, decreased thymus weight, decreased weight gain, and increased basal levels of cortisol (Herman et al., 1995; Cullinan & Wolfe, 2000; & Joëls et al., 2004). The model holds some disadvantages: it is rather labor

intensive, requires more space and resources, and often needs to be carried out over a longer period of time than other models (Kim and Han, 2006; Willner, 1997, 2005); (3) Learned helplessness, which is sensitive to some antidepressants, but requires strong stressors; (4) Social stress: social defeat and social submissiveness, which have been shown to lead to reductions in neurogenesis.

The aforementioned experimenter-applied stress models that employ environmental manipulations, while holding specific advantages, are also confounded by variations in the exact protocols used and difficulties in replication (Forbes et al., 1996; Vollmayr & Henn, 2001; Willner, 2005). Stressful stimuli used in these models can differ between animals in terms of their actual physical qualities and their perceived psychological qualities. These different responses can manifest in disparate CORT levels between rats exposed to the same stressor, leading to increased variability (Sterner & Kalynchuk, 2010). Another concern inherent to these models is the habituation effect that takes place when animals are repeatedly exposed to the same stressors (Galea et al., 1997; Gregus et al., 2005; Grissom et al., 2007). Such individual differences in stress response and adaptation that are characteristic of these models can also be regarded as advantageous in that they more closely resemble the individual differences in stress susceptibility and resilience present in the human population. However, for the purpose of producing reliable and vigorous symptomology that allows for the study of alterations in depression and its etiology, these models do not prove to be as beneficial. To address this need, the exogenous CORT model has been developed whereby CORT is administered exogenously for a period of weeks to months via subcutaneous injection, pellet implantation, osmotic pump infusion, or passive administration through drinking water or food. The exogenous CORT model has the benefit of isolating the effects of glucocorticoids from broader brain and circulatory effects that can result from environmental factors and confound the examination of how stress hormones directly affect the brain. Another advantage of the CORT model is its ability to produce a persistent depressive-like state in rodents, allowing for the

utilization of multiple behavioral tests following CORT wash-out. Additionally, the exogenous CORT model holds validity in that it has been shown to reproduce many of the core features of other stress models (Gourley & Taylor, 2009) and has been shown to be consistently replicable between laboratories. It maintains the ability to observe robust and reliable behavioral symptomology and neurobiological changes in the same animal, with fewer individual differences, when compared to other stress models (Sterner & Kalynchuk, 2010). Furthermore, the behavioral phenotype brought about by CORT is sensitive to reversal by chronic antidepressant treatment. In general, chronic antidepressant treatment increases neurogenesis and counteracts the effects of stress in both rodents and humans (Anaker & Hen, 2017).

Various behavioral measures exist that are used to evaluate “depressive” symptomology in rodents brought about by stress induction and exogenous CORT treatment. These measures include weight loss, sleep disturbances, memory impairments, open field exploration, anhedonia, and behavioral despair. Anhedonia and learned helplessness, a form of behavioral despair, are the most frequently used measures to evaluate depression-like symptoms in rodents. Anhedonia, the lack of interest in pleasurable activities, is most often ascertained by measuring an animal’s preference for sucrose solution over water solution. Rats have a natural preference for sucrose, so a decrease in sucrose preference is thought to be indicative of a depressive state. Notably, antidepressants reverse this behavioral effect in anhedonic rats (Willner, 1997). There are several despair-based paradigms that are used to assess learned helplessness in rats, including the tail suspension test and the forced swim test (FST), but the FST is the most commonly used. The FST protocol first developed by Porsolt and colleagues (1977) included a pretest immersion trial followed by a subsequent immersion test. In this test, rats are placed in a container of water with no possibility of escape, and are forced to swim while their behavior is recorded for a period of time. During the test period, Porsolt found that the onset of immobility was much more rapid, which was interpreted to indicate “despair”, with the reasoning that the animal had realized escape was impossible. Increases in

passive immobility behavior and decreases in active swimming and struggling behavior are considered to be indicative of behavioral despair and a depressive phenotype (Lucki, 1997). The forced swim test holds strong predictive validity, because all forms of antidepressant treatment that are effective in humans decrease immobility behavior on this test (Cryan et al., 2005; Armario et al., 1988; Porsolt et al., 1978). In fact, this test is often used as a screening tool for new antidepressants because there is a strong correlation between the clinical efficacy of antidepressants in humans and their potency on the forced swim test (Borsini & Meli, 1988; Castagne et al., 2011).

2.3 Neurobiological and Behavioral Consequences of Repeated CORT Exposure

2.3.1 Depression-like Behaviour

There is a large body of literature evidencing that exogenous CORT treatment produces reliable and potent changes in various behaviors considered to be symptomatic of depression, evidencing the face validity of the model. Behavioral despair, which holds particular relevance to mood disorders, is one domain that exhibits potent effects as a result of CORT exposure. Specifically, rodents that are administered CORT via injection, pellet implantation, or in their drinking water show a decrease in their latency to immobility, increased time spent immobile, and decreased time spent swimming in the forced swim test (David et al., 2009; Gourley et al., 2008a; Gregus et al., 2005; Hill et al., 2003; Johnson et al., 2006; Kalynchuk et al., 2004; Marks et al., 2009; Murray et al., 2008; Zhao et al., 2008b; Iijima et al., 2010). Importantly, the effects of CORT on depression-like behavior have been shown to be both dose and time dependent (Johnson et al., 2006; Zhao et al., 2008b). For example, daily injections of 10, 20, 40 mg/kg of CORT for 21 consecutive days produces a progressive increase in depression-like behavior on the FST (Johnson et al., 2006). Rats injected with 40mg/kg CORT consistently show increased immobility, decreased struggling, and a shorter latency to immobility than vehicle-injected rats, while rats that receive 20mg/kg CORT show only a shorter latency to immobility, and 10mg/kg CORT-

injected rats do not show any significant increases in depression-like behavior on any of the measures in the FST. CORT administered at 40mg/kg shows a linear progression on immobility behavior when measured at 7, 14, and 21 days, but only begins to reach significance at 14 days (Lussier et al., 2013). However, acute CORT treatment of only a single injection one day before testing has not been shown to have any effect on FST behavior (Johnson et al., 2006). Hence, these findings demonstrate that the amount of CORT, and the duration for which it is administered, have potent effects on behavioral measures indicative of a depressive phenotype (Sterner & Kalynchuk, 2010).

As mentioned, anhedonia is commonly measured with the sucrose preference test. The CORT model holds induction validity for anhedonic behavior, while anhedonia measures themselves hold face validity. In various CORT paradigms, rodents show a reduction in sucrose preference (David et al., 2009; Gorzalka et al., 2003; Gourley et al., 2008a), as well as other anhedonic behaviors that include inhibited sexual and grooming behaviors (Gorzalka et al., 2001; Gorzalka and Hanson, 1998; Hanson and Gorzalka, 1999; David et al., 2009), and decreased food reinforcement response (Gourley et al., 2008a). The CORT model has been previously compared to 21 days of restraint stress at 6 hours per day. The CORT model shows stronger outcomes, evaluated in terms of body weight, sucrose preference, and immobility (Lussier et al., 2009). Significantly, many of the behavioral alterations following CORT administration are reversed with common antidepressant treatment, affirming the predictive validity of the CORT model of depression (David et al., 2009; Ago et al., 2008; Fenton et al., 2015).

2.3.2 Fear response in an open field

Studies show mixed findings in regards to stress-induced or CORT-induced open field test behavior. Male rats exposed to 3 weeks of chronic mild stress have shown reduced exploration in the open field when tested 2 or 24 hours after the last stress exposure (Westenbroek et al., 2003; Westenbroek, Den Boer, & Ter Horst, 2003; Lin et al., 2008). Interestingly, female rats exposed to the same

paradigm have exhibited a different pattern of exploration in the open field compared to males, when tested 2 or 24 hours after the last stressor. Female rats showed increased exploration and distance travelled compared to males (Westenbroek, Den Boer, & Ter Horst, 2003; Westenborek et al., 2003; Westenbroek et al., 2005). Other research demonstrates no effect of CORT on open field exploration (Lussier et al., 2011; Kalynchuk et al., 2004; Brotto et al., 2001; Gregus et al., 2005; Marks et al., 2009), only a sex difference with females exploring more (Kalynchuk et al., 2004), while still others show increased fear response (i.e., decreased exploratory behavior and increased freezing) in an open field following exogenous CORT administration (David et al., 2009; Skórzewska et al., 2006).

2.3.3 Locomotor effects

No differences have been observed between CORT and control rats in terms of the number of lines crossed in an open field test (Gregus et al., 2005), as well as no differences observed in a wire suspension test (Marks et al., 2009), which assesses muscle strength. Hence, CORT effects on behavior are likely not due to nonspecific motoric effects.

2.3.4 Body weight

CORT administration has been shown to affect changes in body weight gain (Barr et al., 2000; Brummelte et al., 2006; Bush et al., 2003; Coburn-Litvak et al., 2003; Gregus et al., 2005; Johnson et al., 2006; Magarinos et al., 1998; Meijer et al., 1997), and changes in body weight are also seen in patients with depression. This effect is dose and time dependent. As the dose of CORT and duration of administration is increased, attenuated weight gain is seen over the course of treatment. However, CORT-treated animals have not been observed to physically eat less, suggesting that the attenuated effect of CORT on weight gain is not due to amount of food consumption, but rather is likely related to increased leptin levels. Nonetheless, the change in weight gain observed in the CORT

model represents a physiological parameter that is also altered in human depression, again lending face validity to the CORT model.

2.3.5 Disrupted HPA axis function

In addition to increased depression-like behavior, CORT administration also produces various physiological changes that are demonstrative of depression. One such change is dysregulated HPA functioning (Johnson et al., 2006). This disrupted stress response has been evaluated using FST as a novel stressor. When introduced to the FST, control animals show low basal levels of serum CORT, a normal peak of serum CORT 30 minutes after testing, and a return to normal levels 60 minutes after the test. However, animals treated with repeated CORT show higher basal levels of CORT, and do not show any CORT response following the forced swim novel stressor. This disrupted stress response remedies when CORT injections are halted for a period of two weeks. Acute CORT treatment has only a minimal effect on CORT response to a novel stressor.

As briefly mentioned earlier, the PVN contains parvocellular neurons that produce CRF to act on the pituitary for the release of ACTH, and eventually cortisol/corticosterone release from the adrenal cortex. Parvocellular neurons receive GABAergic input from other hypothalamic nuclei, but also from the hippocampus (Herman & Cullinan, 1997). GABAergic input to parvocellular neurons is important for the regulation of parvocellular neuron activity (Cole & Sawchenko, 2002). Chronic stress has been shown to reduce GABAergic input to parvocellular neurons, while measured at low circulating corticosterone levels (Joëls et al., 2004). A $GABA_A$ receptor antagonist injected close to the PVN counteracts the inhibitory input to CRF-expressing cells, leading to an increase in CRF and circulating corticosterone levels (Cole & Sawchenko, 2002). The reduced GABAergic inhibition of CRF-expressing neurons following chronic stress may be one of the mechanisms by which CRF and, indirectly, basal CORT levels are enhanced following chronic stress. Neurogenesis may also be implicated in the stress response as it is glutamatergic projections from the

ventral DG of the hippocampus that activate the GABAergic inhibitory interneurons, which inhibit the neurons of the PVN. There is evidence implicating a role for neurogenesis in the maintenance of inhibitory control over the HPA axis, with reductions of neurogenesis further elevating levels of glucocorticoids and exacerbating reduced new neuron birth (Schloesser et al., 2009; Snyder et al., 2011).

2.3.6 Hippocampal alterations

Prolonged stress exposure has been documented to lead to a significant amount of structural remodeling in the adult brain, especially in the limbic system (McEwen, 2000b, 2007). These neurobiological alterations are proposed to underlie depressive pathology, and importantly, these alterations have been well defined using the exogenous CORT administration paradigm. CORT administration in rodents results in structural and functional consequences characterized by hippocampal volume loss, dendritic atrophy of hippocampal pyramidal cells, particularly in CA3 and CA1 apical dendrites (Magariños et al., 1998, 1999; Sousa et al.,1998, 2000; Woolley et al.,1990), and loss of mossy fiber CA3 synapses (Sousa et al., 2000; Tata et al., 2006). This dendritic atrophy is similar to what has been observed in post-mortem tissue of patients with depression (Konarski et al., 2008). While these CORT effects are often not long-lasting once exposure to the stressor has ceased, a high dose and/or extended administration of CORT can lead to hippocampal cell death (Sapolsky et al., 1985). Similar alterations have been found using other chronic stress paradigms. Chronic stress drastically reduces synaptic plasticity in CA1 and DG hippocampal slices in vitro (Joëls et al., 2004) and in vivo (Gerges et al., 2001; Pavlides et al., 2002). Chronic mild unpredictable stress for 21 days has been shown to result in reductions in CA3 surface area of pyramidal cells that does not return to control levels even after 3 weeks recovery. Decreased number of adult generated cells in the hilus, the SGZ, and in the total DG has also been observed after 3 weeks of CMS, showing only partial recovery after 3 weeks (Joëls et al., 2004). In contrast to the hippocampal atrophy observed following stress exposure and

CORT administration, repeated CORT treatment has been observed to cause dendritic hypertrophy in the amygdala (Mitra & Sapolsky, 2008).

Neurogenesis in the hippocampus is another form of structural plasticity that has been associated with depression. Neurogenesis is defined as the generation of new neurons, a process that continues to occur throughout adulthood in the subgranular zone (SGZ) of the dentate gyrus. During the process of neurogenesis, newborn neurons in the SGZ migrate into the granule cell layer of the DG as they mature. They integrate into existing circuitry with dendrites extending into the molecular layer of the DG, and axons projecting to the CA3 region of hippocampal pyramidal cells.

Adult neurogenesis is a key function of the hippocampus that is sensitive to the negative effects of stress (Lucassen, 2015; Anacker, 2014). One of the initial reasons that neurogenesis became a focus in the context of depression was due to the observation that all known antidepressant treatments - antidepressant drugs, electroconvulsive shock, and physical activity - appear to increase the proliferation of newborn cells in the hippocampus (Malberg et al., 2000; Scott et al., 2000; van Praag et al., 1999; David et al., 2009). Notably, many chronic stress paradigms, including chronic CORT administration, have powerful effects on hippocampal neurogenesis in rodents (Fuchs & Gould, 2000; Pham et al., 2003; Santarelli et al., 2003; Sapolsky, 2004; Sheline et al., 1999; Starkman et al., 1992; Surget et al., 2008, 2011; David et al., 2009; Wong & Herbert, 2006, 2004; Tanti et al., 2013; Ramirez et al., 2015), and in non-human primates (Perera et al., 2011; Wu et al., 2014), as evidenced by rapid and potent decreases in the proliferation and survival of newborn cells. The attenuation of cell turnover and maturation of cells in the dentate gyrus following chronic stress may manifest in significant negative alterations in cell connectivity and hippocampal function, such as aberrant maturation and migration of cells in the dentate gyrus. This stress-induced suppression of cell proliferation has been shown to be restored by many different classes of antidepressant drugs in rodents (Czeh et al., 2001; Santarelli et al., 2003; Petrik et al., 2012; Surget et al., 2008; Malberg et al., 2000; Dagyte et al., 2010), non-human primates (Perera

et al., 2011), human post-mortem brain tissue (Boldrini et al., 2009, 2012), and in vitro in human hippocampal progenitor cells (Anacker et al., 2011).

Stress has been shown to increase activity of the dentate gyrus in vivo, and stress and glucocorticoids increase DG synaptic currents in vitro (Shoenfeld et al., 2013; Karst & Joëls, 2003), suggesting that inhibition of DG activity holds relevance for stress-induced neurological disorders, including depression. Specifically, young neurons of the DG serve as potent inhibitors of DG granule cell activity under anxiogenic conditions, and therefore new neuron birth may affect behaviour through the modulation of neuronal networks that are involved in mood and anxiety and affect changes in the hippocampus (Anacker & Hen, 2017). Consistent with this is the finding that blockade of GABA type A receptors in the ventral dentate gyrus prevent the anxiolytic effects of exercise (Shoenfeld et al., 2013). Furthermore, this inhibitory effect of immature DG neurons may be important for the process of cognitive flexibility – the erasing of previously established fear-associated memories to allow for the formation of new non-fear-associated memories (Takahashi et al., 2008; Anaker & Hen, 2017). Ultimately, this may facilitate recovery from anxiety and depressive symptoms by supporting learning for the new context as safe rather than adverse.

Together, these findings provide support for one of the foremost leading hypotheses relating to the etiology of depression - that chronic stress induces pathological remodeling of the hippocampus that leads to a decrease in neurogenesis and subsequent manifestation of depressive symptomology (Duman et al., 2000; Jacobs et al., 2000). Therefore, targeting neurogenesis may pose a promising avenue for combating stress susceptibility and eliciting antidepressant effects. This is particularly relevant given that adaptation to a stressful context is partially mediated by neurogenesis. Under basal conditions of (i) HPA axis activity; (ii) fear response; and (iii) innate levels of anxiety, despair and anhedonia, neurogenesis does not have an influence on behavior. However, when exposed to a stressful event, mice with high levels of neurogenesis show less of an increase in anxiety-like and depression-like behavior compared to mice that have inhibited neurogenesis, by way of transgenic or radiation methods

(Schloesser et al., 2009; Snyder et al., 2011). These neurogenesis-deficient mice also show a suppressed dexamethasone response compared to neurogenesis-intact mice, supporting a role for the hippocampus in regulation of the HPA axis (Jankord & Herman, 2008). Once the stressful experience has dissipated, neurogenesis facilitates reversal learning and cognitive flexibility to aid adaptation and normal functioning in the environment once more, while reducing HPA axis hyperactivity (Mateus-Pinheiro et al., 2013)

Despite findings that support the hypothesis of impaired adult neurogenesis in the pathophysiology of depression, there is in fact no direct evidence that decreased neurogenesis causes human depression. To the contrary, some post-mortem evidence from patients with depression shows no significant reductions in neural stem cell proliferation and cell loss in the hippocampus (Muller et al., 2001; Reif et al., 2006; Stockmeier et al., 2004; Lucassen et al., 2010). In animal models, depression-like symptoms and the restorative actions of antidepressants on behavior are not always concomitant with changes in hippocampal neurogenesis (Vollmayr et al., 2003; Surget et al., 2008; Bessa et al., 2009; David et al., 2009), calling into question a causal role for hippocampal neurogenesis in the pathophysiology of depression. In line with this, interruption of neurogenesis in rodents is not always concomitant with a depressive phenotype (Zhao et al., 2008a). Such findings challenge the hypothesis that reductions in hippocampal neurogenesis are a primary contributing factor in the development of depressive illness. Alternatively, the reductions in hippocampal volumes observed in post-mortem tissue of depressed patients may be attributable to reductions in the complexity of dendrites, rather than a deficit in the proliferation and survival of newborn neurons. To support this, Lussier and colleagues (2013) found that CORT gradually decreases the number and dendritic complexity of immature dentate gyrus neurons, surviving under conditions of elevated glucocorticoid levels, while also eliciting a depressive phenotype. It may also be the case that disruptions in neurogenesis contribute to the manifestation of only specific depressive symptoms. More research is

required to tease apart the influential role of hippocampal neurogenesis in depressive symptomology and antidepressant action.

2.3.7 Disruption of spatial memory

Depression is often associated with impaired cognition, with the most prominent deficits seen in verbal and spatial working memory (hippocampal dependent) and executive functioning. CORT administration has been shown to affect hippocampal dependent memory and executive functioning. CORT impairs object location memory and object-in-place memory (Brymer et al., in preparation). Moreover, cognitive deficits induced by CORT mirror those found in other preclinical models of depression, providing further support for the CORT paradigm as a model for studying depression (Sterner & Kalyncuk, 2010).

To summarize, the CORT model demonstrates face, predictive, and construct validity. For instance, construct validity can be demonstrated by the effects of CORT on behavior, which do not appear to be solely a result of HPA axis disruption, since animals previously treated with CORT do not differ from controls in circulating CORT levels at testing (Gourley & Taylor, 2009). This effect mirrors that seen in human patients with depression and in CMS models. Exposure to CORT produces anhedonia, decreased sensitivity to reward (Gourley et al., 2008a), and increased immobility in the FST (face validity), which represent a depressive phenotype and are sensitive to antidepressant treatment (predictive validity).

3. Reelin as a Putative, Fast-acting Treatment for Depression

3.1 Reelin

In the context of stress and depression, recent research has focused on the activity of the large extracellular matrix glycoprotein, reelin. Reelin plays a critical role in neuronal migration and in the laminar organization of the CNS during embryonic development (D'Arcandelo et al., 1995; Gilmore & Herrup, 2000), as

well as holding important roles throughout adult life. Importantly, impairments in hippocampal plasticity produced by chronic stress and repeated CORT administration show high affinity with the biological activities of reelin.

Reelin received its name from mice that were identified by scientists to display spontaneous mutations that manifested in difficulties with motor behavior. D.S. Falconer was the first to describe the “reeler” mouse in 1951, characterized by its abnormal reeling gate (Falconer, 1951). In the 1960s, histopathological studies showed that reeler mice had a cerebellum significantly decreased in size, and did not display the normal, ordered layering of several brain regions (Hamburgh, 1963). It was later discovered that these reeler mice were homozygous for mutation of the RELN gene encoding the reelin protein. This loss of reelin function through mutation of the RELN gene results in misguided neuronal positioning in the developing central nervous system. Mice that are heterozygous for the reelin gene, having 50% normal levels of reelin, do not display the same neuroanatomical defects, but do display hereditary characteristics that are linked to some psychotic disorders. In human patients, disruptions of the RELN gene have been found to cause a rare form of lissencephaly called Norman-Roberts syndrome (Crino, 2001; Chang et al., 2007). The mutation results in low or undetectable amounts of the reelin protein that results in phenotypical characteristics of ataxia, hypotonia, (low muscle tone), developmental delay, and severe mental retardation. Throughout development and into adulthood, reelin is present not only in the brain, but in the spinal cord, blood, as well as in other bodily organs and tissues (Ikeda & Terashima, 1997; Smallheiser et al., 2000).

3.1.1 Reelin in the CNS

During brain development, reelin is secreted by Cajal-Retzius cells in the immature hippocampus and in the developing cerebral cortex (Meyer, Goffinet, & Fairen, 1999). By controlling cell-cell interactions, reelin plays an important role in the regulation of neuronal migration and positioning during brain development. Reelin affects the differentiation of progenitor cells into radial glia and orients the direction of their fibers, which guide the migrating neuroblasts (Hartfuss, Forster,

Bock, et al., 2003). Newborn neurons travel along radial glial fibers as they move towards their final destinations during development (Rakic, 2009). The layered positioning of reelin-secreting cells is important, since the orientation of radial glial fibers is determined by areas of higher reelin concentration (Nomura, Takahashi, Hara, & Osumi, 2008). To exemplify this importance, the specific connections formed in the layers of the hippocampus during development, and the determination of the compact granule cell layer, are regulated by reelin (Del Rio et al., 1997). Reelin protein has been hypothesized as a stop signal for migrating neuronal precursor cells. It is proposed to control their positioning by sending out a dissociation signal to neuronal groups, allowing them to use radial individual migration to reach their final position (Hack, Bancila, Loulier, et al., 2002).

Reelin is proposed to take part in the “NR2B to NR2A switch” that happens in early postnatal development (Liu, Murray, & Jones, 2004), and this may occur by increased mobility of NR2B-containing receptors as a result of reelin, thus reducing the amount of time they spend at the synapse (Sinagra et al., 2005; Groc et al., 2007).

In the mature brain, reelin is expressed by GABAergic interneurons in corticolimbic structures of adult rodents and primates (Pesold et al., 1998, 1999; Guidotti et al., 2000) and by glutamatergic granule cells in the cerebellum (Pesold et al., 1998). Upon secretion in the extracellular matrix, reelin interacts with two members of the low density lipoprotein receptor gene family: apolipoprotein E receptor 2 (ApoER2) and very-low-density lipoprotein receptor (VLDLR), as well as alpha-3-beta-2 integrin receptors (D’Arcangelo et al., 1999). These receptors are present on both neurons and glial cells. Radial glia, which serve as primary progenitor cells and are capable of generating neurons, astrocytes, and oligodendrocytes (Noctor et al., 2001), contain the same amount of ApoER2, but ten times less VLDLR than neuronal or glial cells (Hartfuss et al., 2003). These two receptors interact with the cytosolic adapter protein, Dab1, encoded by the Disabled-1 (Dab1) gene. DAB1 is a critical regulator of the reelin signaling cascade. Following the binding of reelin to cortical neurons, DAB1

binds to the intracellular part of VLDLR and ApoER2, to affect cell positioning in the developing brain and during adult neurogenesis in a signaling pathway downstream of RELN (Benhayon, Magdaleno, & Curran, 2003). In mice, targeted disruption of Dab1 results in a phenotype similar to that of the reeler mouse.

In adulthood, reelin continues to play important roles in neuronal migration and development. Reelin is highly active at the subventricular zone and the subgranular zone of the dentate gyrus. In some species, such as in rodents, reelin regulates the dissociation of neuroblasts from tangential to individual radial migration (Forster et al., 2006), after they have migrated from the subventricular zone to the olfactory bulb along the rostral migratory stream (RMS). In the adult dentate gyrus, reelin guides the continual migration and integration of immature neurons of the subgranular zone into the granule cell layer.

Reelin participates in several other critical processes of adult brain functioning, including the modulation of synaptic plasticity through the induction and maintenance of long-term potentiation (D'Arcangelo, 2005; Weeber et al., 2002), the regulation of learning and memory (Weeber et al., 2002), synaptogenesis, dendritic spine growth and density (Costa et al., 2002; Niu et al., 2008; Chameau et al., 2009), hippocampal neurogenesis (Pujadas et al., 2010), and glutamatergic transmission, guiding the strength of glutamatergic synapses impinging on dendritic spines. The strengthening of LTP by reelin is due to the interaction of ApoER2 with the NMDA receptor, with the exon 19-containing variant of ApoER2 being produced more during periods of activity (Beffert et al., 2005). The facilitation of dendrite growth by reelin is proposed to occur through Src family kinases, dependent upon the expression of Crk family proteins (Matsuki, Pramatarova, & Howell, 2008).

The stratum lacunosum of the hippocampus has a high level of diffuse reelin immunoreactivity. Here, reelin is released by stratum lacunosum GABAergic interneurons and perforant pathway terminals that impinge onto the distal region of CA1 pyramidal dendrites. Reelin release in the stratum lacunosum is thought to regulate synaptic spine development and contribute to the induction of LTP. In the SGZ, GABAergic cells expressing reelin make

contacts with granule cell somata, regulating the migration and maturation of newborn granule cells. In vitro, addition of recombinant reelin to cortical synaptosomes has been shown to increase membrane protein expression (Dong et al., 2003) and augment the density and clustering of proteins (i.e., neurotransmitter receptors) in postsynaptic membranes (Caruncho et al., 2004). These findings further support a role for reelin in the regulation of glutamatergic input strength onto dendritic spines (Caruncho et al., 2004).

3.1.2 Reelin in Peripheral Tissues

As mentioned, reelin is not only expressed in the brain, but also in peripheral tissues, including the kidneys, liver, and blood (Ikeda & Terashima, 1997; Smallheiser et al., 2000), and in bone barrow (Chu et al., 2014), platelets (Tseng et al., 2010), lymphatic vessels (Samama & Boehm, 2005; Lutter et al., 2012), and the enteric nervous system (Bottner et al., 2014). The peripheral activity of reelin is much less understood than its functional roles in brain development and plasticity in the central nervous system, but its expression has been found to go up sharply following the injury of some organs (Kobold et al., 2002; Pulido et al., 2007). In blood, reelin is likely secreted into the plasma by hepatocytes, platelets and potentially red blood cells. Here, it has been shown to play a role in the formation of red blood cells in bone marrow (Chu et al., 2014). Reelin has also been shown to regulate lymphatic vessel formation (Lutter et al., 2012). In platelets, reelin may be affecting coagulation pattern and inflammatory events. Importantly, alterations in reelin plasma levels have been found in schizophrenia and mood disorders. Specifically, a significant increase in 410 kDa reelin moiety has been observed in schizophrenia (49%), while a non-significant increase has been observed in major depression (34%), and in contrast, a non-significant decrease in bipolar disorder (33%) has been observed. Alternatively, the 180 kDa Reelin values have been shown to be significantly decreased in depression (49%) and in bipolar disorder (29%), compared with controls (Fatemi et al., 2001a).

3.2 Implications for Reelin in Major Depressive Disorder

3.2.1 Reelin in Patients with Mood and Psychiatric Disorders

Several neuropsychiatric disorders, including schizophrenia, bipolar disorder, autism, and major depressive disorder have been proposed to share characteristic biological risk factors. In this context, altered reelin expression has been widely studied for its relevance as one of the factors common to these disorders. Disrupted brain expression of reelin has been frequently implicated in the pathophysiology of these disorders, including schizophrenia (Fatemi, Kroll, & Stary, 2001; Impagnatiello et al., 1998), bipolar disorder (Fatemi, Earle, & McMenomy, 2000; Guidotti et al., 2000), autism (Fatemi, 2002), and major depression (Lussier et al., 2009, 2011, 2013). In post-mortem brain tissue of patients with SZ and BP disorder, reelin expression has been found to be reduced by approximately 50% in the PFC, temporal cortex, hippocampus, and caudate nucleus (Impagnatiello et al., 1998; Fatemi, Earle, & McMenomy, 2000; Guidotti et al., 2000). Similarly, in patients with depression, post-mortem analyses show a general decrease in hippocampal reelin expression (Fatemi et al., 2000; Knable et al., 2004).

3.2.2 Reelin in Animal Models of Depression

Animal models in which reelin expression is genetically decreased can elucidate important information regarding the impact of reelin on cognitive functioning and its implications for understanding the pathophysiology of neuropsychiatric illness. Heterozygous reeler mice (HRM) carry a naturally-occurring mutation that decreases normal levels of reelin protein by approximately 50% in the brain and peripheral tissues. These mice have been widely used to study the impact of impaired reelin expression as it relates to the biological characteristics underlying behavioral impairments present in mood and psychiatric disorders. Although HRM do not exhibit overt differences in phenotype (Teuting et al., 2006; Caruncho et al., 2016), they do display alterations in some endophenotypes that have relevance to these disorders. For instance, HRM mice have been shown to have significantly decreased density of

dendritic spines and deficits in LTP expression (Teuting et al., 1999, 2006), as well as impaired hippocampal-dependent learning and plasticity (Qiu et al., 2006). Importantly, administration of recombinant reelin to hippocampal slices has been shown to enhance hippocampal LTP (Pujadas et al., 2010; Rogers et al., 2011). Other studies have reported deficits in prepulse inhibition (PPI) in HRM, which is considered to measure sensorimotor gating and represent an endophenotype of psychosis (Barr et al., 2008). In relation to the link between stress and depression, adult HRM with impaired reelin signaling appear to be more vulnerable to the negative effects of glucocorticoids (Lussier, Romay-Tallon, Kalynchuk, & Caruncho, 2011; Buret and van den Buuse, 2014; Schroeder et al., 2015). Lussier and colleagues (2011) found that in the absence of CORT, HRM mice do not display any deficits in depression-like behavior, hippocampal neurogenesis, or hippocampal reelin expression. However, in the presence of CORT, HRM mice show dose-dependent increases in depression-like behavior, decreases in neuronal survival and maturation, and decreases in hippocampal reelin expression, that were more pronounced than in wild-type (WT) mice (Lussier et al., 2011). Similarly, Notoras and colleagues (2017) employed an exogenous CORT paradigm in young adult WT and HRM mice wherein CORT (50mg/L) was administered in the drinking water for a 3-week period. HRM mice were more susceptible to the elevated levels of glucocorticoids, displaying increased immobility on the FST as compared to WT mice. These results provide evidence that mice with impaired reelin signaling are more susceptible to the negative effects of glucocorticoids and this evidence supports a role for reelin in the pathophysiology of mood disorders (Fatemi et al., 2000; Guidotti et al., 2000).

It is now well-established that increased levels of glucocorticoids can result in the downregulation of reelin expression in several brain regions, including the hippocampus (Lussier et al., 2009; Fenton et al., 2015). In fact, endogenous reelin expression has been proposed as a marker of stress resilience (Fatemi, 2011) that may also regulate resilience to mood disorders brought on by stress induction (Fatemi et al., 2000). Lussier and colleagues (2009) showed that 21

days of CORT injections produces decreases in reelin-expressing cells in the SGZ (26%), as well as decreases in diffuse reelin expression in the CA1 SLM (21%) of the DG. Given that the SGZ is the site where adult hippocampal neurogenesis takes place, decreases in reelin-expressing cells in this region is particularly relevant regarding a role for reelin in depression and neurogenesis. More specifically, reelin-expressing GABAergic interneurons may be affecting the degree and rate of hippocampal neurogenesis. Our lab sought to investigate this by looking at the parallel time course of hippocampal neurogenesis and reelin expression in the SGZ. CORT-induced increases in depression-like behavior were accompanied by decreases in the number and dendritic complexity of immature dentate granule cells and this was paralleled with decreases in reelin expression in the dentate subgranular zone (26%) (Lussier, Lebedeva, Fenton, Guskjolen, & Caruncho, 2013a). Furthermore, this effect is dose- and time-dependent. Specifically, the gradual increase in depression-like behavior and decrease in newborn neuron maturation rate was concomitant with a decrease in reelin expression that was not observable at 7 days, but showed a 25% decrease at 14 days, and a 26% decrease at 21 days (Lussier et al., 2013a). These data suggest that the downregulation of reelin disrupts hippocampal neuronal maturation, which leads to deleterious behavioral outcomes. Consistent with this is the finding that inactivation of the reelin signaling pathway leads to impairments in adult neurogenesis (Teixeira et al., 2012).

Together, these findings evidence a role for reelin in susceptibility and adaptation to chronic stress and provide support for the investigation of reelin in the context of susceptibility to stress-related mood disorders, such as depression and bipolar disorder (Teixeira et al., 2011). This preclinical body of research opens up an avenue for exploring the potential benefits of increasing endogenous reelin levels in the development of novel therapeutics for depression and other mood disorders. Further support for this proposal comes from preclinical research demonstrating that several antidepressants increase reelin expression in the PFC (Fatemi et al., 2009), and administration of the tricyclic antidepressant imipramine in CORT-treated rats, restores rodent hippocampal

reelin levels, coincident with a reversal in depression-like behavior (Fenton et al., 2015).

In contrast to increased stress susceptibility observed in reelin deficient mice, reelin overexpression has been shown to protect against the development of a depressive phenotype (Teixeira et al., 2011) and reelin supplementation has been shown to enhance synaptic plasticity, dendritic spine density, and cognitive ability in wild-type mice (Rogers et al., 2011), as well as restore sensory motor gating, synaptic plasticity and associative learning deficits in HRM (Rogers et al., 2013).

Given that reelin is downregulated in depression, that chronic stress and chronic CORT paradigms in rodents lead to behavioral and neurochemical deficits that are concurrent with decreases in reelin expression, and that reelin overexpression in animal models has protective effects, our lab was motivated to investigate the effects of recombinant reelin administration on CORT-induced behavioral and neurobiological deficits. Our findings showed that intrahippocampal infusions of reelin restored the behavioral phenotype following repeated CORT, as measured by the FST. Furthermore, this behavioral restoration was paralleled by a restoration of hippocampal neurogenesis (Brymer, unpublished data). While these studies report the behavioral and neurochemical effects of reelin infusions in the brain, much less is known about the effects of peripheral reelin administration on the brain. However, recent findings have reported reelin immunoreactivity in endothelial cells in brain regions showing high levels of extracellular reelin expression (Perez-Costas et al., 2015). Based on these findings, it is possible that peripherally expressed reelin can reach certain areas of the brain in order to affect brain functioning. Furthermore, exogenous administration of peripheral reelin may enhance these effects of reelin on brain functioning and serve a protective role under conditions of compromised functioning.

3.3 Peripheral Reelin: Relation to Stress and Depression

3.3.1 Serotonin Transporter (SERT) Membrane Protein Clustering: Relation to Depression

The plasma membrane is a lipid bilayer that contains many embedded proteins that are able to move freely, to a certain extent, within the membrane. Within this membrane, there are specific domains wherein proteins interact to affect their physiological roles (Rivera-Baltanas et al., 2010). Neurotransmitter transporters and receptors are located in both pre- and post-synaptic sites, and have been shown to assemble in lipid rafts within the membrane (Ostrom & Insel, 2004). Lipid rafts are compartmentalized regions of the membrane that have important ramifications for the interaction of proteins, such that deviations in the clustering of membrane proteins in these regions can contribute to pathophysiology (Romay-Tallon et al., 2017).

In addition to factors that act directly on the brain, there is also evidence to implicate interventions that act upon the immune system as promising avenues for antidepressant targets. To support this, alterations in membrane protein clustering of the serotonin transporter (SERT) and serotonin 2A receptor on the plasma membrane of peripheral lymphocytes has been proposed as a novel biomarker of depression (Rivera-Baltanas et al., 2012). More specifically, patients with depression show a distinct profile of membrane protein clustering (MPC) as compared to healthy controls, evidenced by decreased SERT binding in lymphocytes as well as an increase in the size of SERT and serotonin 2A receptor clusters (Rivera-Baltanas et al., 2012, 2014). Notably, SERT is one of the main targets of antidepressant action, and importantly, this altered pattern of MPC in depression is reversed in patients that are responsive to antidepressant treatment, with alterations of SERT clustering in depression correlating with the remittance of anhedonia symptoms (Rivera-Baltanas et al., 2015). This is important because anhedonia is a fundamental symptom of depression, and if remittance of anhedonic symptoms can be predicted based upon the SERT clustering pattern in patients, this could help inform the course of antidepressant treatment.

Similar to what has been observed in patients with depression, a similar pattern of MPC is also altered in both reelin-deficient mice (Rivera-Baltanas et al., 2010) and in CORT-treated rats (Fenton et al., 2015b), which will be discussed in the following two sections.

3.3.2 Role of Reelin in Membrane Protein Clustering

The pattern of MPC in lymphocytes is altered in reelin-deficient mice, including both HRM and reeler mice. Specifically, HRM show an increase in both the size and number of SERT clusters in lymphocytes, while reeler mice show an increase in SERT cluster size that is more pronounced than that in HRM (Rivera-Baltanas et al., 2010). SERT is primarily responsible for the recapture of serotonin from the extracellular space. The clustering of SERT into lipid raft membrane domains appears to be critical for serotonin (5-HT) reuptake activity and SERT functional roles. A decrease in reelin expression appears to bring about the spread of SERT clusters of larger size, impairing its optimal functioning. HRM are also more susceptible to the depressiogenic effects of CORT, and both HRM and reeler mice show disrupted cytokine secretion from lymphocytes and macrophages (Green-Johnson et al., 1995).

Markers of inflammation hold an important focus in the field of depression research, with levels of proinflammatory cytokines considered indicative of depressive pathophysiology (see reviews listed in Caruncho et al., 2016). This suggests that alterations in serotonin transporter clustering in blood lymphocytes associated with a decrease in reelin expression may be having an effect in immune system alterations that show comorbidity with depression. Thus, decreased reelin expression influences MPC in lymphocytes that may lead to disruptions of cytokine release, holding implications for the pathophysiology of depression.

3.3.3 CORT effects on Membrane Protein Clustering

As just discussed, reelin-deficient mice show altered MPC in immune cells. In CORT-treated rats that show depression-like behavior, reductions in DG reelin-expressing cells are observed (Lussier et al., 2013), with similar reelin reductions seen in depressed patients (Fatemi et al., 2000). This leads to the

question of whether CORT treatment alters MPC clustering in lymphocytes in a similar fashion to that observed in patients with depression. In fact, rats exhibiting a depressive phenotype following 3 weeks of CORT treatment also exhibit a significant increase in SERT and 5-HT 2A receptor cluster size in lymphocyte membranes, as compared to control rats. Furthermore, the increase in cluster size is associated with an increase in depression-like behavior in the FST (Fenton et al., 2015b).

Another interesting finding opens up the possibility for a protective role of the adaptive immune system following chronic stress. Lymphocytes extracted from mice that previously underwent chronic stress were implanted in naïve mice. These implanted lymphocytes went on to produce antidepressant-like outcomes. More specifically, the recipient mice showed reduced anxiety, increased social behavior, and increased hippocampal cell proliferation compared with mice that did not receive donor cells. These recipient mice also had reduced levels of proinflammatory cytokine levels in the blood (Brachman et al., 2015). These results suggest that chronic stress can induce adaptive changes in the immune system, which serve to confer stress resilience.

Together, these findings implicate the peripheral immune system as an important target in the development of antidepressant therapies. Cumulatively, (i) the alterations in reelin expression observed in both the CNS and in the periphery in MDD and in animal models of depression, (ii) the observations of reelin's facilitative actions on various brain functions, and (iii) the observations of behavioral and neurochemical protective effects following reelin supplementation, all provide substantial evidence for targeting the reelin system for the purpose of novel and efficacious antidepressant development.

4. Objectives and Hypotheses

This literature review has described: (i) disrupted HPA axis and various alterations in brain structure and function associated with depression, (ii) the

CORT model as a valid animal model of depression, (iii) reelin alterations in depression and in CORT-treated rats, (iv) reversals of the behavioral phenotype and restorations of neurogenesis following intra-hippocampal reelin infusions in the CORT model, (v) the expression of reelin in blood plasma and peripheral tissues where it affects MPC that may relate to the expression of cytokines, and (vi) altered pattern of MPC in CORT-treated rats and in (vii) patients with depression that is reversed when antidepressant treatment is effective. Expanding on this body of research, we asked whether IV peripheral administration of reelin can rescue both the behavioral and neurochemical phenotype following repeated CORT. In order to investigate the impact of reelin on the behavioral phenotype, we measured depression-like behavior following either repeated CORT, or repeated CORT with the addition of peripheral reelin administration. We also wanted to begin to explore the possible mechanisms underlying reelin's effects by asking whether reelin has direct activity on peripheral immune functioning and/or indirect activity on brain regions by crossing the blood brain barrier. To do so, we investigated (i) the SERT clustering pattern in peripheral lymphocytes as a possible target of reelin's antidepressant action, and (ii) the rate of neuronal maturation in the dentate gyrus of the hippocampus, as a secondary target for reelin's antidepressant potential. In order to address the research aims, a rodent model of depression utilizing chronic corticosterone treatment was employed.

CHAPTER 2

Antidepressant Potential of Peripheral Reelin Administration in a Rodent Corticosterone Model of Depression

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Abstract

Long-term stress exposure significantly contributes to the development of depression. In rodents, chronic exposure to corticosterone (CORT) leads to reductions in hippocampal neurogenesis and expression of the extracellular matrix protein reelin, which facilitates synaptic plasticity, long-term memory, and neurogenesis. We previously found that rats administered weekly intra-hippocampal infusions of reelin (1µl/µg) alongside CORT, displayed a significant reduction in depression-like behavior compared to control rats. Reelin has also been shown to affect the protein clustering pattern of the 5-HT transporter on lymphocyte membranes. Importantly, this clustering pattern is associated with depression and responsiveness to treatment. Both patients diagnosed with depression, and rats administered repeated CORT, show an increase in the size of SERT clusters. The current experiment employed a novel approach to determine whether peripheral reelin injections can reverse behavioral impairments following prolonged CORT exposure. A secondary aim was to explore the peripheral and/or central mechanisms by which reelin injections can impact depressive symptomology. Rats received a single daily subcutaneous injection of CORT (40 mg/kg) or saline for 21 consecutive days, in conjunction with intravenous injection of reelin (3µg/ml or 5µg/ml) or saline, either every 5 or 10 days. The forced swim test (FST) was used to measure depression-like behaviour. CORT- treated rats displayed significantly increased depression-like behaviour on the FST compared to control rats. Importantly, this effect was reversed with addition of peripheral reelin at all doses administered. CORT-treated rats had an increase in the size of SERT clusters reminiscent of the clustering pattern seen in patients with depression, which was reversed with the addition of peripheral reelin. Furthermore, CORT-treated rats showed a decrease in the maturation rate of newborn neurons in the hippocampal subgranular zone, as compared to control rats. CORT-treated rats administered reelin also showed a general decrease in newborn neuron maturation rate. Hence, reelin injections did not reverse the CORT-induced reductions in the dendritic complexity of

immature granule neurons. This finding suggests that decreases in dendritic maturation of newborn hippocampal neurons do not underlie the antidepressant-like effects of peripheral reelin. Alternatively, findings suggest that reelin regulates SERT membrane protein clustering, optimizing its functioning, to reverse depressive symptomology.

1. INTRODUCTION

Depression is a both a common and serious psychiatric illness. According to the World Health Organization (2017), more than 300 million people worldwide are affected by depression. Despite the widespread impact of major depression, there remains major challenges in treatment of the disorder. Remission rates range from approximately 25-40%, even after at least 2 months of treatment (Keller et al., 1998; Trivedi et al., 2006). Furthermore, in a large clinical study of outpatients diagnosed with major depressive disorder, patients who did achieve response or remission did so only after at least 8 weeks of treatment with an SSRI (Trivedi et al., 2006). This finding evidences a substantial therapeutic delay in patients undergoing standard treatment with an SSRI. If left inadequately treated, depression can lead to suicide, a pandemic that claims approximately 800, 000 people each year (World Health Organization, 2017). Hence, there is a clear need for the development of faster-acting and more effective antidepressants.

Stress is a primary risk factor for the development of depression, with prolonged stress posing serious consequences for brain structure and function. The hippocampus is a limbic brain structure that is particularly susceptible to the negative effects of chronic stress. Hippocampal alterations have been implicated in depressive illness. Decreased hippocampal volume has been observed in patients with depression (MacQueen et al., 2003; Campbell et al., 2004). The development of rodent models of depression employing chronic stress paradigms have allowed for observations of hippocampal remodeling coincident with the development of depression-like symtoms. Specifically, both chronic stress and chronic treatment with corticosterone leads to hippocampal volume loss, atrophy of hippocampal pyramidal cell dendrites, loss of CA3 synapses (Magarinos et al., 1998, 1999; Sousa et al.,1998, 2000; Woolley et al.,1990; Sousa et al., 2000; Tata et al., 2006), and reductions in hippocampal neurogenesis (Fuchs and Gould, 2000; Pham et al., 2003; Santarelli et al., 2003; Sapolsky, 2004; Lussier et al., 2011, 2013), that are reversed following antidepressant treatment (Malberg et al., 2000; Scott et al., 2000; van Praag et al., 1999; David et al., 2009).

Chronic CORT treatment has also been shown to lead to reduced expression of the extracellular matrix protein reelin (Lussier et al., 2009). Reelin plays an important role in the hippocampus, where it facilitates synaptic plasticity, long-term potentiation, (D'Arcangelo, 2005; Weeber et al., 2002; Rogers et al., 2013), synaptogenesis, dendritic spine growth and density (Costa et al., 2002; Niu et al., 2008; Chameau et al., 2009), and hippocampal neurogenesis (Pujadas et al., 2010). CORT-induced reductions in hippocampal reelin expression and neuronal maturation rate have been shown to coincide with the development of depression-like behavior in rodents (Lussier et al., 2013). Moreover, direct infusion of reelin into the hippocampus fully restores these behavioral and neurochemical parameters following CORT exposure (Brymer et al., in preparation).

Mice that are deficient in reelin are more susceptible to the depressogenic effects of CORT on behavior, reelin expression, and rate of newborn neuron maturation (Lussier et al., 2011). Reelin-deficient mice also show changes in the pattern of membrane protein clustering of the serotonin transporter in lymphocytes, displaying an increase in the size of SERT clusters on lymphocyte membranes (Rivera-Baltanas et al., 2010). Similarly, CORT-treated rats also show a significant increase in SERT cluster size along lymphocyte membranes, as compared to control rats, and increased cluster size is positively correlated with increased depression-like behavior in the FST (Fenton et al., 2015b). Importantly, patients with depression also show a similar increase in the size of SERT clusters along lymphocyte membranes (Rivera-Baltanas et al., 2012, 2014). SERT is one of the main targets of antidepressant action, and holds important implications for antidepressant efficacy.

To summarize, reelin holds neuroprotective roles in the hippocampus and is also associated with SERT MPC in peripheral lymphocytes, - the pattern of which is altered in depression. As reelin is also expressed peripherally, we were motivated to examine whether injecting reelin into the bloodstream could bring about antidepressant outcomes, by way of direct brain interactions or through the regulation of SERT MPC in lymphocytes.

2. MATERIALS AND METHODS

2.1 Animals

We used 84 male Long-Evans rats (Charles River Laboratories; Montreal, Quebec, Canada), weighing 200-250g upon arrival, in the study. Rats were housed individually in clear plastic cages with free access to food and water. The thermally-controlled colony room was maintained at a 12/12 hr light/dark cycle. All procedures were approved by the University of Saskatchewan Animal Research Ethics Board, and conducted in accordance with the Canadian Council on Animal Care.

2.2 Experimental Design

2.2.1 Handling and Experimental Conditions

Upon arrival to the facility, rats were allowed 48 hours to habituate to the colony room. Following this, all rats underwent a 7-day handling period that incorporated a habituation period with the restraint device that would be used during the tail vein injection procedure. On days 1-3, rats were simply handled in order to habituate them to the experimenter. On day 4, rats were introduced to the restraint device, which was placed in the cage for a period of one minute, while they were allowed to explore. On days 5, 6, and 7 rats were placed in the restraint device for a period of one minute to initiate habituation to the device. Following the handling period, rats were then weight-matched and assigned to one of two experimental conditions based on the subcutaneous administration of 21 days of: (i) saline or (ii) CORT (40mg/kg).

Rats were then subdivided into 12 groups, according to (ii) intravenous saline (every 5 or 10 days) or reelin at four varying doses of reelin (3 µg/ml every 10 days; 3 µg/ml every 5 days; 5 µg/ml every 10 days; 5 µg/ml every 5 days). This resulted in 12 experimental conditions, as illustrated below:

Table 1. Illustration of the twelve experimental subgroups.

	Dose	Frequency	SC Vehicle	SC CORT
IV Vehicle	**N/A**	Every 10 days	N=6	N=6
		Every 5 days	N=6	N=6
IV Reelin	**3µg/ml**	Every 10 days	N=6	N=6
		Every 5 days	N=8	N=8
	5µg/ml	Every 10 days	N=8	N=8
		Every 5 days	N=8	N=8

2.2.2 CORT Injection Paradigm

All CORT and vehicle injections were administered subcutaneously once per day (between 9:00 and 11:00 am). CORT (Steraloids, Newport, RI, USA) was suspended in 0.9% (w/v) physiological saline with 2% (v/v) Polysorbate-80 (Sigma-Aldrich) solution and given at a dose of 40 mg/kg in a volume of 1 ml/kg. Our lab has reported that the 40mg/kg dose reliably increases depression-like behavior in rats (Johnson et al., 2006; Lussier et al., 2013, Fenton et al., 2015). The body weight of each rat was recorded daily from days 1-21 of CORT/vehicle treatment.

2.2.3 Reelin Injection Paradigm

Recombinant reelin (3820-MR-025/CR; R & D Systems, Minneapolis, MN, USA) was reconstituted immediately prior to injections to a working concentration of 3ug/ml or 5ug/ml in 0.1 M PBS (pH = 7.4). Reelin injections were conducted in a different room from those used for CORT injections and housing. Rats were placed in a plastic restrainer and their tail was sanitized and warmed in a beaker of warm water in order to dilate the blood vessels prior to injection. A catheter was then inserted into the lateral tail vein using a 25G needle. The needle was then withdrawn and a syringe was attached to the catheter for blood withdrawal.

Once the appropriate amount of blood was obtained, the syringe was removed and a second syringe containing reconstituted reelin (3ug/ml or 5ug/ml) or saline was attached to the catheter and the appropriate solution was administered at a rate of 0.5 ml over a 30-second period. Both the catheter and the syringe were then withdrawn simultaneously from the vein and a cotton swab was applied to the area and held there until bleeding stopped. The rat was then removed from the restrainer and placed back in its home cage. Figure 3 below illustrates the schedule for the reelin and CORT injections.

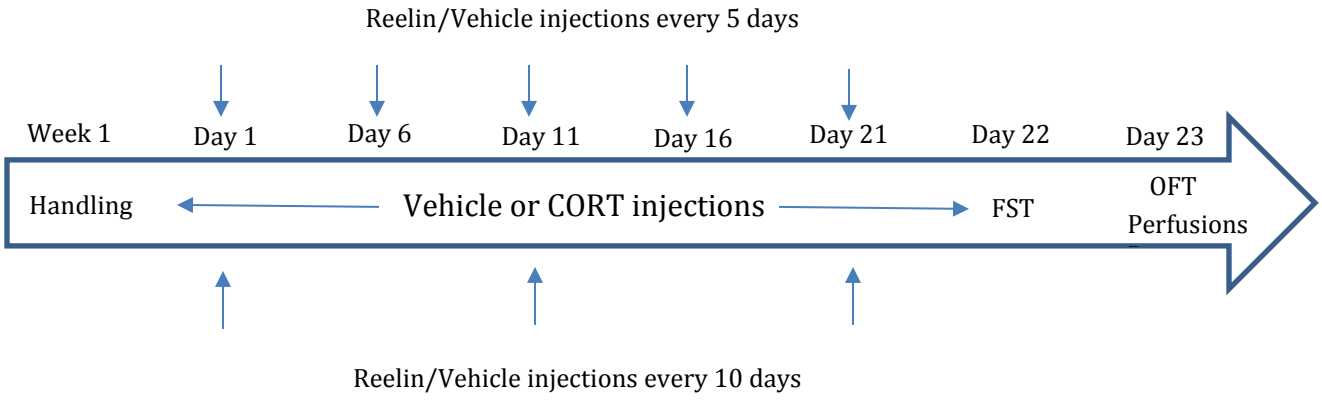

Figure 3. Schematic of experiment timeline, representing the schedule for Vehicle/CORT subcutaneous injections and Vehicle/Reelin intravenous injections.

2.3 Behavioural Testing

Behavioural testing was conducted in a different room from the rooms used for housing, CORT injections, and reelin injections. All rats underwent behavioral testing between 8 am and 5 pm. Behaviour was videotaped and scored at a later time point. The forced swim test was used to assess depression-like behavior and the open field test was used to assess motoric and anxiety-like behaviors.

2.3.1 Forced Swim Test

The FST was conducted on the day after the final injection (Day 22). A modified version of the Porsolt test (1977,1978), employing a single immersion trial was used, as described previously (Gregus et al., 2005). Briefly, each rat was placed individually into a Plexiglas swim tank (25 cm wide X 25 cm long X

60 cm high, 27 ± 2 °C water, 30 cm deep) for 10 minutes. The duration of time each rat spent immobile, and the duration of time spent swimming during the 10-minute test was measured (Cryan et al., 2005). Immobility was defined as the absence of head movement or body propulsion (Strekalova et al., 2004; Cryan et al., 2005; Stevenson et al., 2009), and swimming was defined as the directed movement of the animal's head and body. The water in the container was changed after each rat. More time spent immobile and less time actively swimming or struggling reflects learned helplessness in response to a threat/challenge and is considered to be indicative of a depressive phenotype. Behavior was scored for separately by two different experimenters, each blind to the treatment group the rat belonged to, in order to ascertain inter-rater reliability.

2.3.2 Open Field Test

The open field test was conducted on the day after the forced swim test, and prior to perfusions. Each rat was placed individually into the center of a 50 cm X 50 cm plastic box, which was divided into 16 equal squares. The rat was left in the box for a period of 5 minutes. The inner 4 squares were defined as the center and the outer 12 squares were defined as the periphery. Two behavioral measures were quantified for each rat: the amount of time spent in the center of the arena (in sec), and the total distance traveled (in cm) during the 5-minute test period. The total distance traveled by the rat reflects locomotive behaviour and this was measured in order to ensure that a restoration of immobility deficits with peripheral reelin could not be attributed to a general motoric effect of reelin. The amount of time spent in the center of the arena was measured in order to assess a potential effect of either CORT or reelin on anxiety-like behavior. This measure depends on rats' spontaneous exploration of an open field. Rats will naturally seek protection near the peripheral edges of an open field under conditions of increased anxiety. Hence, greater anxiety-like behavior is reflected by a lesser amount of time spent in the center of the arena. All behaviors were coded using Ethovision software (version 3.0; Wageningen, The Netherlands).

2.4 Blood Work Procedures

Immediately prior to each reelin injection, 0.3 – 0.5 ml of blood was obtained from each rat using a catheter inserted into the lateral tail vein. 2-3 drops of blood from each sample were placed on Super-Frost Plus slides and then gently smeared in a unidirectional manner across the slide using a coverslip. This procedure effectively created a thin blood smear on each slide for subsequent analysis. Three thin smears were obtained for each rat on each day of blood extraction. They were dried at room temperature for at least 1 hr., and then stored at - 80 °C until used for immunocytochemistry. On the final day of the experiment, rats were euthanized and sacrificed. Blood was drawn via heart puncture (up to 3ml in total) immediately prior to perfusions. In addition to 3 thin smears, blood was collected in 5 ml Eppendorf tubes containing ADC solution in a 1:7 proportion before being appropriately stored.

2.4.1 Immunocytochemistry of SERT in blood smears

Immunocytochemistry was conducted directly on smears according to a previously described procedure (Romay-Tallon et al., 2017). The first step was a fixation step. The frozen smears were brought back to room temperature and allowed to dry. They were then fixed with 0.5 ml of 1% PFA for 5 min at room temperature. The smears were comprised of a whole blood sample made up of red blood cells, platelets and white blood cells, but for the purposes of the current experiment, only the lymphocytes were of interest. In order to avoid unspecific labelling in the whole blood sample, a pre-incubation step was performed for 1 h at room temperature with 10% rat IgG diluted in 1% BSA and PBS. For labeling of SERT, a polyclonal primary antibody raised in rabbit was used that specifically recognizes epitopes of both human and rat SERT [anti-serotonin transporter: AB10514P (Millipore, Billerica, Massachusetts)]. Smears were incubated with the primary antibody overnight, diluted 1:250 in 1% BSA and PBS. After incubating, smears were rinsed with PBS 3 times, 10 min each time. The samples were then incubated with a 1:200 solution of fluorescent secondary antibody [Alexa Fluor 568, goat anti-rabbit: (A-11008, Molecular Probes, Eugene, Oregon)] in 1% BSA in PBS for 2 hrs at room temperature. This step is light sensitive, and therefore, it was carried out in the dark. The excess antibody was then rinsed off by washing

the samples with PBS 3 times for 10 min each. To facilitate the identification of the lymphocytes, a nuclear marker was used [Hoechst 33,258 (Invitrogen, Molecular Probes, Carlsbad, California), which was applied at 2 µg/ml for 15 min at room temperature]. After nuclear labeling, the smears were rinsed with PBS for 10 min, left to dry, and coverslipped. The slides were then stored in the freezer at -20 °C.

2.5 Perfusions and Tissue Preparation

On day 22, rats were anesthetized with isofluorane and perfused transcardially with 0.9% sodium chloride solution and 4% (w/v) paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). The brains were dissected keeping the cerebellum and olfactory bulb intact. Tissue was postfixed in 4% (w/v) paraformaldehyde for 48 hours at 4 °C, and then they were placed in a .1 M phosphate buffered saline (PBS) and sodium azide solution at 4 °C until sectioning. Brains were sectioned at 30 um using a cryostat. Brain sections were stored in cryoprotectant solution at – 20 °C until used for immunohistochemistry.

2.6 Image processing and quantification of SERT clusters

SERT membrane protein clustering was analyzed in smears from blood samples obtained on the day of perfusions. SERT immunolabelling is evidenced as immunofluorescent clusters observed primarily in the lymphocyte plasma membrane. Pictures were acquired at 100× magnification from 100 individual lymphocytes per rat using a fluorescence microscope. The pictures were subsequently analyzed using ImageJ software (1.48v, NIH, USA). ImageJ performs measurements in pixels, so the program must be calibrated to reflect the real values of the obtained measurements (i.e., number and size of the SERT clusters). Hence, a known distance is entered into the program to establish equivalency between pixels and distance given in um. Additionally, background conditions were adjusted to optimize the analysis of clusters. To do this, the size of the smallest particle which was not considered background was indicated. Finally, a step to remove outliers was also performed, in order to delete small

specks that might interfere with the analysis and give an overestimation of cluster number and an underestimation of cluster size (Romay-Tallon et al., 2017). At this point, the program creates a binary image for easy quantification of cluster number and size. The minimum size of particle for the program to analyze was indicated as 0.05, and the maximum size was set at ‘infinite’, to provide an accurate measure of the clusters. Once the set of conditions for analysis using ImageJ was established, they were kept constant, so that results could be compared accurately across all conditions. SERT+ immunolabeling was analyzed throughout the entire thickness of the sample (~10µm). As discussed, two sets of measurements were obtained from each lymphocyte: the size of SERT-expressing clusters, and the number of SERT-expressing clusters. For each sample, the measurements from each lymphocyte were averaged.

2.7 Doublecortin (DCX) Immunohistochemistry

Every sixth section was used for immunostaining and 2 sections were quantified per animal. Immunohistochemistry was performed on free-floating sections, as follows: The sections were washed in TBS and incubated in sodium citrate (pH 6.0; 85 °C) for 30 minutes. After 3 TBS washes, the sections were incubated for 24 hours at room temperature with rabbit anti-DCX primary antibody (Cell Signaling, Danvers, MA) at 1:1000 diluted in a solution containing Triton X-100 (.5%, v/v), normal goat serum (NGS; 5%; v/v), and BSA (1%; w/v) dissolved in TBS. After incubation in the primary antibody, the sections were washed in TBS then incubated in 10% (v/v) H_2O_2 in TBS for 30 min to block endogenous peroxidase activity. Sections were then washed in TBS and then incubated with biotinylated goat anti-rabbit IgG (1:500, SigmaeAldrich, St. Louis, MO) secondary antibody diluted in .5% (v/v) Triton X-100, 5% (v/v) NGS, and 1% (w/v) BSA in PBS for 1 hour at room temperature. This was followed by incubation in an avidin-biotin complex (1:500, Vecta Stain Elite ABC reagent, Vector Labs) for 1 h at room temperature. After washes in TBS and 2 washes in .175 M sodium acetate, the sections were stained using .025% DAB and 4.167% $NiSO_4$ dissolved in .002% H_2O_2 and .175M sodium acetate. The reaction was

stopped using .175 M sodium acetate as a rinse. The sections were then mounted onto slides and left to dry overnight and the coverslipped using Entellan resin solution.

2.7.1 Analyses of DCX Immunolabelling

To semi-quantify the dendritic morphology of immature granule cells, dendritic branching in a subset of DCX+ cells from the SGZ and granule cell layer of the DG (from -2.40 mm to approximately -3.94 mm from bregma) was categorized in each rat according to the method of Plumpe et al. (2006) and as previously described (Lussier et al., 2011; Plumpe et al., 2006). One hundred cells per animal were randomly selected using a meander scan method. Each cell was assigned to one of six categories based on the presence and extent of its apical dendrites. Briefly, categories 1 and 2 represented the proliferative stage of development and cells placed into these categories had either no processes (category 1) or one short process (category 2). Categories 3 and 4 represented an intermediate stage of cell development. Category 3 cells showed a medium process that reached the granular cell layer but did not extend into the molecular layer. Category 4 cells were similar except that they had a process that reached the molecular layer. Categories 5 and 6 represented more mature stages of cell development. Category 5 cells had at least one major dendrite branching within the molecular layer. Category 6 cells had a defined dendritic tree with delicate branching within the granule cell layer. The percentage of cells falling into each of these categories was calculated for each animal.

2.8 Statistical Analysis

All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS v 20, Chicago, IL). Separate one-way ANOVAs were used to assess the statistical significance of group differences on each measure (i.e., body weight, FST behavior, OFT behavior, size and number of SERT clusters, and % of DCX+ cells). Significant main effects were followed by Tukey's post-hoc tests where appropriate. The criterion for statistical significance was $p < 0.05$. Error bars on all graphs represent the standard error of the mean (SEM).

3. RESULTS

3.1 Body Weight

Differences in weight gain were analyzed between 4 experimental groups, collapsed across dosage of intravenous reelin/saline administered (i.e., Vehicle-Vehicle, CORT-Vehicle, Vehicle Reelin, CORT-Reelin) by conducting separate one-way ANOVAs with experimental group as the independent variable and weight (grams) at four weekly time-points as the dependent variable. Consistent with past research utilizing an exogenous CORT paradigm (Lussier et al., 2009; Lussier et al., 2011; Lussier et al., 2013), CORT had significant effects on body weight, with a decrease in weight gain observed with repeated CORT. Significant body weight differences were found among the groups on days 1, 7, 14, and 21. There was a significant main effect of experimental group on day 1 of injections, $F(3, 80) = 25.07$, $p < 0.001$. A post-hoc Tukey test revealed that on day 1, CORT-Vehicle and CORT-Reelin rats weighed significantly more than Vehicle-Vehicle and Vehicle-Reelin rats, *ps* < 0.001 (see Fig 4). The reason for this difference was the intentional assignment of heavier rats into CORT treatment groups at the outset of the experiment, given the anticipated weight gain reduction in these rats. On day 7, a significant main effect of group on body weight was observed, $F(3, 80) = 11.34$, $p < 0.001$. Post-hoc Tukey analyses revealed that the CORT-Reelin rats weighed significantly less than the Vehicle-Vehicle and Vehicle-Reelin rats, ps < 0.001 (see Fig 4). Again, on day 14 of CORT injections, a significant main effect of group on body weight was observed, $F(3, 80) = 36.41$, $p < 0.001$. A post hoc Tukey test revealed that both the CORT-Vehicle and CORT-Reelin groups weighed significantly less than both the Vehicle-Vehicle and Vehicle-Reelin groups, ps < 0.001 (see Fig 4). Finally, a significant main effect of group was observed on day 21, $F(3, 80) = 47.98$, $p < 0.001$. Post-hoc Tukey analyses revealed that both the CORT-Vehicle and CORT-Reelin groups weighed significantly less, as compared to both the Vehicle-Vehicle and Vehicle-Reelin groups, ps < 0.001 (see Fig 4).

Differences in weight gain between the four experimental groups were additionally analyzed using a Mixed-design ANOVA, with treatment group (i.e., Vehicle-Vehicle, CORT-Vehicle, Vehicle Reelin, CORT-Reelin) as the between-subjects factor and time as the within-subjects factor. On the Mixed-design ANOVA, spehericity could not be assumed, according to Mauchly's Test of Sphericity ($p < 0.001$), therefore Greenhouse-Geisser correction was employed. There was a significant main effect of time on body weight when averaged across groups, $F(1.42, 113.66) = 141.86$, $p < 0.001$. Additionally, there was a significant main effect of treatment on body weight when averaged across time, $F(3, 80) = 15.19$, $p < 0.001$. Importantly, however, there was a significant interaction effect of treatment group X time, $F(4.26, 113.66) = 132.65$, $p < 0.001$. Following up, Bonferroni pairwise comparisons looking at the effect of each time-point in all treatment groups, revealed the same significant differences between groups for each of the weekly time points, as is reported from the results of the independent one-way ANOVAs above.

Bonferonni pairwise comparisons looking at the effect of all time-points within each treatment group revealed that both Vehicle-Vehicle rats and Vehicle-Reelin rats differed significantly in their weights between all times points (ps < 0.01), showing a steady increase in weight over time. For CORT-Vehicle rats, their weight was significantly lower on days 7, 14, and 21, as compared to their weight on day 1, ps < 0.002. CORT-Vehicle rats did show a significant increase in weight on day 21 as compared to day 14, $p < 0.001$. Similar to CORT-Vehicle rats, CORT-Reelin rats weighed significantly less on days 7, 14, and 21, as compared to day 1, ps < 0.001. CORT-Reelin rats weighed significantly more on day 21, as compared to day 7 and day 14.

In sum, the results of both the Mixed-analysis ANOVA and the independent one-way ANOVAs evidence that CORT-treated rats had significantly attenuated weight gain as compared to Vehicle-treated rats over the course of the 21-day injection period.

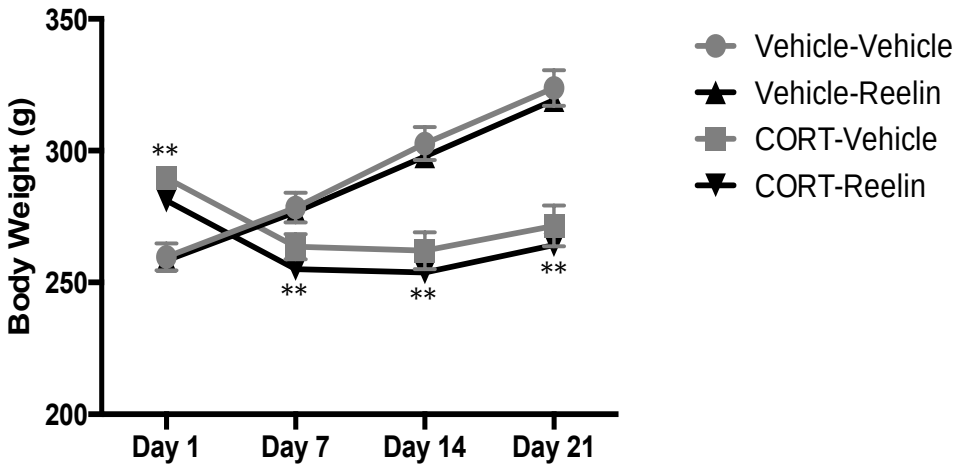

Figure 4. Body weight measured over 21-day injection period. Data is presented as mean ±SEM. Asterisks represent significantly different groups.

3.2 Forced Swim Test

As discussed, previous research has shown that rats treated with CORT display increased behavior symptomatic of depression on the FST (i.e., increased time spent immobile; decreased time spent actively swimming or struggling). In the current experiment, a one-way ANOVA was conducted separately for each behavior on the FST, with experimental group as the independent variable, and immobility and swimming behaviors as the dependent variables. First, it should be noted that an independent samples t-test showed that Vehicle- and CORT-treated rats that received IV saline every 10 days versus IV saline every 5 days did not differ significantly from each other in their depression-like behavior in the FST (i.e., immobility and swimming times) or in their behavior in the open field test. Therefore, these rats were treated in a uniform manner, resulting in Vehicle-Vehicle (N = 12) and CORT-Vehicle (N = 12) groups.

Now, results are reported for immobility behavior when collapsed across dose. A one-way ANOVA showed a significant main effect of CORT on immobility behavior, $F(3, 80) = 14.93$, $p < 0.001$ (see Fig 5). As expected, post-hoc Tukey tests revealed that the CORT-Vehicle rats spent significantly more time immobile

as compared to the Vehicle-Vehicle and Vehicle-Reelin groups, ps < 0.001 (see Fig 5). Importantly, the CORT-Vehicle rats also spent significantly more time immobile as compared to the CORT-Reelin rats, p = 0.002 (see Fig 5). Post-hoc testing also revealed that the CORT-Reelin rats spent significantly more time immobile as compared to the Vehicle-Reelin group, p = 0.006 (see Fig 5). Hence, the CORT-Reelin rats did not statistically differ from the Vehicle-Vehicle rats and showed a decrease in immobility with respect to the CORT-Vehicle rats, but still spent more time immobile than the Vehicle-Reelin rats, demonstrating an almost complete behavioral reversal on the immobility measure of the FST.

Next, results are reported for swimming behavior when collapsed across dose. Again, a significant main effect of experimental group was found on swimming behavior, F(3, 80) = 5.01, p = 0.003 (see Fig 5). As expected, post-hoc Tukey tests showed that the CORT-Vehicle rats spent significantly less time actively swimming than the Vehicle-Vehicle rats (p = 0.012) and the Vehicle-Reelin rats (p = 0.038) (see Fig 5). Importantly, the CORT-Vehicle rats also spent significantly less time actively swimming than the CORT rats treated with reelin, p = 0.002 (see Fig 5). The CORT-Reelin rats did not significantly differ from the vehicle groups in swimming behavior. Hence, a reversal in swimming behavior is observed with the addition of recombinant reelin.

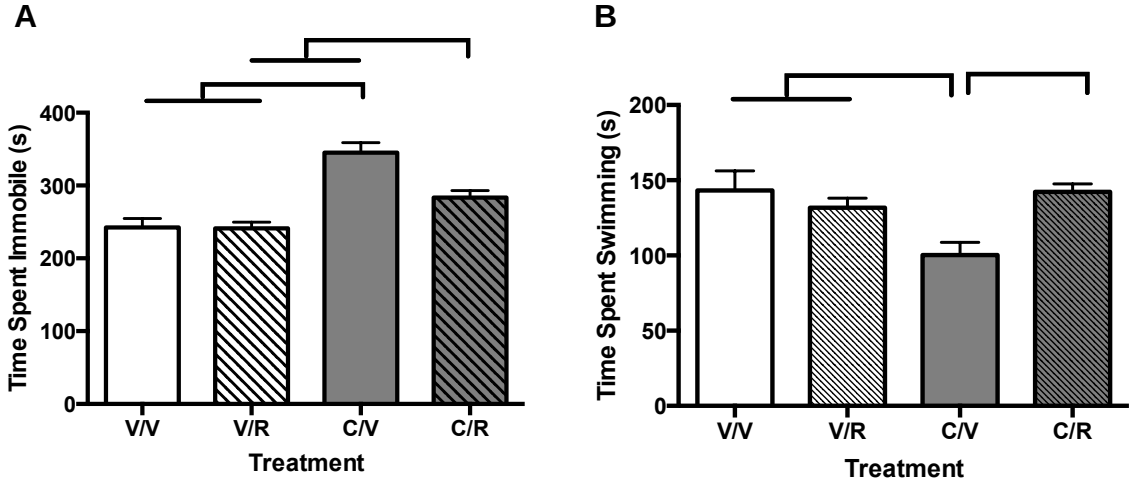

Figure 5. (A) Immobility Time and (B) Swimming Time in the FST. Data is presented as mean ±SEM. Lines represent significantly different groups.

Results are now reported for immobility behavior according to the dose of reelin administered. At the 3µg every 10 day dosage, there was a significant main effect of group on immobility behaviour, $F(3, 32) = 11.04$, $p < 0.001$ (see Fig 5.1). Post-hoc Tukey testing showed that the CORT-Vehicle group spent significantly more time immobile compared to the Vehicle-Vehicle and Vehicle-Reelin groups, $ps < 0.002$ (see Fig 5.1). Additionally, the CORT-Vehicle group spent significantly less time immobile compared to the CORT-Reelin group, $p = 0.020$ (see Fig 5.1).

Similarly, at the 3µg every 5 day dosage, there was also a significant main effect of group on time spent immobile, $F(3, 36) = 11.36$, $p < 0.001$ (see Fig 5.1). Post-hoc tests showed that the CORT-Vehicle group spent significantly more time immobile than all other groups, $p's < 0.03$ (see Fig 5.1).

At the 5µg every 10 day dosage, there was again a significant main effect of group on immobility, $F(3, 36) = 11.91$, $p < 0.001$ (see Fig 5.1). Post-hoc Tukey tests showed that the CORT-Vehicle rats spent significantly more time immobile than both the Vehicle-Vehicle and Vehicle-Reelin groups, $ps < 0.001$ (see Fig 5.1).

Finally, at the 5µg every 5 day dosage, there was a significant main effect of group on time spent immobile, $F(3, 36) = 12.81$, $p < 0.001$ (see Fig 5.1). The CORT-Vehicle group spent significantly more time immobile compared to all other groups, $ps < 0.02$ (see Fig 5.1). Hence, it is important to note that for each dose given, there was a significant effect of CORT, and this is consistently rescued by peripheral reelin administration. Significantly, this effect holds up for the lowest dose of reelin given.

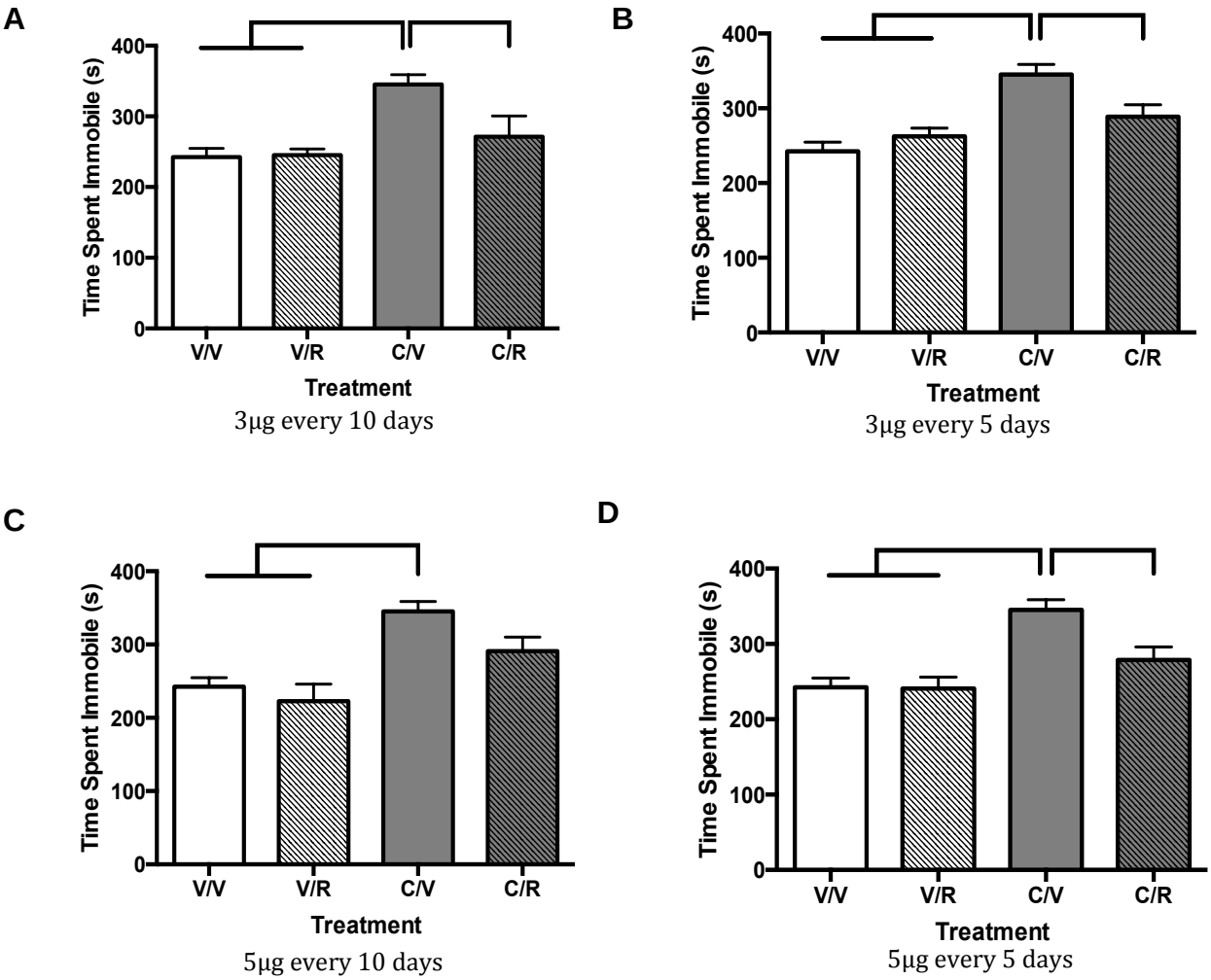

Fig 5.1 Immobility Time in the FST for each dosage administered. (A) 3µg/ml every 10 days. (B) 3µg/ml every 5 days. (C) 5µg/ml every 10 days. (D) 5µg/ml every 5 days. Data is presented as mean ±SEM. Lines represent significantly different groups.

Results are now reported for swimming behavior according to the dosage of reelin administered. At the lowest dose given, 3µg every 10 days, there was a significant main effect of group on swimming behavior, $F(3, 32) = 3.26$, $p = 0.034$ (see Fig 5.2). A Tukey test revealed that the CORT-Vehicle rats spent significantly less time swimming than the Vehicle-Vehicle rats, $p = 0.019$ (see Fig 5.2). No other significant differences between groups were observed for this dosage.

At the 3µg every 5 day dosage, there was a significant main effect of group on swimming time, $F(3, 36) = 3.85$, $p = 0.017$ (see Fig 5.2). The CORT-Vehicle group spent significantly less time swimming than the Vehicle-Vehicle group, $p = 0.044$, and the CORT-Reelin group, $p = 0.024$ (see Fig 5.2). No other significant differences between groups were found for this dosage.

At 5µg every 10 days, a significant main effect of group was found, $F(3, 36) = 3.93$, $p = 0.016$ (see Fig 5.2). The CORT-Vehicle group spent significantly less time actively swimming than the Vehicle-Vehicle group, $p = 0.024$, and the CORT-Reelin group, $p = 0.042$ (see Fig 5.2). No other significant differences between groups were present.

Finally, at 5µg every 5 days, a significant main effect of group on time spent swimming was observed, $F(3, 36) = 3.63$, $p = 0.022$ (see Fig 5.2). A Tukey test showed that the CORT-Vehicle rats spent significantly less time swimming than the Vehicle-Vehicle rats, $p = 0.030$, and showed a trend in this direction when compared to the CORT-Reelin rats, $p = 0.053$ (see Fig 5.2). No other significant differences between groups were observed at this dose.

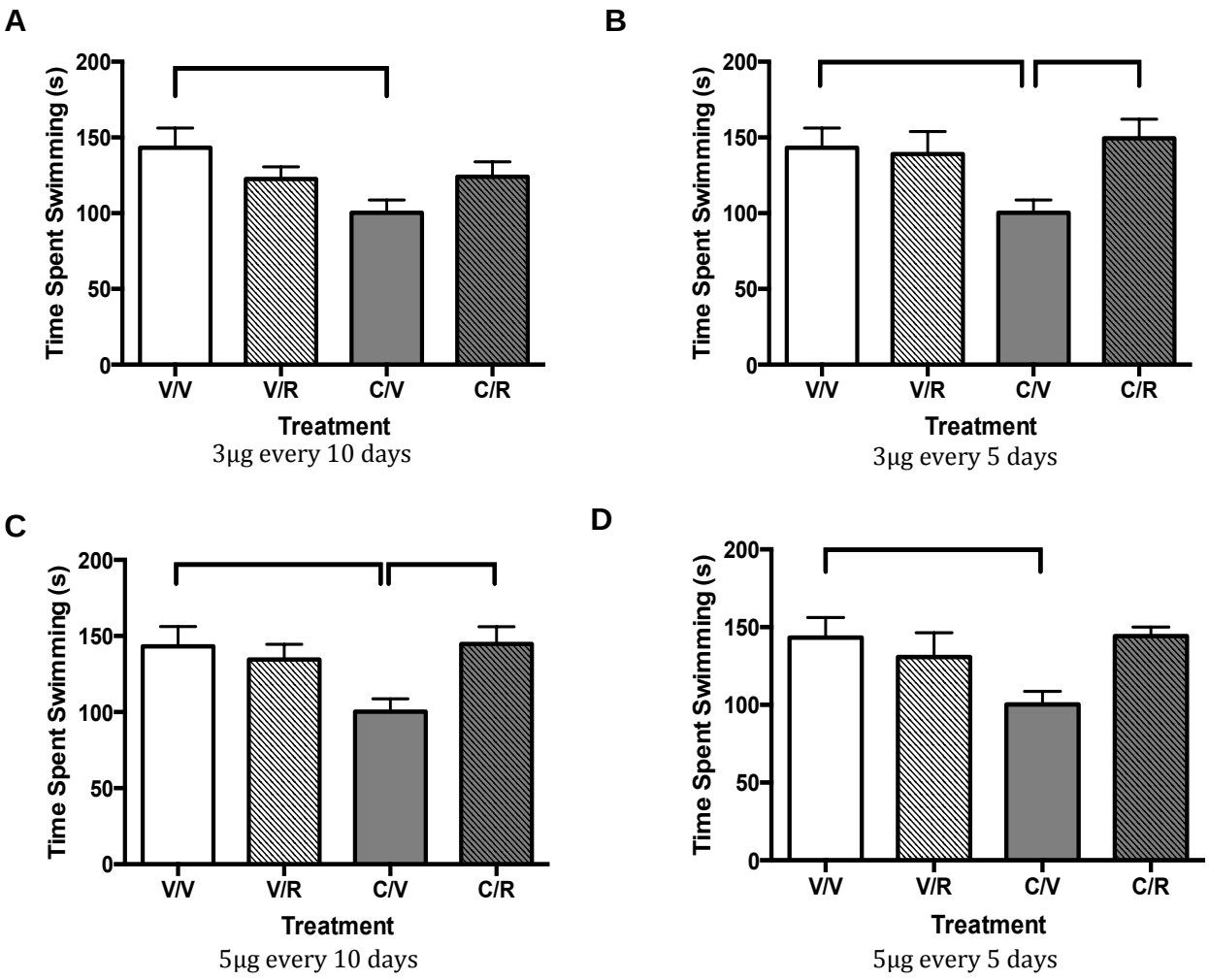

Fig 5.2 Swimming Time in the FST for each dosage administered. (A) 3µg/ml every 10 days. (B) 3µg/ml every 5 days. (C) 5µg/ml every 10 days. (D) 5µg/ml every 5 days. Data is presented as mean ±SEM. Lines represent significantly different groups.

While it is apparent that the pattern of restoration of behavior for the varying doses is less consistent for swimming behavior than for immobility behavior, the CORT-Reelin group does not significantly differ from vehicle groups on this measure and generally spends more time actively swimming than the CORT-Vehicle group. Taken together, the current results support that peripheral reelin administration effectively reversed the depressive phenotype that was brought about by repeated CORT treatment.

3.3 Open Field Test

A one-way ANOVA was conducted separately for each behavior on the OFT, with experimental group as the independent variable and total distance travelled (cm) and duration spent in center of arena (s) as the dependent variables. When collapsed across dose, there was no significant main effect of treatment group on the total distance travelled (cm) during the 5 minute test period, F(3, 78) = 0.53, ns (see Fig 6). Similarly, when collapsed across dose, there was no significant main effect of experimental group on the duration of time spent in the inner zone of the arena (s) in the 5 minute test period, F(3, 78) = 2.50, p = 0.07 (see Fig 6).

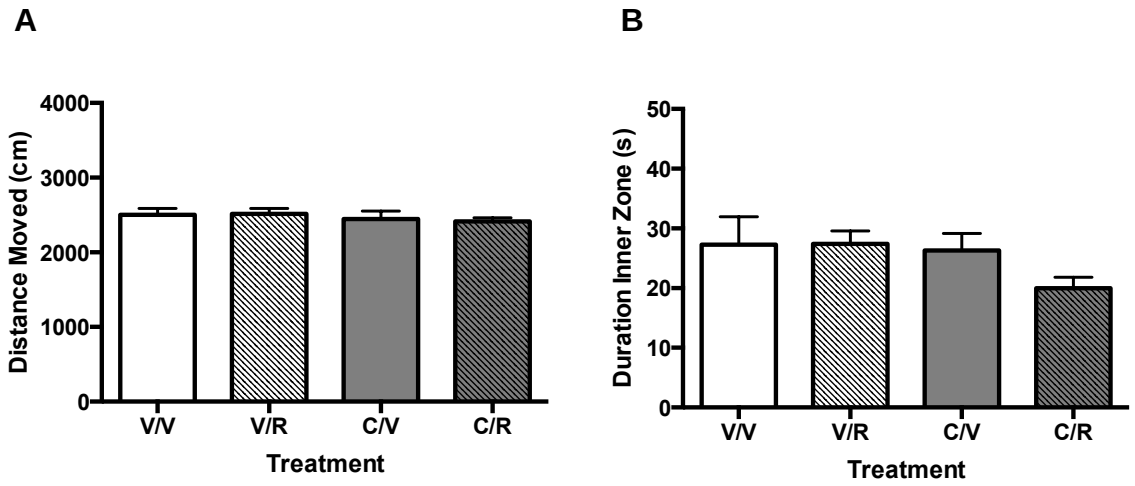

Fig 6. (A) Distance travelled and (B) Time spent in center zone of arena in OFT. Data is presented as mean ±SEM.

When analyzed according to the dose of reelin administered, there were no significant main effects of group for any of the doses administered. Specifically, at the lowest dose of 3ug every 10 days, there was no significant main effect of group on total distance traveled (cm), F(3, 20) = 0.95, ns, nor on duration spent in the center of the arena (s), F(3, 20) = 1.38, p = 0.278 (see Fig 6.1). At the 3µg every 5 day dose, there was no significant main effect of group on total distance traveled, F(3, 22) = 0.37, ns, nor on duration of time spent in center of arena, F(3, 22) = 0.50, ns (see Fig 6.1). Again, at the 5µg every 10 day dose, there was no

significant main effect of group on distance traveled, F(3, 24) = 0.37, ns, nor on duration of time spent in the center of arena, F(3, 24) = 0.85, ns (see Fig 6.1). Finally, at the highest dose given, 5µg every 5 days, there was no significant main effect of experimental group on total distance traveled, F(3, 23) = 1.18, p = 0.34, nor on duration of time spent in center of arena, F(3, 23) = 2.69, p = 0.07 (see Fig 6.1). Hence, neither CORT nor reelin produced an effect on locomotive or anxiety-like behavior, as measured by the OFT. It may therefore be reasoned that the significant behavioral differences observed between experimental groups in the FST are reflective of differences in depression-like behavior, rather than those of locomotive or anxiety-like behavior.

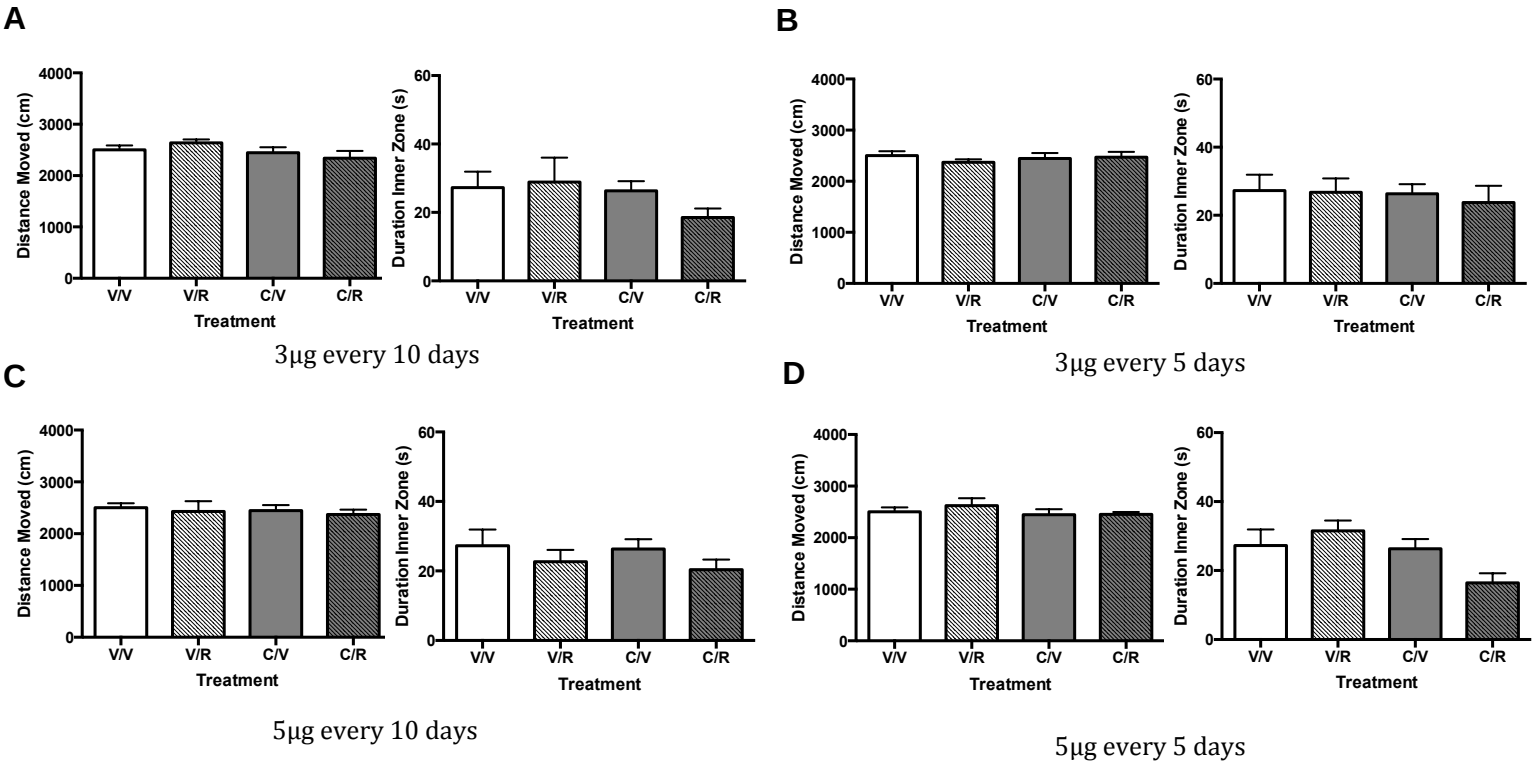

Fig 6.1 Distance travelled and time spent in inner zone of arena in OFT for each dosage administered. (A) 3µg/ml every 10 days. (B) 3µg/ml every 5 days. (C) 5µg/ml every 10 days. (D) 5µg/ml every 5 days. Data is presented as mean ±SEM.

3.4 Effects of CORT and Reelin on Membrane Protein Clustering in Lymphocytes

Thus far, the behavioral findings show that the addition of peripheral reelin rescues depression-like behavior, without producing any alterations in OFT behavior. In addition to these behavioral parameters, biochemical parameters were also measured; specifically, the size (μm^2) and number of SERT clusters on lymphocyte plasma membranes from rat blood smears were analyzed. Figure 7 shows an example of the SERT clustering quantification for each treatment group (i.e., V/V, V/R, C/V, C/R). In each group, you can see the original microscope image of the sample (~10µm thickness), followed by a conversion of the image to a binary colour with subtraction of background, and finally, the automatic analyzation of the binary image by ImageJ to quantify the number of SERT clusters per lymphocyte and the average size of clusters (see Fig. 7).

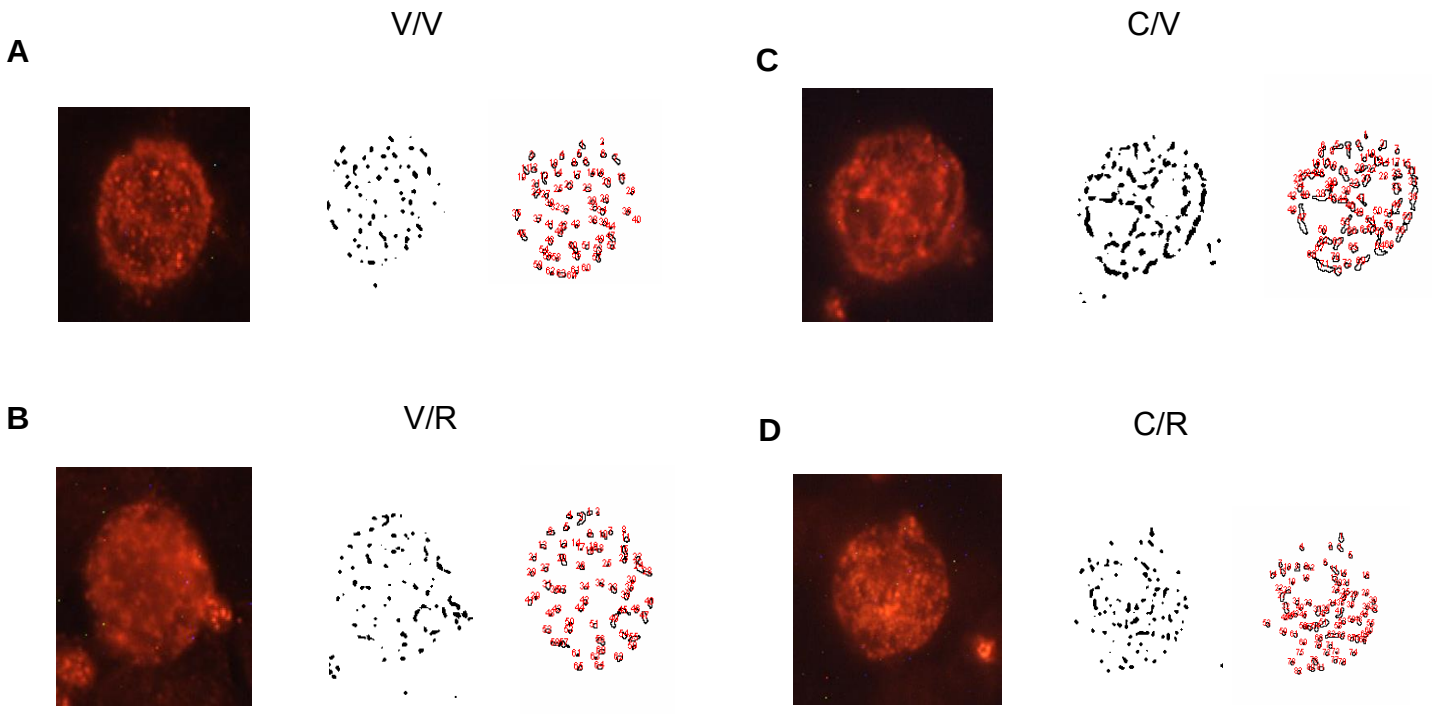

Fig 7. Example of analysis of SERT protein clusters in one lymphocyte from rat blood smears, showing the microscope image of SERT immunostaining, the binary image obtained in ImageJ, and the actual counts of the number and size of clusters as performed by the image analysis system (Romay-Tallon et al., in production). (A) Vehicle-Vehicle (B) Vehicle-Reelin (C) CORT-Vehicle (D) CORT-Reelin.

A one-way ANOVA was conducted separately for both the size (μm^2) and number of SERT clusters, with experimental group as the independent variable, and size and number of clusters as the dependent variables. Results are first reported for SERT cluster size (μm^2) when collapsed across dose of reelin administered. A one-way ANOVA showed a significant main effect of CORT on the size of SERT clusters, $F(3, 91) = 14.87$, $p < 0.001$ (see Fig 8). Post-hoc Tukey tests revealed that, as suspected, CORT treatment increased the size of SERT clusters, such that the CORT-Vehicle rats exhibited significantly larger clusters as compared to the Vehicle-Vehicle and Vehicle-Reelin rats, ps <0.001. Importantly, the CORT-Vehicle rats also showed significantly larger clusters as compared to the CORT-Reelin rats, $p < 0.001$ (see Fig 8). Hence, the effect of CORT treatment on the size of SERT clusters was significantly rescued with addition of peripheral reelin. In regard to the number of SERT clusters, no significant main effect of CORT treatment was observed between groups when collapsed across dose (see Fig 8).

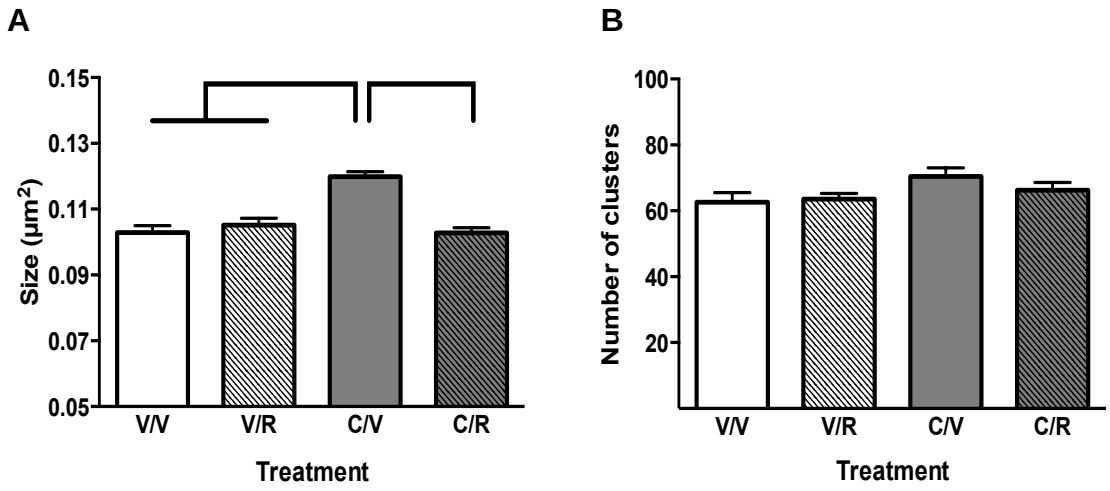

Fig 8. (A) Size (μm^2) of SERT clusters and (B) Number of SERT clusters in lymphocyte membranes. Data is presented as mean ±SEM. Lines represent significantly different groups.

Next, the size of SERT clusters was analyzed according to the dose of reelin administered. At the lowest dose of reelin given, 3µg every 10 days, there was a

significant main effect of treatment on the size of SERT clusters, $F(3, 45) = 15.23$, $p < 0.001$ (see Fig 8.1). Post-hoc Tukey testing revealed that the CORT-Vehicle group showed significantly larger clusters as compared to the Vehicle-Vehicle, Vehicle-Reelin, and CORT-Reelin groups, ps < 0.002 (see Fig 8.1). When reelin was administered at a dose of 3µg every 5 days, a significant main effect of treatment was again observed on the size of SERT clusters, $F(3, 49) = 12.66$, $p < 0.001$. A post-hoc Tukey test showed that the CORT-Vehicle rats had significantly larger clusters as compared to the Vehicle-Vehicle, Vehicle-Reelin, and CORT-Reelin rats, ps < 0.002 (see Fig 8.1). At the 5µg every 10 day dosage, a significant main effect of treatment was again observed on the clustering size of SERT, $F(3, 49) = 21.12$, $p < 0.001$ (see Fig 8.1). A post-hoc Tukey test showed that the CORT-Vehicle group had significantly increased cluster size compared to the three other groups, ps < 0.004 (see Fig 8.1). Finally, when 5µg of reelin was administered every 5 days, there was a significant main effect of treatment on the size of SERT clusters, $F(3, 49) = 13.00$, $p < 0.001$ (see Fig 8.1). Post-hoc Tukey comparisons showed that, just as the case for all other doses, the CORT-Vehicle rats had a significantly larger clusters compared to the Vehicle-Vehicle, Vehicle-Reelin, and CORT-Reelin rats, ps < 0.03 (see Fig 8.1).

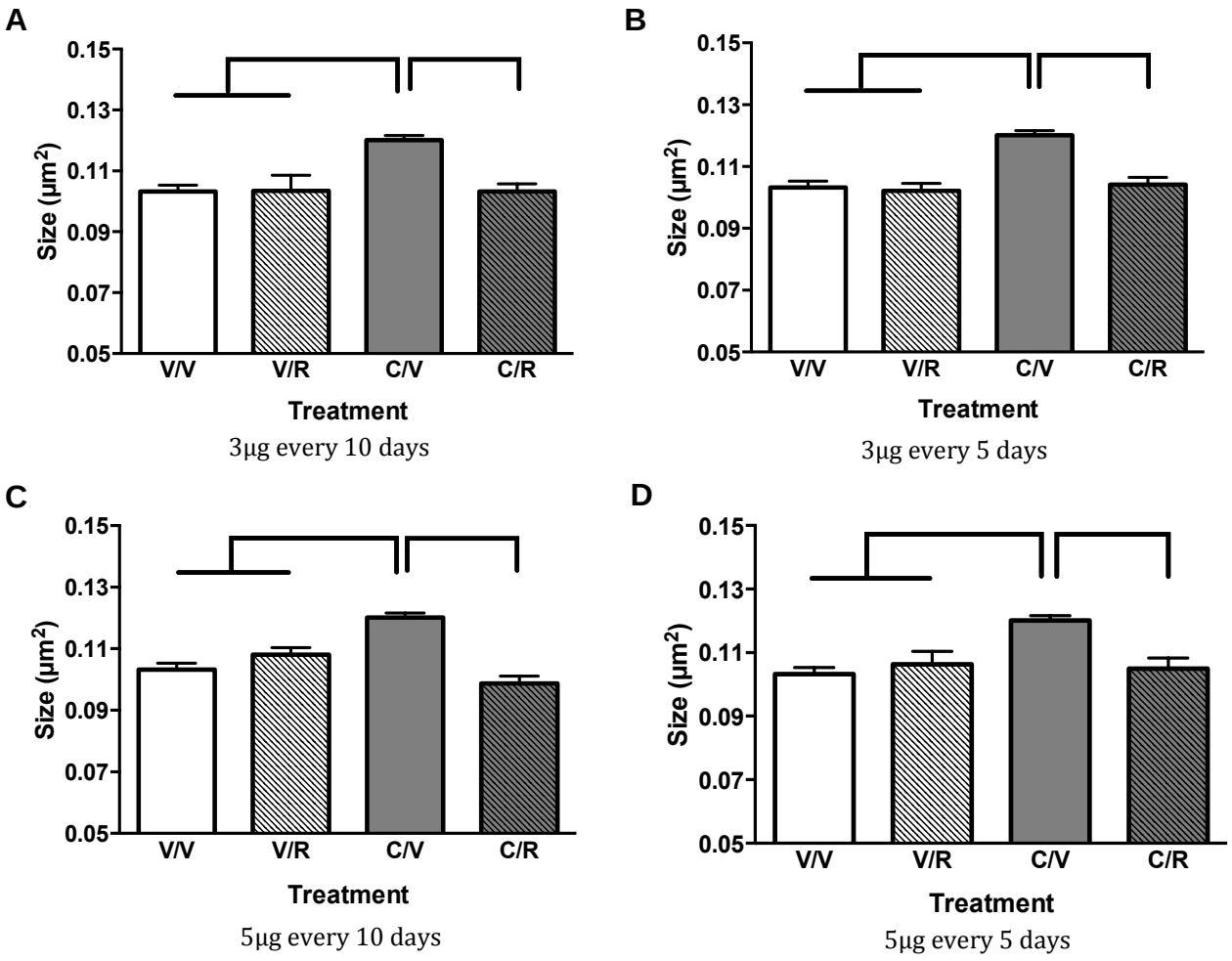

Fig 8.1. Size (µm^2) of SERT clusters in lymphocyte membranes according to each dosage of reelin administered. (A) 3µg/ml every 10 days. (B) 3µg/ml every 5 days. (C) 5µg/ml every 10 days. (D) 5µg/ml every 5 days. Data is presented as mean ±SEM. Lines represent significantly different groups.

Next, the number of SERT clusters was analyzed according to the dose of reelin administered. At the lowest dose of reelin, 3µg, administered every 10 days, no significant main effect of treatment group on the number of clusters was found, $F(3, 45) = 2.18$, $p = 0.10$ (see Fig 8.2). Again, at the 3µg every 5 day dose, there was no significant main effect of treatment on the number of clusters, $F(3, 49) = 1.99$, $p = 0.13$ (see Fig 8.2). At 5µg every 10 days, there remained no significant main effect of treatment on cluster number, $F(3, 49) = 2.01$, $p = 0.11$ (see Fig 8.2). Finally, at the highest dose of reelin given, 5µg every 5 days, no

significant main effect of treatment group on SERT cluster number was observed, F(3, 49) = 2.18, p = 0.10 (see Fig 8.2).

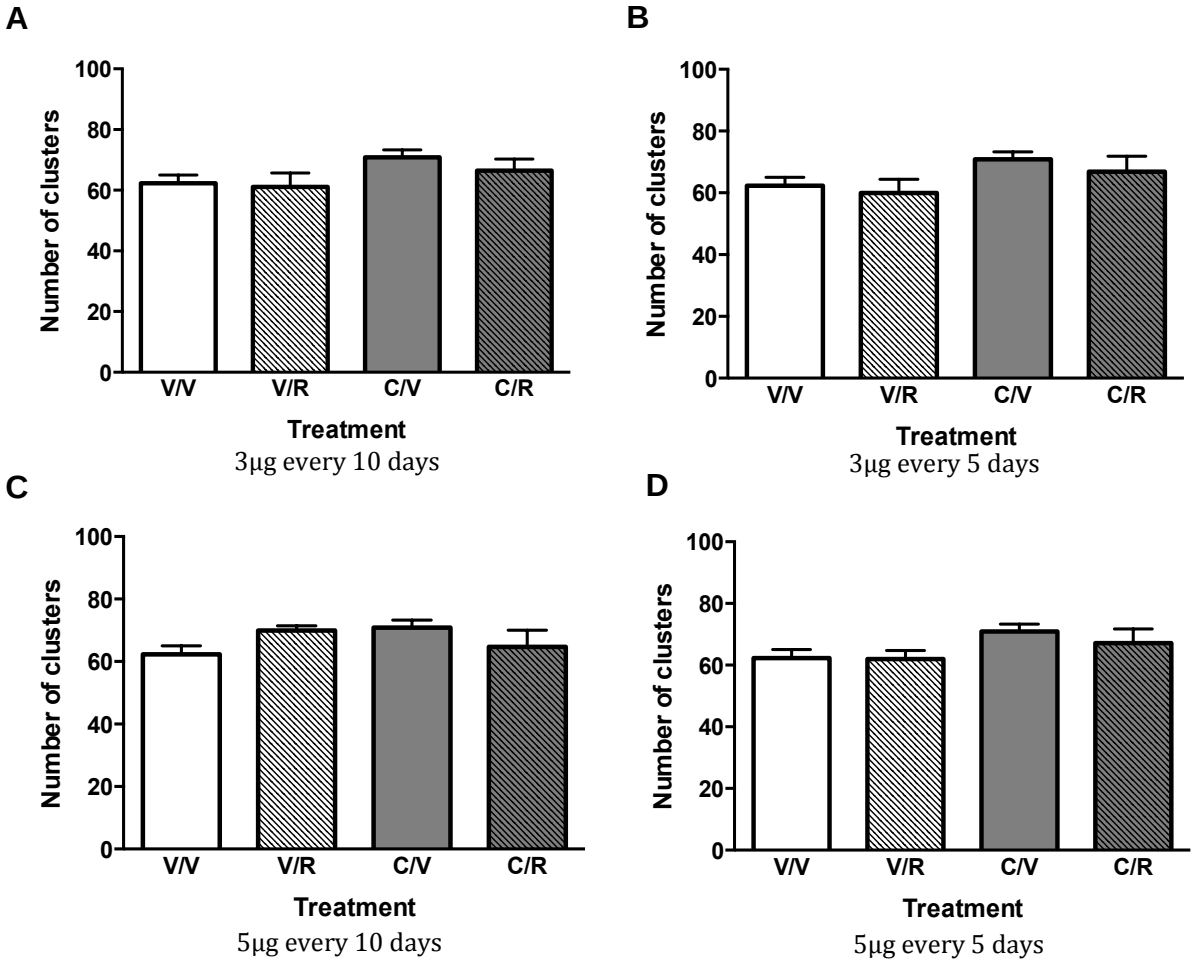

Fig 8.2. Number of SERT clusters in lymphocyte membranes according to each dosage of reelin administered. (A) 3µg/ml every 10 days. (B) 3µg/ml every 5 days. (C) 5µg/ml every 10 days. (D) 5µg/ml every 5 days. Data is presented as mean ±SEM.

These findings are significant because a similar clustering pattern is observed in patients with depression and this particular pattern is restored with common antidepressants when patients are responsive to the treatment (Rivera-Baltans et al., 2012, 2014). Hence, peripheral reelin administration appears to hold promising antidepressant potential through its regulation of MPC in lymphocytes.

3.5 DCX dendritic morphology

In addition to the analysis of neurochemical alterations in the periphery, neurochemical alterations in the CNS, particularly the hippocampus, were also measured. In rodent models of chronic stress and chronic CORT treatment, and in patients with MDD, alterations in hippocampal neurogenesis are observed. In this experiment, doublecortin was used as a marker of neurogenesis. DCX is a microtubule-associated protein that is expressed in immature neurons in the first 2-3 weeks of development. Figure 9 shows the schema devised by Plumpe and colleagues (2006), and used in the current experiment, for the categorization of DCX-positive cells in terms of dendritic morphology. In general, CORT treatment appeared to slow the maturation of DCX+ cells, with more category 1, 2, and 3 cells, and fewer category 5 and 6 cells in the CORT-injected rats.

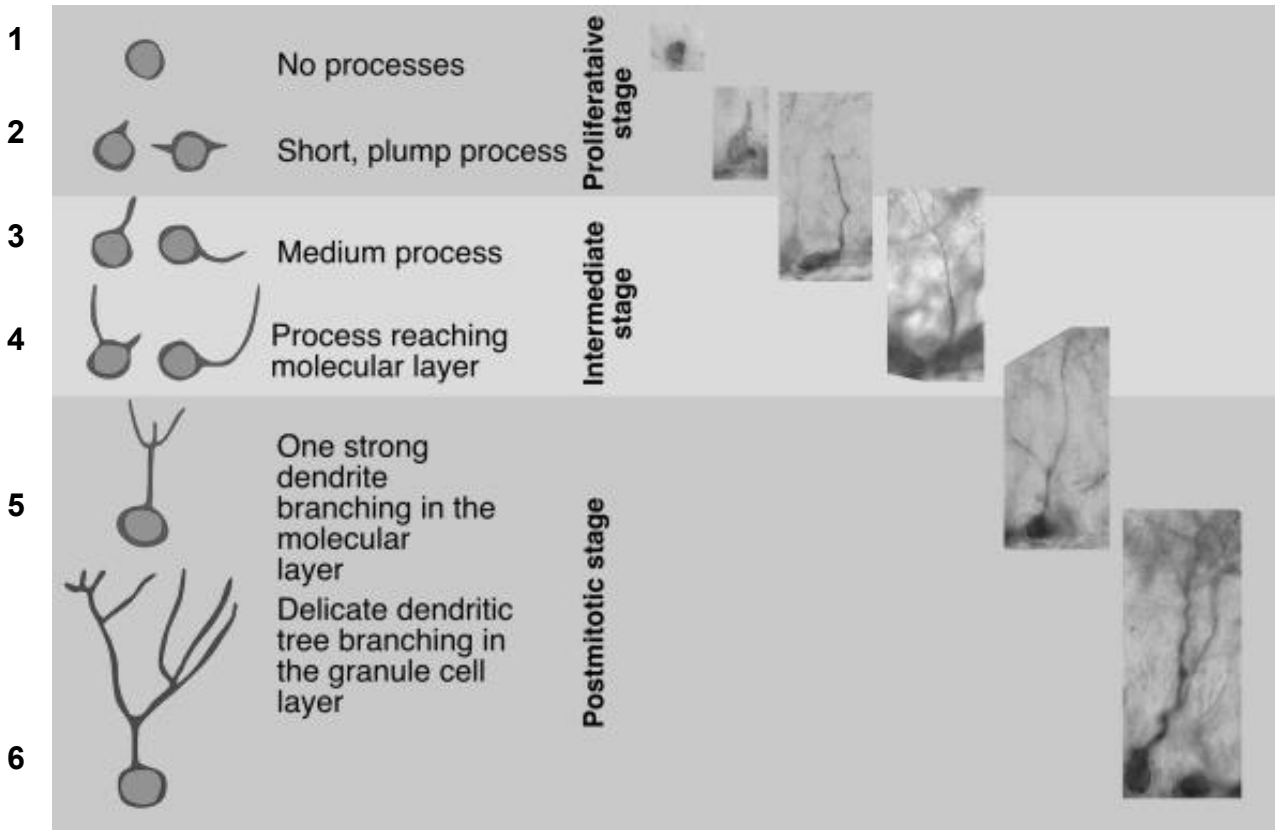

Fig 9. Categorization of dendritic morphology according to the 6 categories of dendritic complexity used in this analysis. Adapted from Plumpe et al. (2006).

A one-way ANOVA was conducted separately for each category of neuronal maturation, with experimental group as the independent variable, and % of DCX+ cells counted as the dependent variable. Results are now reported for the % of DCX+ cells counted in the SGZ, collapsed across dose, for the six categories of neuronal maturation previously described.

In category 1, which represents the most immature stage of neuronal development, there was a significant main effect of group on % of DCX+ cells, $F(3, 70) = 3.91$, $p = 0.012$. Post-hoc Tukey tests revealed that the CORT-Vehicle group showed a significant increase in the % of DCX+ cells in this immature stage of development, as compared to the Vehicle-Vehicle group, $p = 0.026$ (see Fig 10). The CORT-Reelin group also showed a significant increase in the % of DCX+ cells in category 1, as compared to the Vehicle-Vehicle group, $p = 0.013$ (see Fig 10).

In category 2, which reflects an early stage of neuronal maturation, there was again a main effect of group, $F(3, 70) = 9.00$, $p < 0.001$ (see Fig 10). Post-hoc Tukey testing showed that the CORT-Vehicle rats displayed a significant increase in the % of DCX+ cells compared to the Vehicle-Vehicle and Vehicle-Reelin rats, $ps = 0.004$ (see Fig 10). The CORT-Reelin rats also showed a significant increase in the % of DCX+ cells in category 2 as compared to the Vehicle-Vehicle and Vehicle-Reelin rats, $ps < 0.005$ (see Fig 10).

In category 3, which is considered to be an intermediate stage of neuronal development, there was a significant main effect of experimental group on % of DCX+ cells, $F(3, 70) = 16.36$, $p < 0.001$ (see Fig 10). Post-hoc testing revealed that the CORT-Vehicle group showed a significant increase in the % of DCX+ cells compared to the Vehicle-Vehicle and Vehicle-Reelin groups, $ps < 0.002$ (see Fig 10). The CORT-Reelin group showed a significant increase in the % of DCX+ cells in category 3 compared to the Vehicle-Reelin group, $p < 0.001$ (see Fig 10).

In category 4, also considered to be an intermediate stage of neuronal maturation, there was again a significant main effect of experimental group, $F(3, 70) = 3.36$, $p = 0.023$ (see Fig 10). A post-hoc Tukey test showed that the CORT-

Vehicle rats had a significant increase in the % of DCX+ cells compared to the Vehicle-Reelin rats, $p = 0.040$ (see Fig 10).

In category 5, which is regarded as a more mature stage of neuronal development, there was a significant main effect of group, $F(3, 70) = 6.14$, $p = 0.001$ (see Fig 10). Post-hoc Tukey analyses revealed that the CORT-Vehicle group had a significantly decreased % of DCX+ cells, as compared to the Vehicle-Reelin group, $p = 0.002$ (see Fig 10). The CORT-Reelin group also showed a significantly decreased % of DCX+ cells in category 5 as compared to the Vehicle-Reelin group, $p = 0.011$ (see Fig 10).

Finally, in category 6, which represents the most mature stage of neuronal development, there was a significant main effect of group, $F(3, 70) = 19.61$, $p < 0.001$ (see Fig 10). Post-hoc testing revealed that the CORT-Vehicle rats showed the expected significant decrease in the % of DCX+ cells, as compared to the Vehicle-Vehicle and Vehicle-Reelin rats, $ps < 0.001$ (see Fig 10). The CORT-Reelin rats also showed a significant decrease in the % of DCX+ cells in category 6, as compared to the Vehicle-Vehicle and Vehicle-Reelin rats, $ps < 0.001$ (see Fig 10).

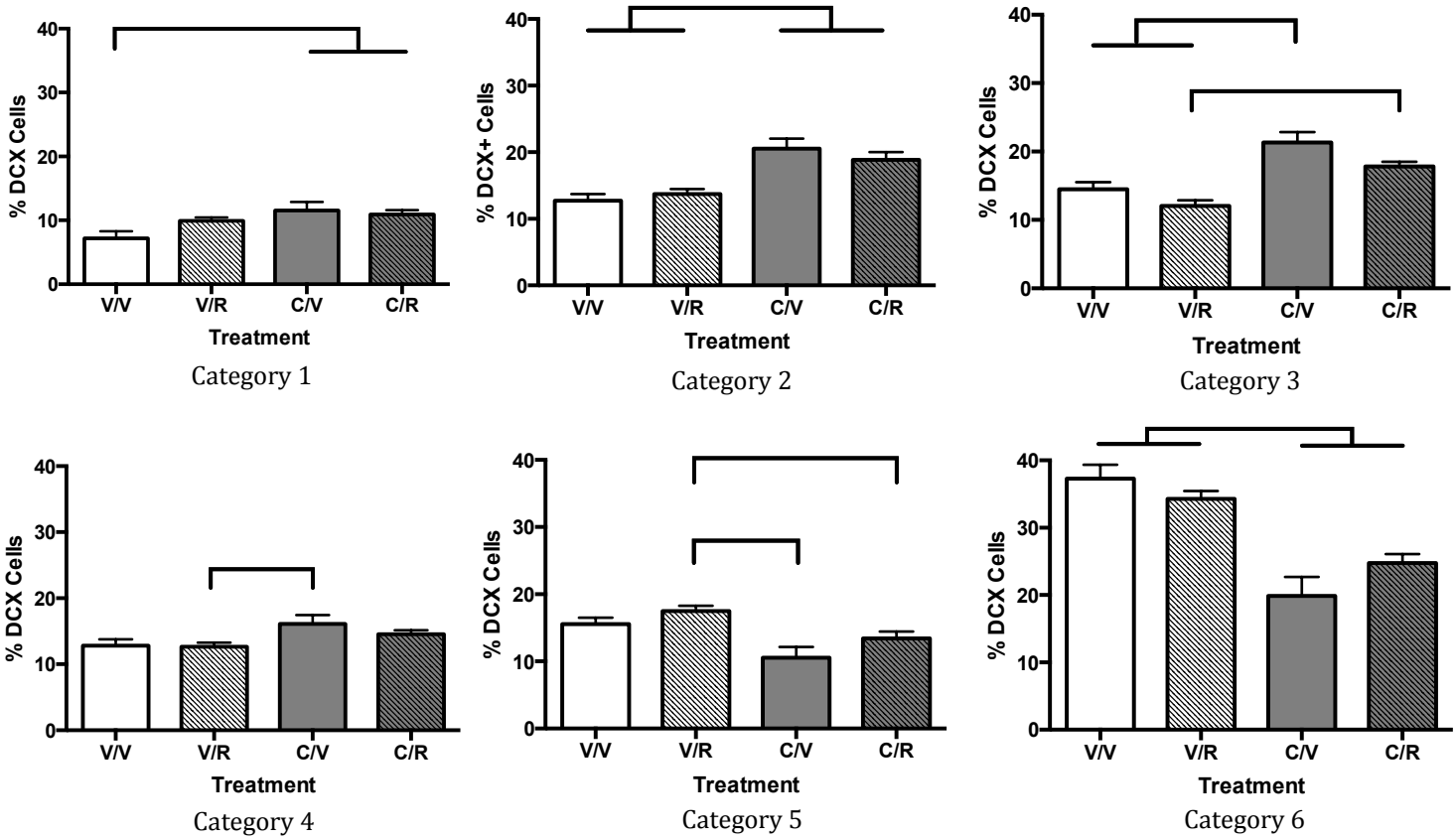

Fig 10. Percentage of Doublecortin cells counted in the subgranular zone of the dentate gyrus for categories 1-6 of neuronal maturation. Data is presented as mean ±SEM. Lines represent significantly different groups.

Figure 11 shows representative photomicrographs taken for each of the four treatment groups, demonstrating DCX+ immunolabelling in the SGZ of the DG. As can be seen in the following images, both the Vehicle-Vehicle and Vehicle-Reelin groups show extensive dendritic branching, reaching into the molecular layer of the DG. However, the CORT-Vehicle group displays significantly reduced complexity of dendritic processes extending from the SGZ into the molecular layer. CORT-Reelin rats (for all doses of reelin administered) also shows diminished dendritic branching when compared to control rats.

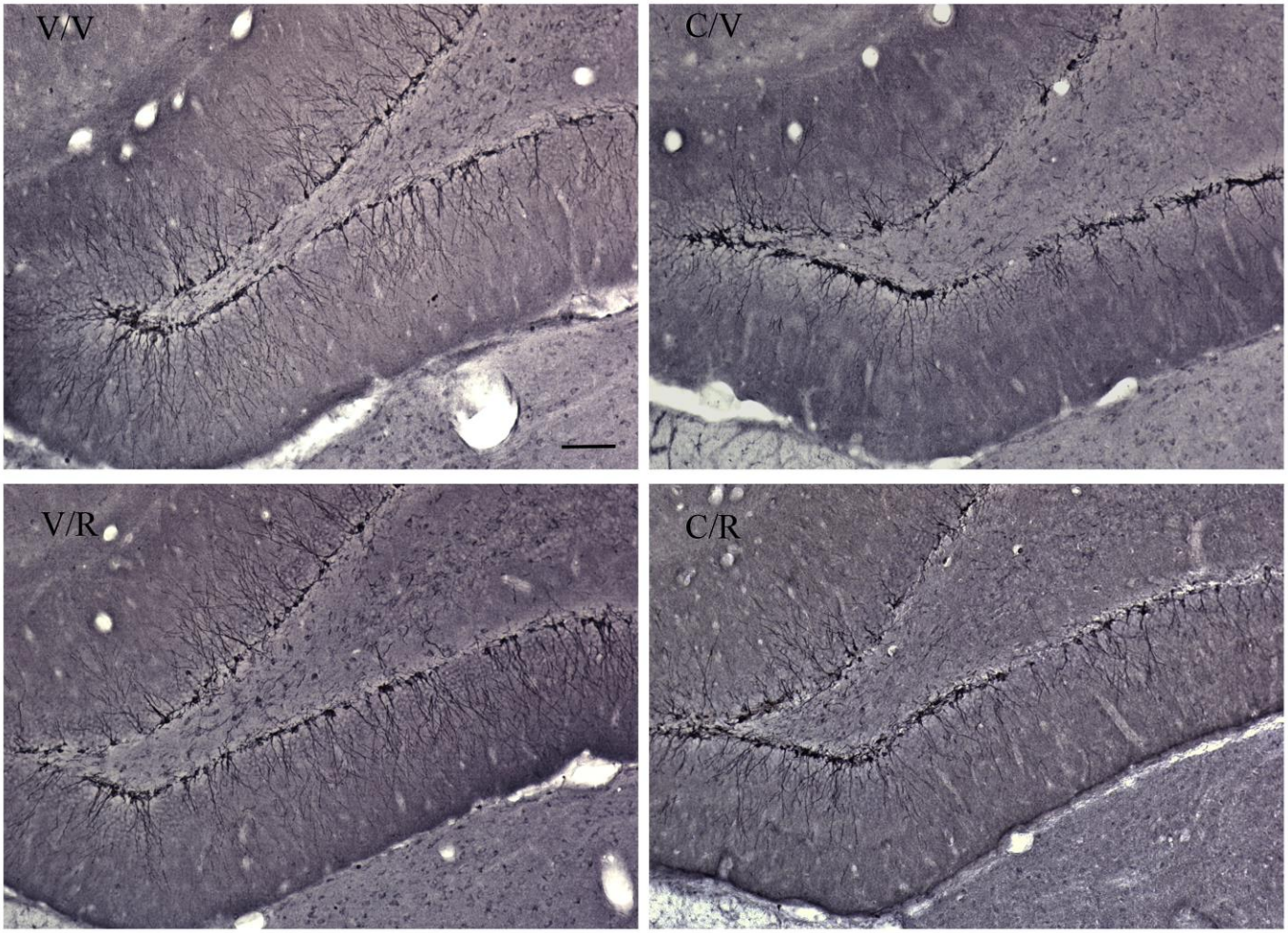

Fig 11. Photomicrographs of DCX+ immunolabelling taken at 10X magnification. (A) Vehicle-Vehicle (B) CORT-Vehicle (C) Vehicle-Reelin (D) CORT-Reelin. Scale bar represents 100µm.

To sum up the current findings, the CORT-Vehicle rats showed an increased number of cells in immature stages of development and a decreased number of cells in more mature stages of development. This result is consistent with previous findings (Lussier et al., 2011, 2013; Brymer et al., in preparation). The addition of peripheral reelin administered alongside CORT treatment shows a trend for a partial restoration of neurogenesis, as illustrated by Fig. 10, but the

CORT-Reelin group continues to display less cell maturation as compared to control groups.

4. DISCUSSION

4.1 Implications for Behavioral Reversals Following Peripheral Reelin

There is a continued need for faster-acting and more effective antidepressants. Standard antidepressant treatment is laden with poor remission rates, therapeutic delays, and significant side effects (Thase et al., 2005; Trivedi et al., 2006; Gaynes et al., 2009; Stahl, 2000). The extracellular matrix protein, reelin, has received attention for its association with mood and psychiatric disorders, including major depression. When administered directly into the brain, chronic and acute reelin infusions have proven efficacious in restoring behavioral and neurobiological CORT-induced deficits (Brymer at al., in preparation). The present study aimed to determine whether peripherally-injected reelin holds antidepressant-like action in terms of behavioral and neurochemical parameters. Consistent with previous findings (Kalynchuk et al., 2004; Gregus et al., 2005; Johnson et al., 2006; Lussier et al., 2011, 2013; Lebedeva et al., 2017), 21 days of CORT treatment produced a depressive phenotype as measured by immobility and swimming behaviors in the forced swim test. Importantly, the addition of exogenous reelin restored the CORT-induced behavioral deficits to that of control rats, which is similar to reversals seen with other antidepressants (Fenton et al., 2015; Rainer et al., 2012). With respect to the CORT-Vehicle rats, a full restoration in immobility behavior was seen with addition of reelin at all doses except for 5µg/ml every 10 days. Rats that received 5ug/ml of reelin every 10 days did not display significantly reduced immobility behavior, as compared to the CORT-Vehicle rats. This finding is somewhat surprising, because rats that received lower doses of reelin (i.e., 3µg/ml every 10 days = 3µg*3 injections = 9µg in total) and higher doses of reelin (i.e., 5µg/ml every 5 days = 5µg*5 injections = 25µg in total) displayed complete immobility reversals. Notably,

however, the 5µg/ml every 10 day group did not differ from vehicle groups, evidencing at least a partial reversal in immobility behavior.

In regards to swimming behavior, the CORT-treated rats spent significantly less time actively swimming than control rats, consistent with previous findings. CORT-Reelin rats that received both 3µg/ml every 5 days and 5µg/ml every 10 days spent significantly more time swimming than CORT-Vehicle rats, evidencing a complete reversal in the behavioral phenotype. However, CORT-treated rats that received reelin at both 3µg/ml every 10 days and 5µg/ml every 5 days did not significantly differ from CORT-Vehicle rats. In this case, the lowest dose of reelin (3µg/ml every 10 days) and the highest dose of reelin (5µg/ml every 5 days) administered did not produce a complete reversal in swimming behavior, while both intermediate doses (i.e., 3µg/ml every 5 days = 3µg*5 injections = 15µg in total and 5µg/ml every 10 days = 5µg*3 injections = 15µg in total) did. This effect does not appear to be a result of frequency of administration because the two different frequencies used (every 5 days and every 10 days) were both successful in fully reversing the behavioral phenotype. Nonetheless, rats administered reelin at 3µg every 10 days and 5µg every 5 days did not significantly differ from vehicle groups, indicating a partial reversal in swimming behavior.

In addition to the FST, the OFT was included as a behavioral index of locomotor activity and anxiety-like behavior. This test was included in order to strengthen interpretation of forced swim test findings. Prior to the current study, the impact of peripherally-injected reelin on behavior, including depression-like behavior, anxiety-like behavior, and more general behaviors, such as locomotor activity, was not known. Therefore, we wanted to assess whether peripheral reelin has an influence in other behavioral domains that could qualify our behavioral findings in the forced swim test. Results demonstrated no effect of either CORT or reelin on distance travelled (an index of locomotor activity) or duration spent in the inner zone of the open field arena (an index of anxiety-like behavior) in the OFT. This finding corroborates the interpretation that the general reversals in immobility and swimming behaviors in CORT-Reelin rats, as

compared to CORT-Vehicle rats, are attributable to a depressogenic effect of reelin, rather than an indirect result of reelin-induced increases in locomotor or anxiety-like behaviors. It is also important to note that no differences were found between the Vehicle-Reelin and Vehicle-Vehicle groups on any of the forced swim or open field behavioral measures, demonstrating that at the doses of reelin administered, there were no negative effects of exogenous reelin in healthy rats under basal conditions.

In line with previous findings reported by other investigators, CORT injections at 40mg/kg administered for 21 consecutive days decreased body weight gain. This significant reduction in weight gain in CORT-treated rats leads to the question of whether the behavioral changes observed in the forced swim test may be a non-specific result of reduced body weight (and concomitant decrease in muscle mass) rather than a direct depressogenic effect of CORT. For instance, it may be argued that CORT-treated rats spend less time swimming and more time immobile due to reduced muscle mass. However, two observations suggest that this is not the case. First, CORT-injected rats do not display differences in their locomotive activity as compared to control rats, as evidenced by similar levels of open field exploration between CORT-injected and Vehicle-injected rats in the open field test, a finding that has been previously reported (Gregus et al., 2005; Brotto et al., 2001; Kalynchuk et al., 2004; Marks et al., 2009). To further corroborate this observation, previous reports demonstrate that rats treated with repeated CORT show similar swim distances to control rats (Sousa et al., 2000). Second, the rescue of CORT-induced increases in immobility behavior in the FST by peripheral reelin did not co-occur with increases in body weight gain. Specifically, peripheral reelin at all doses administered did not have an effect on body weight gain, with both CORT-Vehicle and CORT-Reelin rats demonstrating similarly attenutated weight gain throughout the CORT injection period. Thus, this demonstrates a behavioral restoration following reelin administration that cannot be attributed to an indirect effect of reelin on weight gain. This is particularly significant as provides further support for the CORT model as a valid model for measuring the depressogenic effects of repeated CORT in the FST, while also

supporting the interpretation that reelin is impacting underlying neural circuitry involved in a depression-like state, and not simply affecting depression-like behavior via unspecific actions on body weight gain.

Hence, the behavioral changes in the FST following CORT treatment cannot be attributed to changes in weight gain, but rather support the interpretation for a depressogenic effect of repeated CORT and the antidepressant-like action of peripheral reelin administration.

4.2 Implications for MPC Clustering Restorations Following Peripheral Reelin

Reelin is not only expressed in the brain, but also in peripheral tissues and in blood plasma (Smalheiser et al., 2000), though its roles here are much less understood than in the central nervous system. However, reelin-deficient mice show an altered pattern of protein clustering along the cell membrane of peripheral blood lymphocytes; specifically, an increase in the size and number of serotonin transporter clusters on lymphocyte membranes has been reported (Rivera-Baltanas et al., 2010). In the current experiment, we were motivated to examine SERT cluster size on lymphocyte membranes because not only is it altered in reelin-deficient mice, but both CORT-treated rats displaying depression-like behavior and patients with depression show a similar size increase in SERT clusters (Fenton et al., 2015b; Rivera-Baltanas et al., 2012). We found that CORT-treated rats showed a significant increase in the size of SERT clusters, as compared to control rats, with no change observed in the number of clusters. This effect was completely rescued with the addition of peripherally-injected reelin at all dosages administered. Hence, the CORT-induced increases in the size of SERT clusters in lymphocyte membranes, a pattern also seen in patients with depression, were successfully rescued by peripheral reelin; furthermore, this rescue in SERT clustering corresponds with behavioral reversals on the FST. These results suggest that peripheral reelin administration holds promise for benefiting immune and inflammatory outcomes through its effects on SERT clustering in lymphocytes and the associated optimization of SERT functioning, as well as the associated regulation of pro-

inflammatory cytokine release. The altered pattern of SERT clustering in lymphocyte membranes of rats treated with CORT presents with an increase in depression-like behavior, suggesting that the development of a depressive phenotype may be partially mediated by peripheral SERT activity. Moreover, the restoration by reelin of the altered SERT clustering pattern is concomitant with a rescue of FST behavior, suggesting that the antidepressant action of reelin may also be partially mediated through peripheral SERT activity. It is important to note that the addition of reelin in Vehicle-treated rats did not impact on the size of SERT clusters, indicating that reelin holds a restorative role when glucocorticoid levels are high, regulating the membrane clustering activity of SERT under pathophysiological conditions, and not under normal physiological conditions, thus demonstrating the potential therapeutic efficacy of exogenous reelin administration in the treatment of depressive symptomology.

4.3 Effects of Peripheral Reelin on Hippocampal Neurogenesis

One of the leading hypotheses in the context of depression proposes that prolonged stress produces hippocampal remodeling, specifically reductions in hippocampal neurogenesis, that play a casual role in the development of depression (Jacobs et al., 2000). Findings of reduced hippocampal volume in patients with depression (MacQueen et al., 2003; Campbell et al., 2004), as well as reductions in cell proliferation and survival in animal models of depression which are restored following antidepressant treatment (Santarelli et al., 2003), lend support to this hypothesis. Previous research has shown that reelin positively regulates adult hippocampal neurogenesis (Kim et al., 2002; Pujadas et al., 2010), whereas chronic CORT treatment produces potent reductions in the survival and maturation rates of newborn neurons (Lussier et al., 2011; 2013; Fenton et al., 2015). Furthermore, it has been previously shown that both chronic and acute intra-hippocampal reelin infusions (1µg/ul) completely reverse CORT-induced FST immobility impairments and associated decreases in the dendritic complexity of immature granule cells in the hippocampus (Brymer et al., in preparation).

The current results are consistent with findings from previous studies (Fuchs and Gould, 2000; Pham et al., 2003; Santarelli et al., 2003; Sapolsky, 2004; Sheline et al., 1999; Starkman et al., 1992; Surget et al., 2008, 2011; David et al., 2009; Wong and Herbert, 2006, 2004; Tanti et al., 2013; Ramirez et al., 2015), showing that many forms of chronic stress, including chronic CORT treatment, cause impairments in hippocampal neurogenesis. CORT-treated rats showed a significant increase in the percentage of DCX+ cells in categories 1, 2, 3, which represent the most immature stages of neuronal development, as compared to vehicle rats, and a decreased percentage of DCX+ cells in category 5, when compared to Vehicle-Reelin rats and category 6, when compared to both Vehicle-Vehicle and Vehicle-Reelin rats, which represent the most mature stages of neuronal development.

However, our results also demonstrate that peripheral reelin did not restore the CORT-induced reductions in dendritic complexity. The CORT-Reelin rats showed a similar pattern of neuronal maturation to the pattern seen in the CORT-Vehicle rats. Similar to CORT-Vehicle, the CORT-Reelin rats also had a significantly increased percentage of DCX+ cells in categories 1, 2, and 3, as compared to vehicle rats. The CORT-Reelin rats also showed a significantly decreased percentage of DCX+ cells in category 5, when compared to the Vehicle-Reelin rats and in category 6, when compared to both the Vehicle-Vehicle and Vehicle-Reelin rats. Hence, results of the DCX analyses show that under conditions of elevated levels of glucocorticoids, peripheral reelin does not induce any significant reversals in the disrupted maturation rate of immature neurons in the hippocampus.

5. CONCLUSIONS

In summary, peripheral reelin administration reversed depression-like behavior, as measured by the FST, while having no effect on OFT behavior. This behavioral restoration by peripheral reelin may occur secondarily through brain changes upon crossing of the BBB, but reelin-related restorations of CORT-induced alterations in the SERT clustering pattern suggest that reelin's activity in

the periphery partially subserves its antidepressant-like effects, perhaps through its effects on immune system plasticity in the presence of heightened glucocorticoid exposure. This will be an important avenue for continued exploration, but the current findings demonstrate that peripheral reelin administration holds strong potential for facilitating stress resilience and protecting against the development of depressive symptomology.

CHAPTER 3
General Discussion

1. Overview of Main Findings

The current experiment aimed to extend further support for the existent literature demonstrating that chronic CORT treatment leads to the development of depressive symptomology that is often accompanied with hippocampal alterations, namely reductions in hippocampal cell proliferation and maturation, which have been consistently observed using the CORT model. In addition to utilizing the CORT model as a rodent model of depression, the current research aimed to investigate the effects of the novel administration of peripheral reelin via the lateral tail vein in order to explore whether its antidepressant properties previously demonstrated by way of direct brain infusion extend to its less invasive peripheral administration. At this time, no other known research groups have peripherally injected reelin to explore its potential antidepressant-like effects. In addition, much less is known about peripheral reelin activity than about its roles in the central nervous system. Demonstration by way of peripheral reelin administration of a restoration in depression-like behavior brought about by repeated CORT would be a novel finding indicating that exogenous reelin administration holds promising potential for therapeutic efficacy. To this end, four different dosages of reelin were administered as a preliminary investigation into its therapeutic potential: 3µg/ml every 10 days, 3µg/ml every 5 days, 5µg/ml every 10 days, and 5µg/ml every 5 days.

Another aim of the current experiment was to expand on the existent literature investigating alterations in the membrane protein clustering pattern of the serotonin transporter on peripheral lymphocytes, which has been observed with repeated CORT treatment, and relates to similar alterations seen in patients with depression. We were motivated to determine whether the intravenous administration of reelin could restore any CORT-induced changes in the SERT clustering pattern that might present with CORT-induced increases in

depression-like behavior. This avenue of research would begin to open up investigation into the mechanisms underlying reelin's antidepressant potential.

Finally, previous findings have demonstrated that direct infusion of reelin into the hippocampus, a brain structure particularly susceptible to the negative effects of glucocorticoids, completely reversed the CORT-induced deficits in the dendritic complexity of immature dentate granule cells (Brymer et al., in preparation). We wanted to examine whether the administration of exogenous reelin into the lateral tail vein could similarly reverse the hippocampal impairments brought about by repeated CORT exposure, and possibly elucidate one of the underlying mechanisms for reelin's potential antidepressant-like effects.

2. Effects of Chronic CORT Exposure and Addition of Exogenous Reelin on Depression-like Behavior

Behavioral findings demonstrated the anticipated increase in depression-like behavior brought about by repeated CORT, as measured by the FST. Rats given 40mg/kg CORT once daily for 21 consecutive days spent significantly more time immobile and less time actively swimming in the FST than rats given daily injections of saline. This increase in passive FST behavior displayed by CORT-treated rats as compared to control rats is considered to reflect behavioral despair and the presence of a depressive phenotype, and is consistent with findings from other chronic stress and chronic CORT administration paradigms that utilize the FST as a measure of depression-like behavior. Importantly, in CORT-treated rats, the addition of exogenous reelin at 3µg/ml every 10 days, 3µg/ml every 5 days, and 5µg/ml every 5 days, fully restored the immobility behavioral phenotype in the FST to levels of control rats. This reversal of CORT-induced depression-like behavior following reelin injections, is akin to reversals seen with other antidepressants (Fenton et al., 2015; Rainer et al., 2012). It is not clear as to why rats that received the 5µg/ml every 10 day dosage did not display significantly reduced immobility behavior, as was seen with the other three dosages, but given that this dosage still did not differ from vehicle groups, at

least a partial reversal in immobility behavior is evidenced. Results from the swimming behavioral measure of the FST show less of a consistent and robust pattern when compared to the immobility measure. However, for all of the dosages administered, CORT-treated rats spent significantly less time actively swimming than the vehicle group, which is again consistent with previous findings using the FST as a measure of learned helplessness. Importantly, at 3µg/ml every 5 days and 5µg/ml every 10 days, reelin injections in CORT-treated rats caused a complete reversal in the behavioral phenotype, such that CORT-Reelin rats spent significantly more time swimming than CORT-Vehicle rats. However, this difference was not observed in CORT-treated rats that received reelin at both 3µg/ml every 10 days and 5µg/ml every 5 days. Again, the reason for this distinction is unclear, but CORT-Reelin rats demonstrated comparable swimming behavior to vehicle groups at all dosages, indicating a partial or complete reversal in all cases.

The OFT was administered as a second behavioral measure in order to examine changes in locomotor and anxiety-like behavior following CORT treatment and treatment with exogenous reelin. Findings are mixed in the existent literature regarding both stress and CORT effects on anxiety-like behavior in the open field test, with some paradigms showing stress-induced increases in locomotor and anxiety-like behavior (David et al., 2009; Skorewska et al., 2006; Ivy et al., 2008), while others show no changes (Lussier et al., 2011; Marks et al., 2009; Gregus et al., 2005; Kalynchuk et al., 2004; Lebedeva, unpublished data). For the purpose of the current experiment, it was important to investigate whether reelin injections could induce changes in locomotor activity or anxiety-like behavior that might confound interpretation of the depression measures evaluated using the FST. The total distance the rat traveled in the 5-min test period served as a simple measure of locomotor activity. Results demonstrated no effect of either CORT or reelin on locomotor behavior in the OFT. This finding is important because it supports the interpretation that the decreased immobility time displayed by CORT-Reelin rats as compared to CORT-Vehicle rats, is due to a precise influence of reelin activity on the

development of depressive symptomology, rather than a general effect of reelin on locomotor activity. The duration of time that rats spent in the center zone of the arena in the 5-min OFT period was used to evaluate the presence of anxiety-like behavior. Similar to that observed for total distance travelled, there was no effect of either CORT or reelin on this measure. Hence, addition of exogenous reelin in otherwise normal physiological conditions does not lead to increases in anxiety-like behavior as measured by the OFT. While future research should incorporate additional tests that measure anxiety-like behavior in order to corroborate this finding, the current results are important in providing initial support for the proposal that reelin holds antidepressant potential in the absence of additional negative side effects.

There is a continued need for the development of faster-acting and more efficacious antidepressant treatments, given that less than 1/3 of patients with depression experience remission with standard antidepressant treatment, and only 50% achieve remission with implementation of two different pharmacotherapies for 6 months (Thase et al., 2005; Trivedi et al., 2006). Not only does it take approximately 3 weeks for patients to experience therapeutic efficacy with common antidepressants (Stahl, 2000), but many patients experience significant negative side-effects which introduces significant discouragement for the continuation of treatment. For such reasons, the current research has attempted to address an important and necessary limitation in pharmacotherapy development for depression. Current behavioral findings provide promising support for investigation into this novel avenue of antidepressant development.

As mentioned, the functional activity of reelin in the periphery is much less understood than its roles in the brain and during development of the central nervous system. However, reelin is expressed in blood plasma (Smalheiser et al., 2000), and plasma levels of reelin have been shown to be altered in mood and psychotic disorders (Fatemi et al., 2001a). In reelin-deficient mice, the clustering of proteins along the cell membrane has been shown to be altered in peripheral blood cells (i.e., lymphocytes). Specifically, Rivera-Baltanas et al.,

(2010) observed alterations in the size and number of serotonin transporter clusters on lymphocyte membranes in both HRM and reeler mice, which was particularly evident in the reeler mice, which exhibit null reelin expression. Moreover, rats treated with CORT for 3 weeks also show an altered pattern of serotonin transporter and serotonin 2A receptor clustering on lymphocyte membranes, as compared to control rats. An increase in FST depression-like behavior in these CORT-treated rats positively correlates with larger membrane protein clusters (Fenton et al., 2015b). Lymphocytes from patients with depression show a very similar increase in the size of serotonin transporter and serotonin 2A receptors along the plasma membrane (Rivera-Baltanas et al., 2012). Given these findings, we were motivated to examine whether addition of exogenous reelin could reverse the altered pattern of SERT clustering in lymphocytes induced by repeated CORT treatment. In line with previous studies, we found that CORT-treated rats showed a significant increase in the size of SERT clusters, as compared to control rats, with no change observed in the number of clusters. Importantly, when reelin was given to CORT-treated rats, a complete reversal in the size of SERT clusters was observed. Furthermore, this reversal was found for all dosages of reelin administered. These findings are significant because they show for the first time that peripheral reelin can reverse the pattern of SERT membrane protein clustering observed in CORT-treated rats displaying depression-like behavior – the same pattern that is exhibited in patients with depression. This observation also suggests that the antidepressant-like action of peripheral reelin in our CORT model of depression may be at least partially mediated by reelin's regulation of SERT clustering in peripheral lymphocytes. While future studies are required to further investigate the effects of exogenous reelin in peripheral cells, this initial and novel finding suggests that peripheral reelin may hold therapeutic efficacy for the treatment of depression by way of restoring altered protein expression in depressed patients.

In addition to investigating the effects of reelin administration in peripheral lymphocytes, we also aimed to explore whether peripheral reelin administration could affect aspects of brain structure and function, particularly in the

hippocampus, where it is known to hold important functional roles. Specifically, we wanted to examine whether peripheral reelin could partially restore CORT-induced deficits in hippocampal newborn neuron maturation rate that have been previously observed (Lussier et al., 2011, 2013). Previous research has evidenced a facilitative role for reelin in hippocampal neurogenesis (Kim et al., 2002; Pujadas et al., 2010) and previous findings from our lab have demonstrated that both chronic and acute intra-hippocampal reelin infusions (1µg/ul) completely reverse CORT-induced FST immobility impairments and associated decreases in the number and maturation rate of newborn neurons, when rats are sacrificed one week after the last reelin infusion (Brymer et al., in preparation). An observation of even partial restoration in neurogenesis with addition of reelin would provide evidence that exogenous administration of reelin into the bloodstream is capable of producing structural and functional consequences for brain functioning, whether by direct crossing of the BBB, or indirectly through other mechanisms. To this end, DCX, which is highly expressed in immature neurons in the first 2 weeks of development, was used as a marker of neurogenesis. Results showed the anticipated effect of chronic CORT treatment on hippocampal neurogenesis. CORT-treated rats showed a significant increase in the percentage of DCX+ cells in categories 1, 2, 3, which represent the earlier stages of neuronal development, as compared to vehicle rats, and a decreased percentage of DCX+ cells in category 5, when compared to Vehicle-Reelin rats and category 6, when compared to both Vehicle-Vehicle and Vehicle-Reelin rats, which represent the most mature stages of neuronal development. These results are in line with many previous studies demonstrating that chronic stress and chronic CORT treatment produce robust reductions in hippocampal cell proliferation and maturation in rodents (Fuchs and Gould, 2000; Pham et al., 2003; Santarelli et al., 2003; Sapolsky, 2004; Sheline et al., 1999; Starkman et al., 1992; Surget et al., 2008, 2011; David et al., 2009; Wong and Herbert, 2006, 2004; Tanti et al., 2013; Ramirez et al., 2015; Lussier et al., 2011, 2013). The results are also not surprising given that the hippocampus is particularly susceptible to the negative effects of chronic stress. The

hippocampus contains a great number of glucocorticoid receptors and as mentioned earlier, there is evidence implicating a role for neurogenesis in the maintenance of inhibitory control over the HPA axis; neuronal projections from immature neurons of the ventral dentate gyrus ultimately inhibit neurons in the PVN to prevent the release of CRF. Reductions in neurogenesis have been shown to further elevate levels of glucocorticoids and exacerbate reductions in new neuron birth (Schloesser et al., 2009; Snyder et al., 2011).

The examination of hippocampal neurogenesis was motivated by the goal of determining whether reelin administered peripherally could have effects on brain functions which have been implicated in depression, and may contribute to reelin's antidepressant effects. Results of the DCX analysis demonstrate that peripheral reelin did not restore the CORT-induced reductions in dendritic complexity. CORT-Reelin rats showed a similar pattern of neuronal maturation as CORT-Vehicle rats. Specifically, CORT-Reelin rats had a significantly increased percentage of DCX+ cells in categories 1, 2, and 3, as compared to vehicle rats. CORT-Reelin rats also showed a significantly decreased percentage of DCX+ cells in category 5, when compared to Vehicle-Reelin rats and category 6, when compared to both Vehicle-Vehicle and Vehicle-Reelin rats. In spite of this observation, CORT-Reelin rats do show a slight increase in the percentage of DCX+ cells in the more advanced stages of neuronal maturation, (i.e., in categories 5 and 6), as compared to CORT-Vehicle rats, though this does not reach significance. It is possible that some reelin crossed the BBB to affect dendritic maturation in immature neurons, but was unable to produce any significant changes at the dosages given. While it cannot be determined based on the results of this experiment, it is unlikely that the antidepressant-like effects of reelin observed on FST behavior are due to an involvement of peripheral reelin in the maturation of newborn neurons in the hippocampus. In order to investigate this in the future, radioactive-tagging of the recombinant reelin protein administered exogenously should be considered, as it would allow for the determination of whether injected reelin is capable of crossing the BBB.

As discussed earlier on, reelin-deficient mice are more susceptible to the depressiogenic effects of CORT (Lussier et al., 2011), overexpression of reelin has been shown to protect against the development of a depressive phenotype (Teixeira et al., 2011), and direct infusion of reelin into the hippocampus restores behavioral and neurochemical deficits induced by chronic CORT treatment (Brymer et al., in preparation). The current behavioral, and neurochemical findings observed in the periphery support this previous research and a protective role for peripheral reelin in the face of deleterious stress effects, providing evidence for reelin's antidepressant potential.

3. Limitations

3.1 Validity of the Repeated Exogenous CORT Model for the Study of Depression

Many studies have established the exogenous CORT model as a valid tool for recapitulating the depressive phenotype characteristic of human depression. Nonetheless, the CORT model cannot provide a fully realistic simulation of the human disorder. One of the limiting factors of the CORT paradigm is that the artificial administration of exogenous CORT does not resemble the range of stressful experiences that take place in daily life. Furthermore, the 40mg/kg dose of CORT that is needed to reliably and consistently produce a depressive phenotype is supraphysiological, since this level of glucocorticoid release is not produced by natural stressors (Johnson et al., 2006).

Such limitations are not unique to the CORT model, however; the human experience of depression is very difficult to reproduce and other stress models suffer similar shortcomings. For instance, repeated restraint stress consistently enforces a single physical stressor, to which animals readily adapt, and which does not reflect the variety of stressor types encountered in daily life. Due to the progressive habituation that occurs in the chronic restraint model, extensive variability between animals can often occur, and sometimes depression-like behaviors are not observed at all (Lussier et al., 2009; Gregus et al., 2005; Dunn

& Swiergiel, 2008). A 10-year review provides evidence that the preclinical CMS model can most adequately capture the human experience of depression (Willner, 1997), but this model is limited with difficulties of implementation, and is still subject to individual differences in stress susceptibility and HPA axis response. An advantage of the chronic exogenous CORT model is that it does not carry the same problems of stressor habituation and individual variability in stress response. Importantly, the repeated CORT model produces similar impairments in behavior, neuronal morphology, and neurobiology as is seen in human patients and in other stress models.

In choosing an animal model of depression it is necessary to consider the research questions that need to be addressed, and which model can most efficiently address them. In the current experiment, we wanted to replicate the direct effects of exogenous CORT on depression-like behavior and neurochemical factors that have been previously observed. In using this approach, we could then simply and effectively evaluate the effects of reelin on CORT-induced behavioral and neurochemical changes, devoid of any habituation and psychosocial factors that could confound any effects of reelin we may observe. Importantly, use of the exogenous CORT model allowed us to effectively address this research aim.

3.2 Sex-Differences in Depression-like Behavior

Another limitation of the present study is the sole use of male Long-Evans rats for all testing procedures, thus limiting the generalizability of the current findings to the human population. This is particularly relevant given that female rats have been shown to have higher basal and stress-induced levels of circulating glucocorticoids compared to male rats (Mashoodh et al., 2008). As well, both glucocorticoid levels in females and processes of neuronal plasticity, including dendritic density of CA1 pyramidal neurons, depend upon stage in the estrous cycle (Carey et al., 1995; Shupnik, 2002; Wooley et al., 1990; Shors et al., 2001). Sex differences in behavior have also been observed following exogenous CORT treatment. There is evidence to suggest that female rats

confer greater stress resilience compared to male rats, as they display less depression-like behavior in the FST than males after chronic CORT exposure (Kalynchuk et al., 2004). This is a relevant limitation of the current study, because extensive literature reveals that women in their reproductive age are overrepresented in depression, the prevalence being approximately 2-3 times higher in women than men (Nolen-Hoeksema, 1987; Young et al., 1990; Kessler et al., 1993; Williams et al., 1995; Korstein, 1997; Bracke, 1998; Maier et al., 1999; Frackiewicz et al., 2000). This higher prevalence normalizes once women reach menopause (Weissman & Olfson, 1995), suggesting that ovarian hormones throughout reproductive life play a role in the sex difference, as has been stress models incorporating female rodents. As such, it is important that preclinical findings can generalize to female patients as well as males.

Given that the cyclic release of endogenous female sex hormones adds an extra, sensitive factor to the CORT model, we did not include female rodents in this preliminary investigation into the antidepressant potential of peripheral reelin administration. Yet, as mentioned, female rats have been shown to be differentially affected by CORT treatment compared to males, and as such may show a differential response to reelin injections in the presence of high circulating glucocorticoid levels. This limitation will need to be addressed in future studies in order to investigate reelin's potential antidepressant actions in the opposite sex, and the ways in which exogenous reelin activity may be differently mediated.

3.3 Validity of the Forced Swim Test for Measuring "Behavioral Despair"

One of the major limitations concerning the FST as a measure of depression-like behavior in rodents, is whether this behavioral despair test first developed by Porsolt and colleagues in 1977 actually measures what it claims to. As discussed, many paradigms that employ 'depressogenic' manipulations, such as chronic stress and chronic levels of glucocorticoid induction, increase the amount of time rats spend immobile, speed the onset to immobility, and decrease the amount of time rats spend actively trying to escape (Bielajew et al., 2003; Cancela et al., 1991; Molina et al., 1994). Furthermore, this stress-induced

condition of immobility that mice and rats assume in the face of escape failure, termed “behavioral despair”, has been consistently prevented with different antidepressant treatments (Porsolt et al., 1977, 1978; Borsini et al., 1989; Cryan et al., 2005; Detke et al., 1997), yet some criticisms have been made regarding the FST as a test of behavioral despair indicative of a depressive phenotype.

As discussed earlier, the FST protocol used by Porsolt and colleagues (1977) included a pretest immersion trial followed by a subsequent immersion test. During the immersion test, Porsolt found that the onset to immobility was much more rapid, interpreted as “behavioral despair”, with the reasoning that the animal had realized escape was impossible. However, there is also evidence to suggest that the immobility behavior displayed by mice and rats in the FST is not reflective of “despair”. O’Neill and Valentino (1982) questioned this assumed relationship between inescapability and immobility when they found that there was no difference in immobility time during the test period between animals which had been able to escape during the pretest and those which had not been able to. Borsini and colleagues (1986) used Porsolt’s version of the forced swim test to show that rats behave quite differently depending on the levels of water (or no water) used in both pretest and test sessions. Regardless of the pretest condition, immobility time was not affected during the test period when rats were placed in just 4 cm of water, a finding that is in opposition to the learned helplessness, and hence “despair”, that Porsolt argued would be produced. Instead, Borsini et al. (1986) suggest that it is familiarity with the environment rather than despair that affects immobility, since even rats that were pre-exposed to an empty cylinder prior to test spent more time immobile in 15 and 30 cm of water as compared to rats that were not pre-exposed. In spite of this, familiarity with the environment does not appear to be the only factor, since no difference in immobility was found during test in 4 cm of water between rats with prior environmental experience and those with none. Instead, it is suggested that the presence of “fear” or a “life-threatening experience” in rats exposed to greater water depths plays a role in immobility time, since regardless of pretest condition rats exposed to 15 and 30 cm of water climbed, jumped, and dived the most, with

the most vigorous activity seen in the 30 cm water condition. These results offer an alternative interpretation to immobility behavior in the FST – one that suggests immobility in water is caused by a feeling of danger in a recognizable environment, rather than a sense of despair – and are supported by research proposing immobility behavior as an adaptive stress response (Hawkins et al., 1978). While it is important to remain critical in the interpretation of animal behavioral measures that attempt to model complex aspects of human disorders, the fact that the FST is consistently responsive to multiple classes of antidepressant drugs and non-pharmacological antidepressant treatments, including transcranial magnetic stimulation, REM sleep deprivation, and electroconvulsive shock (Borsini & Meli, 1988), supports its validity in the development of novel antidepressants. Future work should continue to explore reelin's antidepressant potential by incorporating multiple behavioral tests alongside the FST, in order to measure a range of depression-like behavior and strengthen the current findings.

3.4 Limitations of the Reelin-Injection Paradigm

One of the concerns that arose when evaluating the efficacy of the paradigm used for reelin injections was a concern regarding the stressful effects of the reelin injection procedure itself. The experience of being placed in the restraint device, having blood taken, and receiving a tail vein injection, introduced an added stressor for rats already subjected to the CORT paradigm. In addition, some rats required more pricks, and hence longer time spent in the restrainer, in order to access the vein and administer the reelin. This inconsistency may have introduced greater variability between rats. However, at the outset of the experiment, control measures were taken in order to address this foreseen concern. Specifically, all appropriate control groups were included in the experimental design (i.e., Vehicle- and CORT- treated rats that received intravenous saline injections every 5 and 10 days in order to match both frequencies of reelin administration). This was done to control for confounding effects of time spent in the restraint device. On this note, Vehicle-treated rats that

received IV saline every 10 days, and Vehicle-treated rats that received IV saline every 5 days did not significantly from each other in their depression-like behavior in the FST or their anxiety-like behavior in the OFT. Furthermore, an additional two groups of rats were included in the experiment, which received subcutaneous CORT or Vehicle injections, but did not receive lateral tail vein injections in the restraint device. The data from these two groups of rats was not included in the reported findings, because these rats were added as a precautionary measure in order to evaluate any potential impact of the restraint device on behavior. We found that Vehicle- and CORT-treated rats that did not undergo the lateral tail vein procedure, and Vehicle- and CORT-treated rats that did receive intravenous saline injections in the tail vein did not significantly differ from each other in their depression-like or anxiety-like behavior. This signifies that exposure to the restraint device and the tail vein injection procedure itself, whilst likely a stressful experience, did not have any significant impact on behavior that could confound the current results. Despite this, future research should consider measuring basal circulating glucocorticoid levels and glucocorticoid levels following the injection period, in order to determine whether exposure to the restraint device significantly impacts glucocorticoid release.

4. Future Directions

There are several important directions that future work should take in order to strengthen and develop the current model. First of all, multiple behavioral tests should be conducted in order to gain a broader understanding of exogenous reelin's antidepressant-like effects, as well as its potential influence on memory processes. As mentioned earlier, reelin holds important facilitative roles in the hippocampus, including the facilitation of long-term memory formation. Recent work in our lab found that intra-hippocampal reelin infusions (1µg/ul) directly into the dorsal hippocampus completely restored the CORT-induced impairment in hippocampal-dependent object-location memory. It will be important to explore whether IV reelin injections similarly hold a protective role for hippocampal-

dependent memory in the face of deleterious CORT effects. To address this, future work should look to incorporate the object-location memory test as well as other hippocampal-dependent tasks that are sensitive to chronic CORT exposure, such as the Morris Water Maze. It will also be important to employ behavioral tests that can provide additional measures of depression-like behavior, in order to examine the potential impact of exogenous reelin on other depression-like symptoms. In this vein, the sucrose preference test could provide a valuable measure of anhedonia, a cardinal symptom of depression.

As discussed, the prevalence of depression is approximately 2-3 times higher in women than in men, and stress models with female rodents demonstrate that females are affected differently than males in terms of both behavior and neurobiology. For this reason, it is particularly relevant to explore gender influences in the reelin injection paradigm under conditions of chronic CORT treatment, on both depression-like and anxiety-like behaviors as well as on underlying neurobiology.

Based on previous findings reporting an altered pattern of SERT clustering on lymphocyte membranes in both patients with depression and in reelin-deficient mice and CORT-treated rats, we focused our analysis on this transporter in the present study. However, future work should begin to explore additional transporters, such as the dopamine transporter, to determine how their clustering patterns in peripheral blood cells may be impacted by increased glucocorticoid levels and addition of reelin. This vein of research opens up investigation into the neurobiology underlying reelin's amelioration of depression-like behavior. The identification of various neurochemical markers is important in order to guide future investigation into the underlying mechanisms that contribute to negative stress effects and the restorative effects of reelin.

At the same time, it would be valuable to look at the parallel and temporally dynamic effects of CORT and reelin on the development, expression, & progression of depression-like symptoms in rodents, and the regionally-specific mechanisms that might be having an influence. To this end, behavioral testing can be incorporated throughout the CORT and reelin injection period to examine

whether acute doses of reelin can protect against the progressive development of CORT-induced deficits. Blood sampling can be acquired at corresponding time points so that in addition to examining any protective effects of reelin on behavior, we can see how these effects may coincide with alterations in peripheral transporters, allowing us to more confidently ascertain the mechanisms underlying the antidepressant-like effects of exogenous reelin.

As briefly mentioned earlier, reelin has been shown to be expressed in endothelial cells of the BBB (Perez-Costas et al., 2015), but whether reelin is able to cross the BBB is still not known. While results from the current experiment evidence that peripheral reelin impacts FST behavior, they do not provide any indication that reelin crossed the BBB to do so. This will be important to determine in future research, as the crossing of the BBB means that reelin can directly impact brain changes to have neuroprotective effects. Radioactive-tagging of the recombinant reelin protein prior to injection in the lateral tail vein would allow subsequent identification of the injected protein via immunohistochemical analyses. This would ultimately allow the determination of whether or not reelin reached the brain.

5. Conclusions

The main aim of the current experiment was to determine, for the first time, whether reelin administered peripherally holds antidepressant-like action, without negatively impacting in other domains. The second aim of the experiment was to examine whether two neurobiological markers, hippocampal cell maturation and pattern of serotonin transporter clustering in peripheral lymphocytes, both of which are implicated in the context of depression, are positively impacted by the addition of peripheral reelin following a period of prolonged exposure to stress hormone. All doses of reelin administered reversed the depression-like behavior brought about by treatment with stress hormone, while restoring the associated changes observed in serotonin transporter clustering in lymphocyte membranes.

These findings demonstrate the antidepressant potential of reelin administered peripherally. In conclusion, the preclinical body of research outlined in this thesis provides novel and valuable findings that hold strong potential for addressing the limitations inherent in current pharmacotherapeutic treatments with standard antidepressants.

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