

# **INTERACTIONS OF STRESS AND MOTOR SYSTEM FUNCTION**

Nafisa M. Jadavji  
Bachelor of Science, University of Lethbridge, 2006

A Thesis  
Submitted to the School of Graduate Studies  
Of the University of Lethbridge  
In Partial Fulfillment of the  
Requirements for the Degree

**MASTER OF SCIENCE**

Neuroscience  
University of Lethbridge  
LETHBRIDGE, ALBERTA, CANADA

© Nafisa M Jadavji, 2008

“Stress is an aspect of our daily lives and conversations and yet there is considerable ambiguity in the meaning of this word.”

Bruce S. McEwen, 2000.

## **Thesis Abstract**

Stress is one of the most critical influences on behavior, performance and disease. Recent findings from our laboratory have shown that stress represents a major modulator of motor function in the intact and damaged brain. The mechanisms by which stress and stress hormones affect motor system function, however, have not yet been determined. The objective of this thesis was to determine the route of action of stress and stress hormones on the motor system in a rat model. The first experiment investigates whether corticosterone is involved in mediating stress-induced motor impairments. The second experiment compares the role of glucocorticoid and mineralocorticoid receptors in regard to modulating the motor response to stress. The third experiment determines the differential effects of stress on motor function in males and females. The final experiment systematically describes changes in neuronal cell signaling that affect normal function of motor areas. The results indicate that disturbance of fine motor control by stress is not associated with stress hormone increases. Furthermore, it is modulated through the glucocorticoid and mineralocorticoid receptors. Stress differentially impairs motor function in males and females. These changes in motor behaviour could possibly be the result of changes in neuronal cell signaling within the motor system. This research provides new insights into physiological influences in motor system function and disorders of the motor system.

## Acknowledgements

There are a number of people I would like thank. I hope I don't forget anyone.

I want to thank my supervisory committee. Firstly, Dr. Kathy Hegadoren for taking time out her busy schedule to be my external examiner. Secondly, I would like to thank Dr. Olga Kovalchuk and Dr. Robbin Gibb for all their help and guidance over the past two years. I would also like to thank Dr. Darren Hannesson for his comments and guidance with experiments and experimental data during the summer and fall of 2006.

Thank you to specific members of the Metz Lab. Rebecca Supina, for helping me test animals and develop a Western Blotting protocol. Dora Capatos, for her help in troubleshooting and running numerous gels for the Western blotting chapter in this thesis. Thank you to Jamshid Faraji for being a great office mate and answering all my questions about statistics. I would like to thank Dr. Alexander Klein for providing me feedback on the first drafts of chapter one and three. Thank you to some past members of the Metz lab, Dawn Merrett and Scott Kirkland for their assistance with experiments.

Rocio Rodriguez, Mike Baker, Kristi Kutanzi and Igor Koturbash, thank you for answering all my questions in regards to Western Blotting.

I also would like to thank Karen Dow-Cazal, Charlotte Holmes, Linda Pickering and Tyler Barrows for taking such great care of the animals used in this experiment.

Thank you to Naomi Cramer and Donna McLaughlin for answering all my questions and helping me with things around the CCBN.

Thank you to a number of friends that have provided me with the support I have needed to complete the experiments, writing this thesis and in my pursuit of leading a balanced life. They include Carly Turner, Norah Moshkovits, Michael A.P. Smith, Afra Foroud, Bev Robinson, Brooke Rakai, Kelli McAllister, Kimberley Finn, Julia Wasilewski, Jennifer Anne Stenbeck, Anne Burnett and Dr. Jennifer Mather. You have been there through the good and the bad and it means the world to me.

I want to thank Dr. Tracy D. Farr for taking me on as a volunteer during her MSc. degree, your continued support, patience and for teaching me almost everything I know to this day.

Lastly and most importantly, I want to thank my supervisor, Dr. Gerlinde A. Metz, for a number of things. Firstly, I want to thank you for taking a chance on me. Secondly, thank you for challenging me by giving me the opportunity to work in her lab for over 6 years. Thirdly, thank you for giving me the freedom to work on my own and grow as a scientist. Lastly, thank you for all your support and patience – I know I have challenged both but you stuck with me in the end and I greatly appreciate it. I hope that one day we can work together again, as colleagues.

## Table of Contents

Title page	i
Signature Page	ii
Quotation	iii
Thesis Abstract	iv
Acknowledgements	v
Table of Contents	vi - vii
List of Tables and Figures	viii
List of Abbreviations	ix
1. Introduction	1
1.1. Introduction to Thesis	
1.2. Introduction to Stress	
1.3. Corticosteroid Receptor System	
1.4. Introduction to Motor System	
1.5. The Phenomenon of Plasticity	
1.6. Modeling Stress in the Rodent	
1.7. Testing Behaviour in the Rodent	
1.8. Introduction to Experiments and Thesis Hypothesis	
2. <b>Experiment 1:</b> Elevated Corticosterone Levels Are Not Associated with Stress Induced Motor Disturbances	16
3. <b>Experiment 2:</b> Blocking Glucocorticoid and Mineralocorticoid Receptors Neutralizes Motor Function Impairment Associated With Stress	38
4. <b>Experiment 3:</b> Sex Differences in Skilled Movement in Response to Restraint Stress and During Recovery from Stress	65
5. <b>Experiment 4:</b> Restraint Stress Impairs Skilled Motor Function by Disrupting Cell Signaling in Motor Circuits	93
6. Discussion	107
6.1. Summary of Results	
6.2. Review of Stress	
6.3. Significance of Results	
6.4. Clinical Relevance and Future Direction	

6.5. Thesis Summary

7. References

113

## **List of Tables and Figures**

- Figure 1.** Hypothalamic-pituitary adrenal (HPA) axis.
- Figure 2.** Triple labeled sections of the rat substantia nigra seven days after unilateral 6-hydroxydopamine lesion.
- Figure 3.** Time chart illustrating the order of manipulations and tests.
- Figure 4.** Skilled reaching task photograph and results.
- Figure 5.** Skilled walking task photograph and results.
- Figure 6.** Plasma circulating corticosterone concentration.
- Figure 7.** Scatter plots of correlation results.
- Table 1.** Comparison of major characteristics of linear correlation between plasma circulating CORT and skilled motor function.
- Figure 8.** Time chart illustrating the order of manipulations and behavioural tests.
- Figure 9.** Skilled reaching task photograph and results.
- Figure 10.** Number of attempts to grasp a single food pellet.
- Figure 11.** Qualitative skilled reaching performance.
- Figure 12.** Open field activity measurement, number of rears.
- Figure 13.** Time chart illustrating the order of manipulations and behavioural tests.
- Figure 14.** Skilled reaching task photograph and results.
- Figure 15.** Qualitative skilled reaching task photographs and results.
- Figure 16.** Skilled walking task photograph and results.
- Figure 17.** Open field activity measurement, number of rears.
- Figure 18.** Plasma corticosterone concentration prior to and at chronic stress levels.
- Figure 19.** Time chart illustrating the order of experimental manipulations.
- Figure 20.** PI-3 protein expression in cerebellum and AKT protein expression in cerebellum and brain stem.

## List of Abbreviations

ACTH – adrenocorticotrophic hormone  
AKT – protein kinase B  
CORT – corticosterone  
CREB – cyclic- responsive element-binding protein  
CRF – corticotrophin releasing factor  
DNA – deoxyribonucleic acid  
ELISA – enzyme linked immunosorbent assay  
ERK – extracellular regulated kinases  
GABA – gamma-aminobutyric acid  
GC – glucocorticoid  
GR – glucocorticoid receptor  
HPA – hypothalamic-pituitary-adrenal (axis)  
Inc – incorporation  
LTP – long-term potentiation  
MAPK – mitogen activated protein kinase  
MEK1, MEK2 – MAPK/ERK kinase 1/2  
MR – mineralocorticoid receptor  
mRNA – messenger ribonucleic acid  
PCNA – proliferating cell nuclear antigen  
P13-K – phosphatidylinositol-3 kinase  
p53 – protein 53  
SAM – sympathetic-adrenomedullary

## **1. Introduction**

### **1.1. Introduction to the thesis**

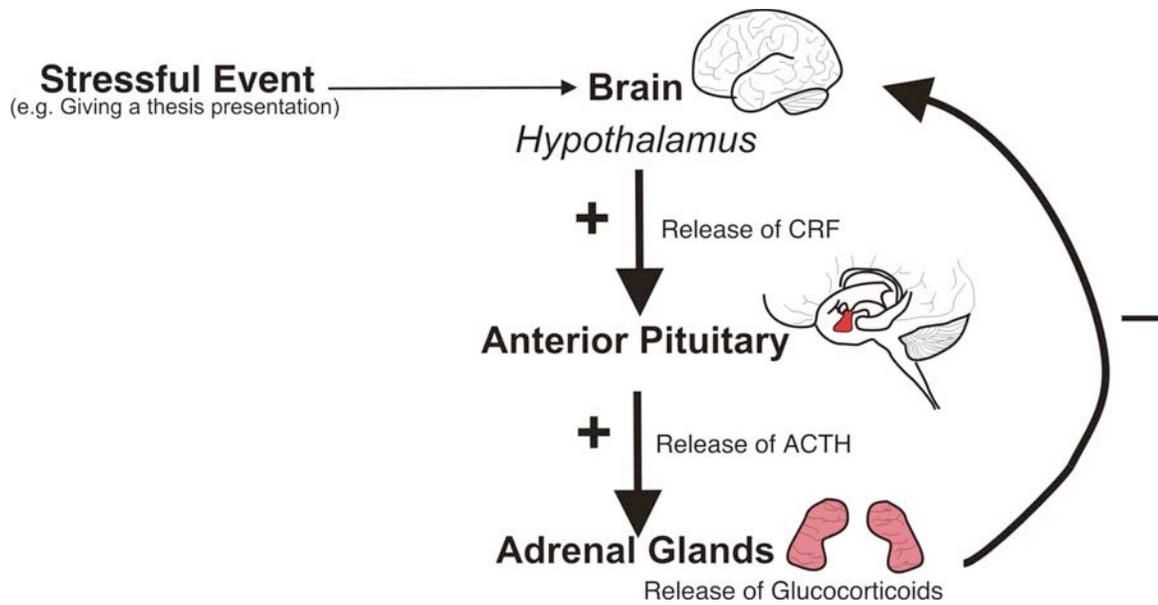
Previous studies have shown that exposure to stress can impair both skilled and unskilled motor function (Metz et al., 2001, 2005; Metz, 2007). The mechanisms by which stress and stress hormones affect motor system function, however, have not yet been determined. The purpose of this thesis is to elaborate the route of action of stress and stress hormones on the motor system in a rodent model. The following seven sections comprise the introduction portion of the thesis. The first section defines the term stress and describes associated physiological responses. The second section emphasizes the role of mineralocorticoid and glucocorticoid receptors in the stress response. The third section will discuss the impact of mineralocorticoid and glucocorticoid receptors on motor system function. The fourth section will elaborate on the phenomenon of neuronal plasticity, plastic changes and changes in morphology in response to stress. The fifth section discusses modeling stress in the rodent. The sixth section describes behavioural test strategies in rats used to assess changes in movement abilities in response to stress. The final section of the introduction outlines the experiments described in this thesis.

### **1.2. Introduction to Stress**

The stress response consists of a complex set of behavioural, physiological and biochemical reactions that serve to re-establish homeostasis (Selye, 1976; Sapolsky, 1992; Lopez et al., 1999; Pedersen et al., 2001). A stressor, such as writing a final exam, initiates the stress response, which includes the activation of the hypothalamic-pituitary-adrenal (HPA) axis (Figure 1). The HPA axis serves as a monitor of demands in the

environment and begins in the brain with the hypothalamus, which releases corticotrophin releasing factor (CRF; Akil et al., 1999). CRF in turn stimulates the anterior pituitary, which in response releases adrenocorticotrophic hormone (ACTH). The end-product of the HPA axis is the release of stress hormones also referred to as glucocorticoids (GCs), which are released by the adrenal gland once it has been stimulated by the release of ACTH (McEwen, 2000). GCs are present in all living organisms and are a type of steroid hormone, which exert effects on multiple target tissues (Sapolsky, 1992). In humans the main GC is cortisol and in rats it is corticosterone (CORT; Akil et al., 1999; Sapolsky, 2000). Regulation of the HPA axis occurs through negative feedback (Sapolsky, 1992; Baccan et al., 2004).

The sympathetic-adrenomedullary (SAM) system is the second component of the endocrine reaction to stress. The SAM system is a component of the sympathetic division of the autonomic nervous system, which causes a release of epinephrine. The increased levels of epinephrine facilitate the rapid mobilization of other systems during the stress response.



**Figure 1.** Hypothalamic-pituitary-adrenal (HPA) axis activated during the stress response to a stressful event (e.g., writing a final exam). The hypothalamus is stimulated by a stressful event to release CRF, which in turn stimulates the anterior pituitary to secrete ACTH. ACTH stimulates the adrenal glands to release GCs (e.g., CORT). Increased levels of GCs work through negative feedback mechanisms to turn off the HPA axis.

Stress can have both beneficial and devastating effects on behaviour. It has been suggested that the effects of stress depend on the stressor's strength and duration (McEwen and Sapolsky, 1995). Previous work has shown that it can improve or impair performance in learning tasks (Selye, 1976; Sapolsky, 1992; McEwen and Sapolsky, 1995; Albeck et al., 1997; McEwen, 2000; Gunnar and Quevedo, 2007). The beneficial responses include energy diversion to the areas in the body needed to escape the stressful situation (Sapolsky et al., 2000). The negative consequences of stress include impairment in cognitive function such as spatial abilities, specifically impairments in learning seen in the water maze task (Holscher, 1999). Previous research has also shown a decrease in exploratory behaviour in animals exposed to chronic stress (Berridge and Dunn, 1989). Recent research in our laboratory has revealed that chronic stress causes impairments in skilled and non-skilled motor function in intact rats (Metz et al., 2001, 2005; Metz, 2007). For example, animals exposed to oral CORT treatment, cold swim or restraint stress show poorer performance on the skilled reaching task when compared to baseline performance (Metz et al., 2005).

The negative effects associated with stress are mainly linked to chronic activation of the stress response, which in turn has been linked to disease. The body's response to stress not only involves the brain but also endocrine, autonomic and immunological systems. There is an overall weakening of the system during chronic stress, making it more prone to disease (Selye, 1976; Sapolsky, 1992; Albeck et al., 1997; Lopez et al., 1999; Gunnar and Quevedo, 2007; Metz, 2007). For example, previous studies have shown that GCs impair the capacity of nervous tissue to recover from injury by reducing cell function, plasticity and ability for regrowth (Selye, 1976; Sapolsky, 1992; Albeck et

al., 1997; Gunnar and Quevedo, 2007; Metz, 2007). GCs bind to the glucocorticoid and mineralocorticoid receptors in the brain, which help facilitate subsequent molecular actions (de Kloet et al., 1999).

### **1.3. Corticosteroid Receptor Systems**

There are two types of receptors that GCs bind to in the brain. The first is the mineralocorticoid (type-I) receptor and the second is the glucocorticoid (type-II) receptor. Both receptors are members of a super-family of ligand-regulated transcription factors, which mediate slow genomic actions and are necessary for maintenance of homeostasis (Meaney et al., 1996; Lupien and McEwen, 1997). The receptors reside in a complex containing heat shock proteins, which is located in the cytoplasm of the cell (Akil et al., 1999). Once the ligand has bound to the receptor, the whole complex will move into the nucleus of the cell and begin interactions with deoxyribonucleic acid (DNA). The interactions include interfering with transcription rates and translation of messenger ribonucleic acid (mRNA) into proteins (Sapolsky, 1992; Lupien and McEwen, 1997; Akil et al., 1999; Baccon et al., 2004).

#### *Mineralocorticoid Receptor*

Mineralocorticoid (MR) or type-I receptors bind to endogenous CORT with high affinity (de Kloet et al., 1999). Aldosterone also binds to the MR and is involved in conserving sodium, secreting potassium and modulating blood pressure (Quinn and Willams, 1998). The regulatory function of MR can be blocked by specific antagonists including RU-28318 (spironolactone) and RU-40555 (de Kloet et al., 1999, 2005). Within the central nervous system MRs can be found in high density within the

hippocampus, lateral septum and medial amygdala. They are expressed less densely in other brain regions, such as the cerebellar cortex, brain stem, spinal cord and pituitary (Ahima and Harlan, 1990; Ahima et al., 1991; Spencer et al. 1995; Joels et al., 2004). Within the cell MR is loosely bound to the nuclear membrane (Sapolsky, 1992). Previous research has shown that the MR is involved in mediating various behaviours including spatial navigation and anxiety (Korte et al., 1995).

### *Glucocorticoid Receptor*

Glucocorticoid (GR) or type-II receptors bind CORT with a lower affinity when compared to MR (de Kloet et al., 1999). Thus, GRs are activated mainly by high CORT levels (Baccan et al., 2004). GRs are found in nearly all cell types throughout the central nervous system and peripheral nervous system. GRs are located in both neurons and glial cells within the motor cortex, cerebellum, hippocampus, striatum, amygdala and thalamus (Ahima and Harlan, 1990; Sapolsky, 1992; Pedersen et al., 2001; Pace and Spencer, 2005). The function of GRs has in part been determined by using antagonists, for example RU-486 (mifepristone), which exerts activity at two levels of receptor action. First, it reduces the amount of GR converted to the DNA-binding state (Beck et al., 1993). Then, it abolishes down-stream events involving interaction of the DNA-bound receptor with the transcription complex (Beck et al., 1993).

Furthermore, GRs have been proposed to be involved in the consolidation process of learning and memory (Korte et al., 1995). Recent studies have suggested that the GRs participate in the stress response along with MRs (Feldman and Weidenfeld, 1999).

A combination of GR and MR antagonism prior to stress exposure causes no increase in circulating CORT levels of animals, indicating that acting together both the

MR and GR play a role in negative feedback within the HPA axis (Feldman and Weidenfeld, 1999; Moldow et al., 2005). Since the MR and GR have different affinities for GCs, it has been hypothesized that they could possibly play different roles in HPA axis regulation and facilitates the regulation of each other (Sapolsky, 1992; Spencer et al., 1998). Because MR and GR have been found in the motor system (Ahima and Harlan, 1990), they could potentially modulate the motor response to stress.

#### **1.4. Introduction to Motor System**

The motor system includes a number of brain structures that are responsible for coordinating motor functions (Kaas, 1991; Thach, 1999; Ghez and Krakauer, 2000). The motor system is organized hierarchically and begins with the cortex with outputs to the brain stem and to the spinal cord (Ghez and Krakauer, 2000). Each component of the hierarchy contains differential concentrations of MRs and GRs (Ahima et al., 1990, 1991). The motor cortex is involved in executive function of movement. Interestingly, GRs have been shown to be present in the motor cortex (Ahima et al., 1990). The cortex communicates with the brain stem and spinal cord to control complex voluntary movements (Thach, 1999; Ghez and Krakauer, 2000). GRs and MRs are also present in the brain stem (Ahima et al., 1990, 1991). The brain stem contains two main systems, the medial and the lateral pathways. The medial pathway provides basic postural control and influences the motor neurons that innervate axial and proximal muscles. The lateral pathway is concerned with goal-directed limb movements, such as reaching.

The motor neurons in the spinal cord are primarily responsible for execution of movement, such as skilled movements and communication with muscles (Ghez and Krakauer, 2000). A study by Marlier et al. (1995) showed that GRs are present in high

density within the spinal cord. Another study by Ahima et al. (1991) showed that the spinal cord also has MRs. Although the function of human and rodent motor systems is well-defined, it can change quite rapidly as a result of neuronal plasticity (Kaas, 1991; Kolb and Whishaw, 1998).

### **1.5. The Phenomenon of Plasticity**

Brain plasticity refers to the brains' ability to change in response to environmental influences or injury (Kolb and Whishaw, 1998). Experiences such as stress, environment, drugs and learning are catalysts for brain plasticity in human and rodent (Kolb and Whishaw, 1998; Weiller and Rijntjes, 1999). Neuronal plasticity is reflected by change in molecular processes, cellular function, and changes at the behavioural level. For example, at the molecular level there might be differences in the expression of neurotrophic factors (Pham et al., 2002), which is reflected at the behavioural level as improvements in motor tasks after injury. These improvements can be attributed to recovery or compensation (Kaas, 1991; Kolb and Whishaw, 1998).

If a stressor lasts for weeks, the brain copes by adaptive plasticity. This includes changes in hormone secretion that alter levels of local neurotransmitters, which then produce structural as well as functional changes in the central nervous system (McEwen, 2000; Radley et al., 2005; Riva, 2005; Steckler, 2005). Structural changes can also occur, which results in altered neuronal activity or vice versa (Sandi, 2004). A study by Suanada et al. (2000) revealed that chronic exposure to restraint stress causes atrophy of apical dendrites and alterations in density of dendritic spines in the hippocampus. Another study found shortening of apical dendrites within the hippocampus (Sandi, 2004). Neurogenesis

in the hippocampus can be abolished by both acute and chronic stress (McEwen, 2000). Lastly, an example of how molecular processes can affect neuronal plasticity is by modulating protein production. For example, Thome et al. (1999) have demonstrated that exposure to stress leads to reduced expression of synaptophysin but increased expression of synaptotagmin in the hippocampus. Both proteins are integral to the synaptic vesicle membrane and required for vesicle fusion and neurotransmitter release. It has been hypothesized that the differential regulation of synaptic vesicle proteins could be involved in the morphological and behavioural changes observed after stress exposure (Thome et al., 2001). This phenomenon could thus also account for motor impairments caused by stress.

Exposure to chronic stress has been suggested to lead to detrimental effects on the brain. For instance, animals exposed to chronic stress have shown neuronal structural remodeling and cell death in the hippocampus, amygdala and the prefrontal cortex (Suanada et al., 2000; Bogolepov et al., 2001; Joels et al., 2004; de Kloet et al., 2005; Froc and Christie, 2005). Within the sensorimotor cortex Bogolepov et al. (2001) showed cortical cell death after exposure to emotional stress and suggested that it is most likely due to ischemia-like mechanisms. Smith et al. (2008) have shown that exposure to stress exaggerates cell death in the substantia nigra in the 6-hydroxydopamine rat model of Parkinson's disease (Figure 2). One mechanism of cell death might be related to long-term potentiation (LTP). Specifically, an electrophysiological study by Sandi (2004) showed that long-term stress impairs LTP, which can be translated into disturbances of neural function. Another study has shown that CRF has depressant-like actions in the sensorimotor cortex *in vivo* (Froc and Christie, 2005). The findings of stress-induced

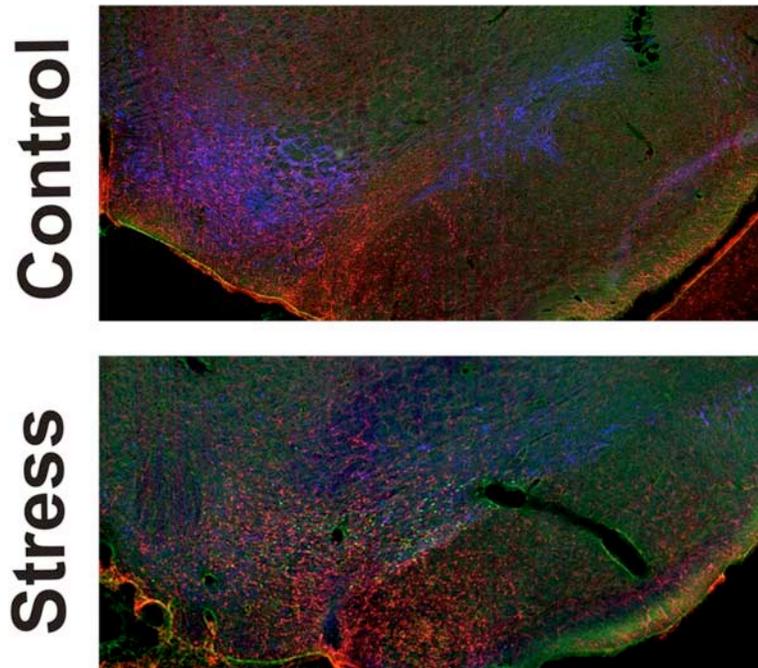
changes, however, might also depend on many variables, including the model of stress used (Sapolsky, 1992).

## **1.6. Modeling Stress in the Rodent**

Understanding the mechanisms underlying stress-induced disturbances will allow for improved clinical therapies and treatments (Tamashiro et al., 2005). Therefore, laboratory animal models have been used for a number of years to examine the behavioural and physiological responses to stress. Some examples of stress paradigms used in the laboratory include restraint, social, predator smell, forced swim, foot shock, noise and light or cold swim stress (Katz et al., 1981; Metz et al., 2005; Tamashiro et al., 2005; Zhao et al., 2007). Rodent models of stress are widely used in the laboratory in part because of their relatively short lifespan, enabling investigators to conduct longitudinal studies in a short time, in addition to conducting studies at critical periods such as neonatal or adolescent stages (Tamashiro et al., 2005). Stress treatments in animals usually occur in random order to reduce predictability, or daily in a predictable way for varied durations. Most commonly, two key time points, acute and chronic stress, are compared to parallel human stress conditions in the investigation of behavioural and physiological changes (Metz et al., 2005; Tamashiro et al., 2005).

The experiments described in this thesis will use three stressors, restraint and cold swim stress, in addition to administering CORT orally to mimic the main physiological correlate of stress. The duration of these treatments is classified as chronic, since treatments took place daily for 15 days.

## Lesion Hemisphere 7 Days Post-Lesion



**Figure 2.** Triple-labeled sections of the rat substantia nigra seven days after unilateral 6-hydroxydopamine lesion. Red cells labeled with glial fibrillary acidic protein, blue cells labeled with tyrosine hydroxylase and green cells labeled with Fluoro-Jade. Note the decrease in tyrosine hydroxylase-positive cells and increase in Fluoro-Jade-positive cells in animals exposed to stress. Modified from Smith et al. (2008).

## **1.7. Behavioural Responses to Stress**

As discussed previously, there are several ways that stress can directly or indirectly influence behaviour. There are a variety of behavioural tests available for testing stress-induced changes in sensorimotor function in rats. During the course of the experiments for this thesis, three main behavioural tests were utilized, based on earlier studies. The single pellet reaching task and ladder rung walking task test skilled motor function in rodents (Whishaw, 2000; Metz and Whishaw, 2000). Furthermore, the open field task was used, which represents, a standard measurement of exploratory activity that is commonly used in stress research (Roth, 1979; Sousa et al., 2006).

The single pellet reaching task has the advantage of providing both a quantitative and qualitative measure of fine motor performance in rats (Whishaw et al., 1992a; Whishaw et al., 1992b; Whishaw, 2000). In this task, a laboratory rat reaches for a single piece of food. The entire reach sequence can be subdivided into eleven stereotypical movement components (Metz and Whishaw, 2000). These movement components can be evaluated using a scale adapted from a movement notion that expresses the limbs as axes and documents movement by observing change in the limb and body axes (Eshkol and Wachmann, 1958). Notably, the reaching movement involved in the single pellet reaching task is similar to reaching in humans (Eshkol and Wachmann, 1958; Whishaw et al., 1992a; Whishaw et al., 1992b)

The ladder rung walking task provides both a quantitative and qualitative measure of skilled walking in rodents (Metz and Whishaw, 2002). Animals are trained to walk across a horizontal ladder, which has rungs set in an irregular pattern. The numbers of

errors are counted (quantitative), as well as scores of how the animal places its limbs on the rungs (qualitative; Metz and Wishaw, 2000).

The open field task is a standard test used to measure exploratory activity. It is one of the standard tests used to assess activity after animals are exposed to stress (Roth and Katz, 1979). The animals avoid novel and potentially dangerous environments and explore new situations with respect to availability of food. Thus, this test reflects a measurement of anxiety in laboratory rodents (Sousa et al., 2006).

In addition to behavioural tests, blood samples were collected to analyze for corticosterone and glucose concentrations. Corticosterone concentrations were determined to confirm whether treatments led to elevation of stress hormone levels (Metz et al., 2005). Blood glucose levels were determined since stress has been linked to alterations in glucose levels (Pederson et al., 2001; Bates et al., 2007; Garcia-Bueno et al., 2007). Decreases in glucose utilization within the brain have been shown in animals exposed to stress (Garcia-Bueno et al., 2007), whereas cells within the periphery require increased levels of glucose when responding to a stressor (Sapolsky, 1992).

## **1.8. Introduction to Experiments**

The main purpose of this thesis was to elaborate the route of action of stress and stress hormones on the motor system in the rodent model. This thesis contains four chapters each of which addresses specific aspects of stress-induced modulation of motor function. The four questions that these chapters address are the following. **(1) Does the severity of motor impairment during stress correlate with circulating levels of corticosterone?** This objective is to examine the dose-response relationship of the level of stress hormones with the degree of motor impairment by detailed qualitative and

quantitative behavioural analysis. **(2) Does stress exert its detrimental effect on motor function mainly via action on the glucocorticoid receptor (GR) or the mineralocorticoid receptor (MR)?** While previous data pointed out that corticosterone mediates at least some of the effects of stress on the motor system, it needs to be determined if these effects are mediated by its main receptor, GR, or possibly by MR. The objective will be investigated by using GR and MR agonists to activate and antagonists to block the function of these receptors. **(3) Are there sex differences in the effects of stress on motor system function?** This objective is to investigate the interaction of stress and stress hormones with sex hormones by exploring possible sex differences in the susceptibility of the motor system to the influence of stress. **(4) What are the effects of restraint stress on cell signaling proteins?** The objective is to investigate changes in apoptotic cell signaling proteins in areas of the brain involved in motor function using neurochemical techniques.

### **Thesis Hypothesis**

The stress response activates a number of systems within the body to release different hormones, which in turn have varying effects on the central nervous system. The system then interprets and integrates these signals to formulate a behavioural response. Recent studies suggest that there are multiple contributors to the behavioural effects of stress, however, the role of most of these individual factors has not been determined yet. Nevertheless, it is accepted that corticosterone represents a key player in the rodent stress response and thus it is a prime candidate to initiate a line of research into the mechanisms of stress-induced motor impairment. Therefore, the guiding hypothesis

for this thesis is that **other receptors and routes than traditionally described are involved in modulating motor performance after exposure to stress.**

## **2. Experiment 1**

### **Elevated Corticosterone Levels Are Not Associated with Stress-Induced Motor Disturbances**

#### **Abstract**

Chronic stress has been shown to alter behaviour in both humans and rodents. For example, previous studies have shown that chronic stress can impair skilled motor function in animals. The stress response is defined by an increase in stress hormones, cortisol in humans and corticosterone (CORT) in rodents, both secreted by adrenal glands. CORT can cross the blood brain barrier easily to regulate homeostasis. The purpose of this study was to quantitatively investigate the relationship between circulating CORT and performance of skilled motor function in animals exposed to three different stress manipulations. Male and female rats were trained in skilled reaching and ladder rung tasks. Once baseline measurements were completed, rats underwent daily sessions of either swimming in 5°C cold water, restraint stress, or oral CORT treatment. Manipulations lasted for 15 days and animals were tested in the skilled reaching task daily and twice in the ladder rung walking task. Blood samples were collected during baseline and on day 15 of manipulations. The results showed that successful reaching decreases in animals exposed to swim and restraint. Animals administered oral CORT showed a significant increase in circulating plasma CORT concentration but no decrease in skilled reaching success. Correlation analysis revealed a negative relationship between plasma CORT concentration and ladder rung walking score in swim stress animals, as well as reaching success in restrained animals. The results from this study suggest that

CORT is not the main factor mediating loss of skilled motor function after stress. Therefore, other endocrine and psychological factors may be involved in modulating skilled motor function in response to stress.

## **Introduction**

Stress is one of the most critical influences on behaviour, performance and disease (Selye, 1976). The stress response has been characterized by an increase in circulating stress hormones, which are referred to as glucocorticoids (GCs), specifically cortisol in humans and CORT in rodents (Lucas et al., 2007). CORT is released when the hypothalamic-pituitary-adrenal (HPA) axis is activated. The adrenal glands release CORT into the blood and the effects of this hormone are widespread, and include actions on the brain (Dronjak et al., 2004). CORT crosses the blood brain barrier easily and binds to two types of receptors, the mineralocorticoid and glucocorticoid receptors. Once bound, CORT coordinates the organism's ability to cope with stress by diverting energy supplies to challenged tissues. In addition, CORT promotes the interpretation of information and the discontinuation of behaviour that is no longer needed (de Kloet et al., 1999). The release of CORT is controlled by the HPA axis, which also stops its release of CORT through a negative feedback mechanism (de Kloet et al., 2005).

The dose-response relationship between the severity of the stressor and the magnitude of the stress response has led to the conclusion that the HPA axis is able to distinguish between stressors of different severity and durations (Sapolsky, 1992; Dayas et al., 2001; Metz et al., 2005). It still remains to be determined if CORT is the main mediator of the behavioural changes in response to stress.

Current evidence suggests that stress alters emotion, cognitive and motor function (McEwen and Sapolsky, 1995; Metz et al., 2001, 2005). Previous work has shown that increases in CORT cause impairments in skilled and non-skilled motor function, including performance in a reaching task (Metz et al., 2001, 2005). However, it still remains unclear if these impairments are mediated by CORT. The objective of the present study was to investigate whether CORT is a modulator of the motor impairments seen after exposure to stress. Cold swim stress, restraint stress and CORT treatment were used to investigate the relationship of circulating plasma CORT to performance in skilled motor function, such as the skilled reaching and skilled walking tasks.

## **Materials and Methods**

### *Subjects*

Subjects were 18 male and 48 female adult Long-Evans Hooded rats, raised at the University of Lethbridge vivarium and weighing 280-600g at the beginning of the experiments. The animals were housed in groups of two or three in standard polycarbonate shoebox cages (45.5 x 25.5 x 20 cm) on corn cob bedding (Bed O Cobs 1/8"). The light cycle was 12:12h with lights on at 07:30 h. The housing room was maintained at a temperature of 20°C and 30% relative humidity.

Prior to the experiments, rats were food deprived to 95% of their initial body weight to encourage participation in the reaching task. Supplementary food was given daily in their home cages five hours after behavioural testing to maintain body weight.

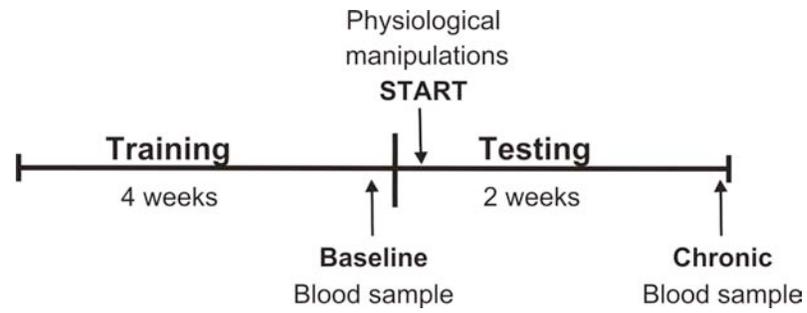
Water was available *ad libitum*. The animal experiments were approved by the University of Lethbridge Animals Welfare Committee.

### *Experimental Design*

Animals were trained and tested in a skilled reaching task and a ladder rung task (Figure 3; 4A; 5A). Then they were assigned to one of three experimental groups: swim stress (n=5), restraint stress (n=47) and 5 mg oral CORT treatment (n=14). The stress-inducing procedures and CORT treatments were performed daily at the same time of day over a period of 15 days, during which animals were tested daily in the skilled reaching task. Ladder rung testing occurred before manipulations and on day 14 of daily treatments. Blood samples were collected at two time points during the experiments; on baseline and on day 15 of stress or CORT treatment. Blood was collected at either 10 min or 60 min after exposure to restraint stress to determine short-term changes in CORT levels.

### **Physiological Manipulation**

*Swim Stress.* Animals were individually placed in a bucket filled with ice-cold water (5°C) for 5 min (Armario et al., 1987, 1995). The water was deep enough so that the animals' feet or tail did not have contact with the bottom. After swimming, animals were dried with a towel and returned to their home cage, which was placed on a heating pad.



**Figure 3.** Time chart illustrating the order of manipulations and tests. Training and testing included skilled reaching and skilled walking performance. Blood sample collection took place before physiological manipulation commenced and on day 15 of physiological manipulations.

*Restraint Stress.* Animals were placed in a transparent Plexiglas container (5 cm inner diameter) for a period of 20 min each day (Garcia et al., 2000; Faraday, 2002; Mercier et al., 2003). The container had perforated ends to allow ventilation. The container maintained the animals in a standing position without compression of the body.

*CORT Administration.* One group of animals received 5 mg of oral CORT (Sigma, Oakville, ON) treatment. Administration of 5 mg of oral CORT was previously shown to cause impairments in motor function (Metz et al., 2005). The CORT was administered once daily mixed with cookie crumbs, water and peanut oil for consumption.

## **Behavioural Testing and Analysis**

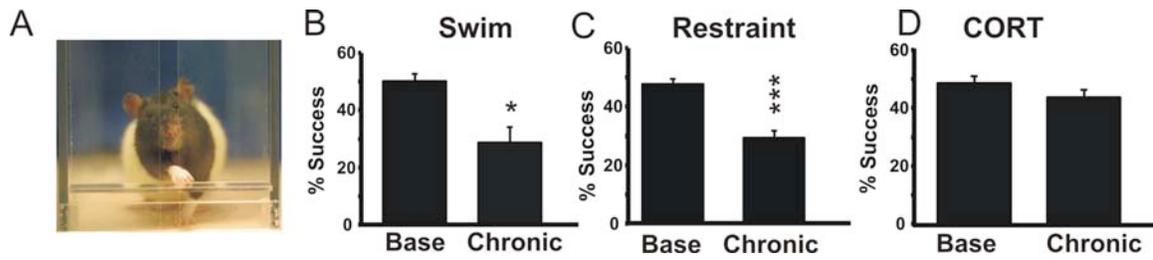
### *Skilled Reaching Task*

*Skilled Reaching Task Apparatus.* The reaching box was made of clear Plexiglas (40 x 45 cm and 13.1 cm wide). Animals were trained to extend their forelimbs to reach for food pellets through a 1.3 cm wide vertical opening in the middle of the front wall (Figure 4A). The vertical opening extended from the floor to a height of 15 cm. To hold the food pellet, a 2 cm wide by 4 cm long shelf was positioned outside the front wall of the box. The shelf was mounted 4 cm above the floor. Food pellets (45 mg banana flavoured Dustless Precision Pellets, Bioserv Inc., Frenchtown, NJ) were placed in one of two small indentations on the shelf. The indentations, each 5 mm in diameter and 1.5 mm deep, were 2 cm away from the inside wall of the box and were centered on the edges of the slit through which the rats reached (Metz and Whishaw, 2000).

*Training and Testing.* Once rats began to reach for food, food was placed in the indentation contralateral to the limb that the rat used for reaching. Between individual reaching movements, rats were required to leave the food aperture and walk to the rear of the box in order to reposition themselves prior to the next reach. Each training and testing session required the rats to reach for 20 food pellets. Reaching performance was scored by counting misses and successful reaches (Metz and Whishaw, 2000). An ‘attempt’ was defined as a repeated forelimb movement towards the pellet and obtaining the pellet after more than one reach. A ‘success’ was recorded if an animal grasped a food pellet on the first attempt and withdrew the paw with the pellet through the slit to consume it (Metz and Whishaw, 2002a). A ‘miss’ was recorded if an animal touched and missed the pellet using more than one attempt to grasp it. Additionally, if the animal lost the pellet in the cage after grasping it, a ‘drop’ was scored. Percent reaching success was calculated by counting the number of successful reaches divided by the number of pellets given in each session (20) multiplied by 100. Typical success rates at baseline were approximately 50%.

### *Skilled Walking Task*

*Ladder Rung Walking Task Apparatus.* The apparatus was made of two side walls (1 m long and 20 cm high) of clear Plexiglas with metal rungs (3 mm in diameter), inserted at random distances ranging from 1 to 5 cm to create a floor (Metz and Whishaw, 2000; Figure 5A). The irregular pattern was used to maintain the difficulty of the task in repeated test sessions. The apparatus was elevated 30 cm above the ground with a neutral start box and the animals’ home cage at the end.



**Figure 4.** Skilled reaching task. (A) Photograph illustrating the skilled reaching task in which animals are required to grasp and obtain food pellets. Mean percent success of animals before (Base) and after (Chronic) exposure to swim stress (n=5). (B), restraint stress (n=47). (C) and oral CORT administration (n=14). (D). Note the significant decreases in percent success of animals exposed to swim and restraint stress. Significances are given as comparison to baseline values. \*  $p < 0.05$ , \*\*\*  $p < 0.001$ .

*Training and Testing.* Animals were trained in five trials. Each trial required an animal to cross the length of the ladder to reach the home cage placed at the end of the apparatus. One training session was administered, with the baseline test session on the following day. One test session was performed at baseline and chronic time points. Each test session consisted of three trials during which the animals' performance was videotaped.

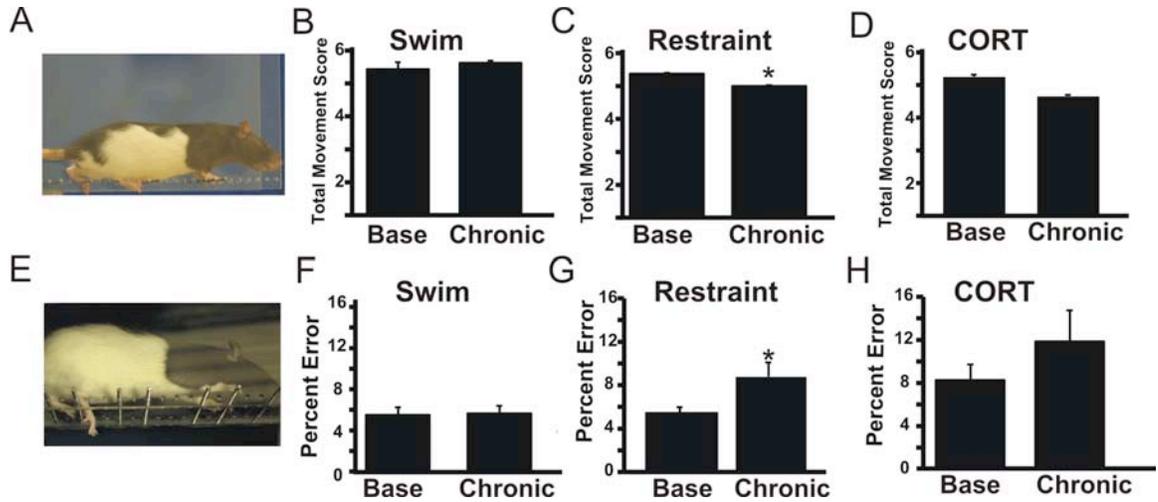
*Video Taping and Analysis.* The ladder rung walking performance was video-recorded from a lateral perspective (Metz and Whishaw, 2002b). The camera was positioned at a slight ventral angle, so that both sides and the paw positions could be recorded simultaneously from a ventral view. The tapes were analyzed frame-by-frame for quantitative and qualitative analysis.

Quantitative analysis was based on the number of errors in each crossing. Based on the limb placement scoring system (see below), an error was defined as each limb placement that involved missing the rung or slipping off the rung (score of 0, 1 or 2 points according to the scale). The mean number of errors per step of each fore- and hind limb was calculated and averaged for three trials.

The qualitative analysis of forelimb and hind limb placements was performed using a foot fault scoring system developed earlier (Metz and Whishaw, 2002b; Figure 5E). Consecutive steps were analyzed, excluding the last step before a pause and the first step after a pause. The last stepping cycle at the end of the ladder rung apparatus was also excluded from scoring. Limb placement was scored by categorizing the placement of the limb on a rung and the limb protrusion between rungs when a miss occurred by using a 7-category scale (Metz and Whishaw, 2002b). A score of 0 indicates a total miss and was

given when the limb completely missed the rung. A score of 1 point indicates a deep slip, as the limb was initially placed on the rung, but then slipped off when weight bearing and caused the limb to fall in-between rungs. A score of 2 indicates a slight slip, as the limb was placed on a rung, but slipped off when weight bearing without causing a fall that interrupted walking. A score of 3 indicates a replacement, as the limb was placed on a rung, but withdrawn before weight bearing and placed on another rung. A score of 4 indicates a correction, with the limb aiming for one rung, but it was then placed on another rung without touching the first one. Alternatively, a score of 4 was given when the limb was repositioned on the same rung. A score of 5 indicates a partial placement, as the limb was placed on the rung with either the wrist or digits of the forelimb or the heel and toes of the hind limb. A score of 6 indicates a correct placement with the mid-portion of the palm weight bearing (Metz and Whishaw, 2002b).

An error was counted when animals missed a rung or slipped off (error scores of 0,1 and 2). Percent error was calculated by dividing the number of errors by total number of steps and multiplying by 100. The number of foot placement errors was calculated as a percentage of the total number of steps in a respective trial.



**Figure 5.** Skilled walking task. (A) Photograph illustrating the skilled walking apparatus in which animals are required to walk across a horizontal ladder. Mean Total Movement Score of animals before (Base) and after (Chronic) exposure to swim stress (B), restraint stress (C), and oral CORT administration (D). (E) Photograph illustrating an animal making an error while crossing the apparatus. Mean percent error of animals before (Base) and after (Chronic) exposure to swim stress (F), restraint stress (G), and oral CORT administration (H). Note the significant decrease in the total movement score and significant increase in the percent error of animals exposed to restraint stress. Significances are given as comparison to baseline values. \*  $p < 0.05$ .

### *Blood Sample Collection*

Rats were individually transported to the surgical suite and immediately put under 4% isoflurane anesthesia. Anesthesia was maintained for approximately 5 min in which 1.0 ml of blood was collected from the tail vein. Blood was sampled using a heparinized butterfly catheter. The blood was transferred to centrifuge tubes, and plasma was obtained by centrifugation at 10,000 g for 8 min. The samples were stored at -20°C. Plasma CORT concentrations were determined by radioimmunoassay using commercial kits (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA; Ishida et al., 2003).

### *Statistical Analysis*

Statistical Analysis was performed using a StatView software package (Version 4.5, Abacus Concepts, Inc., CA, USA, 1996). The blood sample, skilled reaching and skilled walking data were subject to ANOVA and paired t-tests. In addition, significant data are presented in bivariate plots displaying the relationship between two variables (CORT concentration and behaviour). Regression lines and Pearson's correlation coefficients were calculated. A p-value of less than 0.05 was chosen as the significance level for all statistical analyses. All data are presented as mean  $\pm$  standard error.

## **Results**

### *Skilled Reaching Task*

Reaching success is shown in Figure 4. Figure 4B illustrates the reaching success before and during exposure to swim stress. Animals had a significantly lower percent success at the chronic testing time point ( $28.7 \pm 5.3\%$ ) when compared to baseline test

time point ( $50 \pm 2.6\%$ ;  $t(4) = -6.19$ ,  $p < 0.05$ ). Furthermore, restraint stress significantly decreased the reaching success rate by half when compared to baseline (Figure 5C;  $t(44) = -5.95$ ,  $p < 0.001$ ). Figure 5D shows percent success in animals exposed to CORT. Chronic CORT caused moderate reduction of reaching success.

#### *Ladder Rung Walking Task*

*Number of Placement Errors.* Swim stress animals had no change from baseline to the chronic stress testing time point (Figure 5F). Restraint animals made significantly more errors at the chronic testing time point when compared to baseline testing (Figure 5H;  $t(43) = 2.389$ ,  $p < 0.05$ ). Animals administered CORT had no changes in the number of errors from baseline to chronic treatment (Figure 5G).

*Foot Fault Scoring.* Animals exposed to swim stress had no change in their total movement score (Figure 5B). Restraint exposed animals had a significantly lower total movement score during the chronic testing time point when compared to baseline testing (Figure 5C;  $t(44) = -0.596$ ,  $p < 0.05$ ). Animals administered CORT had no change in their total movement score (Figure 5D).

#### *Blood sample Analysis.*

Circulating plasma CORT levels are illustrated in Figure 6. No changes in CORT levels were observed when baseline and chronic stress samples were compared for swim stress (Figure 6A) or restraint stress (Figure 6B). The CORT-treated animals had significantly higher plasma circulating CORT concentration after chronic treatment ( $539.4 \pm 38.3$ ) when compared to baseline ( $368.6 \pm 28.9$ ; Figure 6C;  $t(9) = -4.374$ ,

$p < 0.05$ ). No difference in plasma CORT concentration was observed between 10 min collection and 60 min time points.

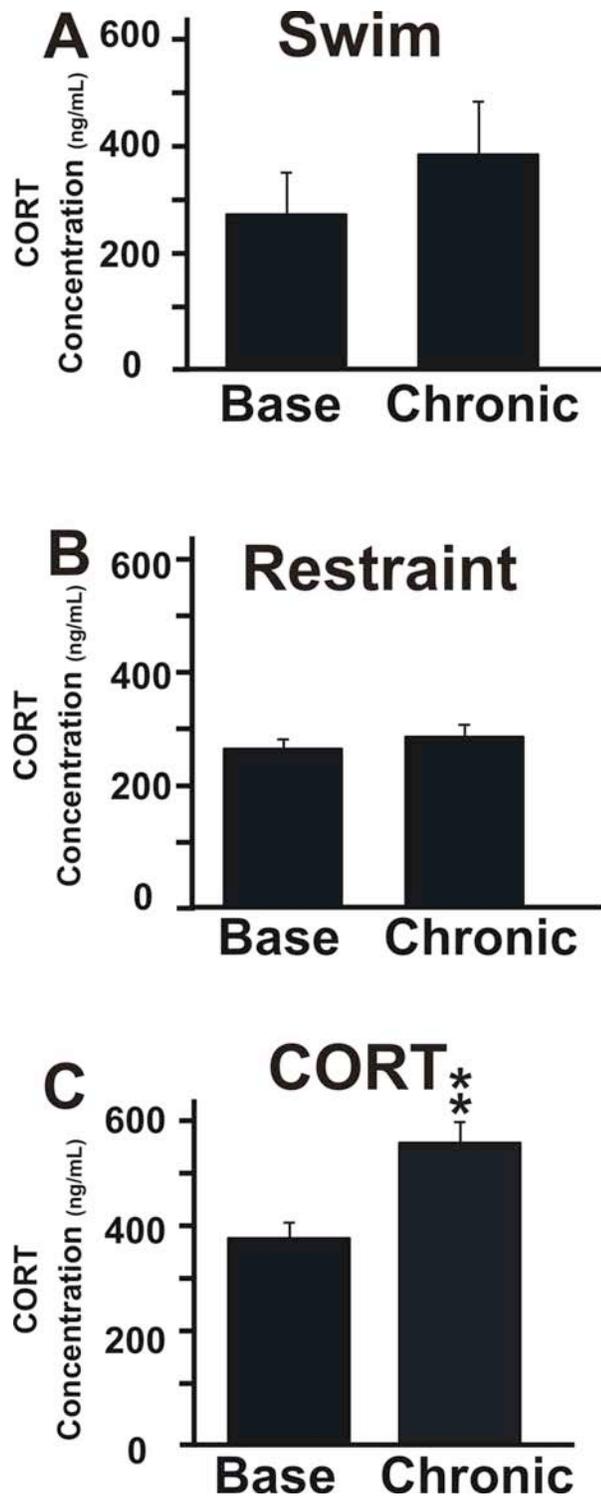
### *Correlation Analysis*

Circulating plasma CORT concentrations underwent correlation analysis with behavioural results (skilled reaching or walking) to determine whether the concentration of circulating CORT was influential on behavioural outcome. Table 1 and Figure 7 highlight the main relationships.

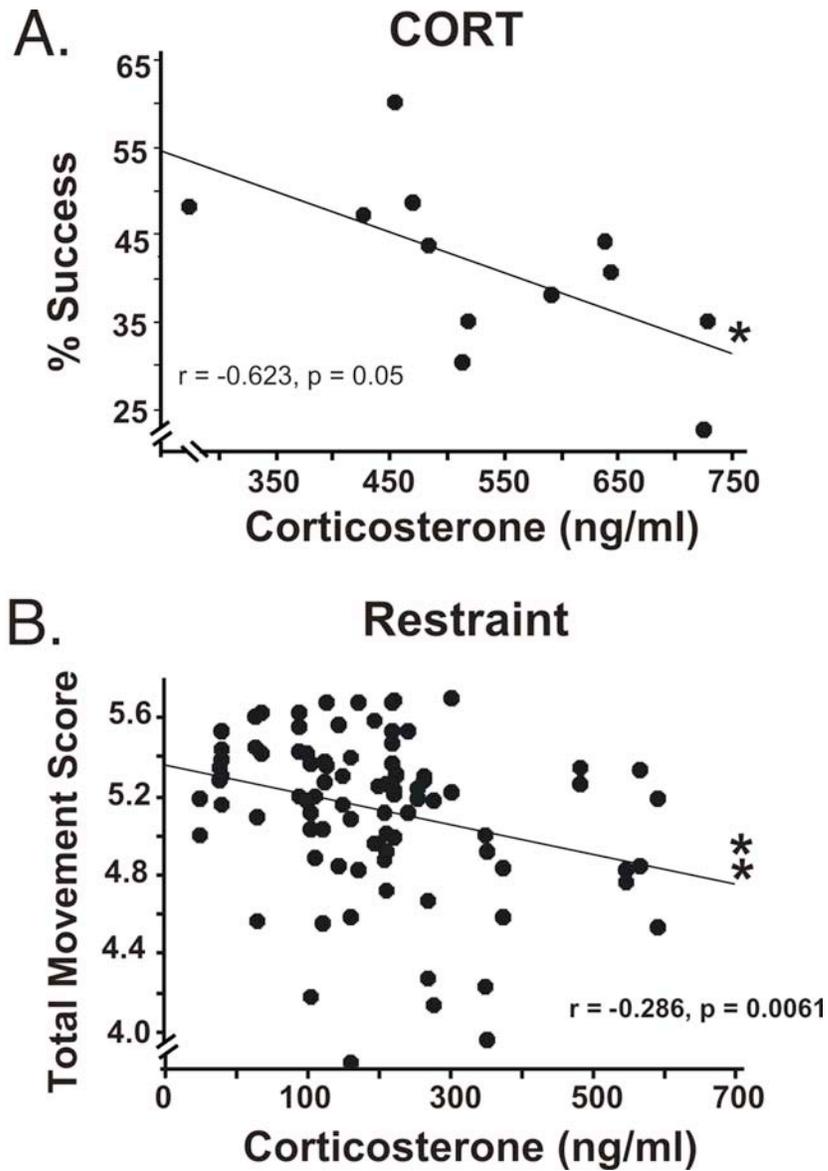
*Effects of Circulating CORT on Skilled Reaching.* There was no relationship between restraint or swim stress and reaching success. Oral CORT administration and percent success were negatively correlated ( $r = -0.623$ ,  $p < 0.05$ ), indicating that animals with higher plasma CORT levels had lower reaching success.

*Effect of circulating CORT on the Number of Errors in Skilled Walking.* There was no correlation between circulating CORT levels and number of errors in any of the groups.

*Effect of Circulating CORT on Total Movement Score in Skilled Walking.* Animals exposed to both swim stress and oral CORT treatments did not have a significant relationship with total movement score. Animals exposed to restraint stress had a negative relationship between total movement score and circulating CORT levels ( $r = -0.286$ ,  $p < 0.01$ ).



**Figure 6.** Plasma CORT levels. Mean CORT concentration of animals before (Base) and after (Chronic) exposure to swim stress (A), to restraint stress (B), and oral CORT (C). Note the significant increase in CORT concentration in animals administered CORT. Significances are given as comparison to baseline values. \*\* $p < 0.01$ .



**Figure 7.** Scatter plots of correlation results. (A) Correlation of CORT concentration and percent success in the skilled reaching task after CORT treatment. (B) Correlation of CORT concentration and total movement score after exposure to restraint stress. Note that the higher CORT concentrations are significantly related to decreased success rates and lower movement scores.

**Table 1.** Comparison of correlation between circulating plasma CORT and skilled motor function. Significant correlations are identified in table. X indicates no relationship.

Behavioural Test	Plasma Corticosterone Concentration		
	Restraint	Swim Stress	CORT.
<b>Reaching</b>	×	Negative $r = -0.6229, p = 0.05$	×
<b>Ladder Beam Movement Score</b>	Negative $r = -0.2864, p = 0.0061$	×	×
<b>Ladder Beam % Score</b>	×	×	×

## **Discussion**

The objective of this study was to investigate whether endogenous CORT serves as the main modulator of motor impairments in response to stress. The study used two models of stress and oral CORT treatment. Animals exposed to swim and restraint stress had a significant decrease in the number of pellets that they obtained successfully. Skilled walking in restraint stress-treated animals was also impaired, however, this stressor did not result in CORT elevation. Although animals treated with oral CORT showed no impairments in skilled movement performance, these animals had elevated CORT levels and revealed a negative significant relationship with reaching success. A significant negative correlation between restraint stress and skilled walking movement score was also observed.

The present study found that both swim and restraint stress caused a significant decrease in skilled reaching success while only restraint stress caused a significant decrease in skilled walking. Restraint stress is referred to as a relatively mild stress, since it mainly represents a psychological stressor, while cold swim stress is often thought to be a moderate stress (Dronjak et al., 2004). The difference in the severity of stress could serve as a possible explanation for the differences in skilled motor impairments. Work by Lucas et al. (2007) has shown that restraint stress exerts effects on neurochemical markers of the motivational system in the rats' midbrain, specifically the dopamine transporter and dopamine receptors. In addition, another study by Kawahara et al. (1999) showed that mesocortical dopamine release is stimulated by the locus coeruleus during the experience of hemodynamic or emotional stress. Dopamine is a key neurotransmitter that is involved in motor function, as shown by the progressive loss of dopamine that

leads to dysfunction in Parkinson's disease (Liss and Roeper, 2007). Exposure to stress has been suggested to change dopamine properties, which may lead to the motor impairments seen in response to stress (Liss and Roeper, 2007; Metz, 2007).

In this study, circulating plasma CORT levels were not significantly elevated in swim stress or restraint-treated animals, whereas CORT concentrations were significantly elevated in animals treated with CORT. Although the classic concept of stress has been based on an increase in stress hormones, recent findings have suggested that an elevation in CORT may not be the single component causing behavioural alterations in response to stress (Smagin et al., 2001; Lucas et al., 2007). Studies have shown that swim and restraint stress cause changes in other components of the stress response, such as increased levels of CRF (Smagin et al., 2001; Dronjak et al., 2004). Furthermore, the motor impairments may also be due to activation of the mineralocorticoid receptor (MR), since CORT binds to the receptor and recent data has suggested that MRs participate in the stress response (Pace and Spencer, 2005).

The data from this experiment suggest that chronic swim and restraint stress does not lead to elevated circulating plasma CORT. A study done by Rodgers et al. (1999) showed an insignificant relationship between elevated plasma CORT levels and duration of time spent in the closed arms of an elevated plus maze, suggesting that circulating plasma CORT concentrations are not related to behavioural changes. However, the study by Rodgers et al. (1999) found a significant positive correlation between risk assessment behaviour and CORT levels. Risk assessment in rats can be measured by how many times the rats explore the open arms of an elevated plus maze (Rodgers et al., 1999). Specifically, both rats and mice had higher circulating plasma CORT concentrations

when they navigated through an elevated plus maze. The elevated plus maze is widely used to study anxiety-related processes, which are often associated with a stress response (Rodgers et al., 1999; Benabid et al., 2007; Metz, 2007). As mentioned previously, Metz (2007) suggests that the anxiety associated with the stress response maybe linked to the skilled motor impairments observed after exposure to stress. Previous work by Metz et al. (2005) has shown that administration of diazepam, an anxiolytic drug, significantly improves skilled motor behaviour and significantly decreases plasma circulating CORT concentrations. Therefore, it can be suggested that increased anxiety associated stress modulates the motor impairments seen after exposure to swim and restraint stress.

Exposure to chronic stress in particular has been shown to cause major changes in animal behaviours, such as learned helplessness. Learned helplessness usually occurs as a result of exposure to uncontrollable stress and is associated with decreases in behavioural function (Shors, 2004; Henn and Vollmayr, 2005; Greenwood and Fleshner, 2008; Wood et al., 2008). Learned helplessness was first observed by a group of psychologists at the University of Pennsylvania that noticed experience of stress can impede the ability to learn new tasks (Shors, 2004). In addition, these animals exhibited symptoms such as sleep and eating disturbances, ulcers and decreased immune functions, leading to the suggestion that learned helplessness is an analog of human depression (Shors, 2004; Greenwood and Fleshner, 2008). Learned helplessness could possibly help explain the motor impairments associated with exposure to stress described in this thesis.

Many of the behavioural changes in stressed rats might be related to alterations in transmitter systems or other endocrine variables. For instance, levels of expression of gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter, and related receptors

were found to be affected by exposure to stress (Doherty and Gratton, 2007; Skillbeck et al., 2008). Furthermore, changes to GABA receptor activity after exposure to restraint and swim stress were shown to be dependent on sex (Skillbeck et al., 2008; Zheng et al., 2007). A study by Doherty et al. (2008) indicated that GABA regulates the release of dopamine within the nucleus accumbens after exposure to stress. At the molecular level, the GABA A receptor has been suggested to regulate phosphorylation of proteins involved in cell signaling in both the hippocampus and prefrontal cortex, after exposure to stress (Zheng et al., 2007).

Aside from providing interesting implications of the effects of stress in motor system function, the present study also had a number of limitations. Firstly, the assessments made in the present study might have overlooked some possible sex differences. Previous works has shown that stress exerts differential effects on male and female Long-Evans rats (Faraday et al., 2002). Sex differences might depend on stressor type and interval, as well as on the kind of behavioural test used. A separate comparison of male versus female rats in the present study might have provided further insight into the effect of stress on motor performance. Secondly, the present study used a relatively small number of animals in the swim stress group (n=5), whereas the restraint stress and oral CORT administration groups had a larger group size (n=10). A follow-up study to increase the number of animals in the swim stress group might enhance the statistical power in order to detect more subtle differences between groups.

## **Conclusion**

The present study extends the current knowledge of mechanisms that cause motor disruptions after exposure to stress. The results suggest that elevated CORT levels are not associated with the motor impairments caused by stress. Increased anxiety might lead to direct GC actions on the motor system as well as indirect effects by affecting non-motor areas that might modulate motor function.

### **3. Experiment 2**

#### **Blocking Glucocorticoid and Mineralocorticoid Receptors Neutralizes Motor Function Impairment Associated With Stress**

##### **Abstract**

Stress is one of the most critical influences on behaviour and performance. Most research has focused on the effects of stress on limbic system functions, including learning and memory, however, recent findings have shown that stress also is a potent modulator of motor control. For instance, stress can impair skilled and non-skilled movements in intact rats. The mechanisms by which stress and stress hormones exert these effects, however, have not been determined yet. Previous studies have suggested that the glucocorticoid receptor (GR) plays a major role in mediating the stress response, however, recent studies have shown the mineralocorticoid receptor (MR) may participate in stress-induced motor impairments. The purpose of this study was to compare the role of GR and MR in stress-associated disruption of motor function in a rat model of stress. Five groups of male and female rats were tested in a skilled reaching task daily throughout the treatment period. The first group was tested on the skilled reaching task and in an open field while administered agonists for MR (aldosterone; 500 ug/kg) or GR (dexamethasone; 750 ug/kg) p.o. each for 3 days. The remaining four groups received daily treatments of either restraint stress or oral corticosterone for 14 days. On day 1 and day 13 and 14 of either treatment, rats were administered antagonists for either GR (RU-486, mifepristone; 50 mg/kg) or MR (RU-28318, spironolactone; 100 mg/kg) p.o. While both acute and chronic stress and corticosterone treatments reduced skilled reaching

success, administration of both the GR and MR antagonists neutralized these effects by protecting skilled reaching success. In addition, administration of aldosterone and dexamethasone caused a significant decrease in the success rate of animals when compared to baseline testing. There was no difference between male and female rats in the response to any of the treatments. These observations suggest that both GR and MR activation plays a central role in modulating motor system function.

## **Introduction**

One of the main features of the stress response has been characterized as an increase in circulating glucocorticoid (GC) levels (Seyle, 1976; de Kloet et al., 1999). GCs include the main stress hormone in humans, cortisol, and corticosterone (CORT) in rodents. GCs readily cross the blood brain barrier and affect the central nervous system by binding to two types of receptors, the high-affinity mineralocorticoid or type 1 receptor (MR) and the low affinity glucocorticoid or type 2 receptor (GR; de Kloet et al., 1999; de Kloet et al., 2005).

The MR has a ten-fold higher binding affinity for GCs as compared to the GR (Moirmoto et al., 1996; Spencer et al., 1998; de Kloet et al., 1999; Rogerson and Fuller, 2000). Thus, the MR is mainly involved in binding GCs during non-stress conditions (Cole et al., 2000; Pace and Spencer, 2005). For example, low levels of GCs act via MR to maintain low basal activity of the hypothalamo-pituitary-adrenocortical (HPA) axis (Pace and Spencer, 2005). Recent findings, however, suggest that the MR may also become activated during a stressor challenge. The classical notion suggests that the MR is not activated during the stress response as MRs become saturated during basal CORT

levels (Smythe et al., 1997; Spencer et al., 1998; Pace and Spencer, 2005; Derijk et al., 2006). In this case GRs, but not MRs, would become activated when CORT is elevated. A recent study by Pace and Spencer (2005), however, indicated that MR mediates negative feedback to downregulate HPA axis activity in response to a mild stressor, but not in response to a moderate stressor, such as restraint. Accordingly, using restraint stress Cole et al. (2000) showed that an MR antagonist does not alter CORT secretion, whereas a GR antagonist was found to modulate CORT secretion in animals exposed to restraint. Based on rates of MR and GR occupancy, Spencer et al. (1998) concluded that MR activation is critical in facilitating GR-dependent regulation of HPA axis activity when GC levels are high. Furthermore, MRs have been suggested to be involved in identifying stress severity during onset of the stress response, yet the role of MR in feedback inhibition of the HPA axis still remains unclear (de Kloet et al., 2005).

GRs, which have a lower affinity for GCs than MR, are thought to be mainly activated when GC levels are elevated. GRs constrain HPA axis activity during and after presentation of stressors via negative feedback in order to downregulate GC secretion (Spencer et al., 1998; Bamberger et al., 1996; Bauchman et al., 2003). Thus, blocking the GR may abolish a rise in CORT levels in response to stress (Moldow et al., 2005). Because of the prominent role of GR in the stress response, it is generally believed that stress-induced behavioural changes are mainly mediated by this receptor. Behavioural alterations associated with chronic stress include altered sensory function (Swiergiel et al., 2007), diminished learning and memory (Holscher, 1999), anxiety and depression (Pohl et al., 2007), and motor function (Metz et al., 2001, 2005).

Recent evidence suggest that both GR and MR play a role in normal HPA axis functioning and behaviour (Devenport et al., 1990). For example, studies have confirmed a role for MR in stress-associated emotional changes. Smythe et al. (1997) showed that blocking hippocampal MR can alleviate CORT-induced anxiety in the Black-White box task, indicating that MRs are directly involved in anxiety. The differential role for GR and MR in behaviour, however, has not been analyzed in other behavioural paradigms.

While most previous studies comparing the function of GR and MR focused on the limbic system, the present study extends these studies to elaborate the role of GR and MR in affecting motor system function. The study compared the effects of GR and MR activation on motor performance, and blocking GR and MR function during the response to acute and chronic restraint stress, and supplemental CORT treatment. Animals were tested in skilled reaching and exploration of an open field. The results revealed that acute and chronic treatment with either MR or GR blocks motor impairments observed in animals exposed to restraint stress or oral CORT administration. Activation of the MR or GR causes similar motor impairments seen in animals exposed to restraint stress and oral CORT administration.

## **Materials and Methods**

### *Subjects*

Subjects were 64 adult male and 64 adult female Long-Evans hooded rats, raised at the University of Lethbridge vivarium and weighing 250-600g at the beginning of the experiment. Animals were initially housed in groups of two or three in shoebox cages

under a 12:12 h light/dark cycle with light starting at 07:30 h. The housing room was maintained at a room temperature of 20°C and 30% relative humidity.

Prior to the experiment, rats were food deprived to 95% of their initial body weight to encourage participation in the reaching task. To maintain body weight, supplementary food was given daily in their home cages five hours after behavioural testing was completed. Animals were weighed daily before testing commenced. Water was available *ad libitum*. The animal experiment was performed according to the standards set by the Canadian Council of Animal Care.

### *Experimental Design*

Testing and training of the animals was performed during the light phase of the cycle each day. Figure 8 illustrates the time course of manipulations and behavioural assessments. Two experiments were performed, each involving animals pre-trained in the skilled reaching task. Baseline measurements began after 21 days of daily training and were taken on 5 consecutive days. Video recordings of skilled reaching and open field exploration were taken the day before the onset of stress (baseline). Experiment 1 investigated the effects of GR and MR activation on motor performance (Figure 8A). Animals were administered either a selective MR agonist (aldosterone; 10 males, 10 females) or a selective GR agonist (dexamethasone; 10 males, 10 females). Video recordings of skilled reaching and open field were taken the day before the administration of the drugs. Animals were administered aldosterone from day 6 to day 8 of testing. On day 6 and 7 video recordings of skilled reaching and open field were taken. On day 8 blood samples were collected. From day 9 to 15, animals were given a treatment break

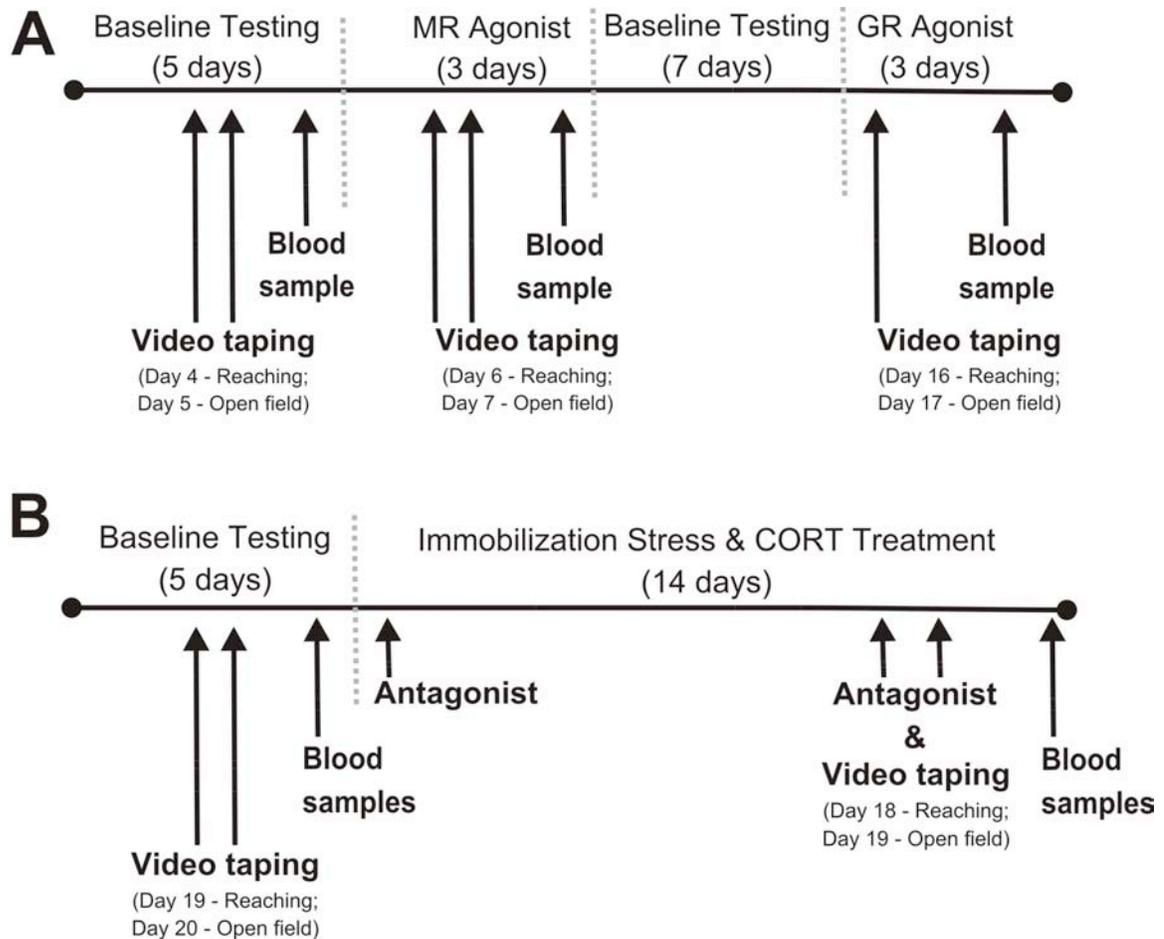
during which their reaching success remained at baseline levels. Animals were then administered dexamethasone from day 16 to 18. On day 16 and 17 video recordings of skilled reaching and open field were taken. On day 18 blood samples were collected again.

Experiment 2 investigated the role of GR and MR in mediating stress-induced disturbance of motor function (Figure 8B). Animals were divided into six groups, each with an equal number of male and female rats: Restraint and GR antagonist RU-486 (n=20); Restraint Stress and MR antagonist RU-28318 (n=20); CORT and GR antagonist RU-486 (n=20); CORT and MR antagonist RU-28318 (n=20); Restraint only (n=20); CORT only (n=8). All animals were exposed to 14 days of physiological manipulations (restraint or CORT treatment). On day 18 and 19, skilled reaching and open field tasks were video recorded. On day 20, of treatment, blood samples were collected.

### **Physiological Manipulations**

*Restraint Stress.* Animals were placed in a transparent Plexiglas container (5 cm inner diameter) for a period of 20 min. each day (Garcia et al., 2000; Faraday, 2002; Mercier et al., 2003). The container had perforated ends to allow for ventilation. The container maintained the animals in a standing position without compression of the body.

*Oral CORT Administration.* Animals received 5 mg of oral CORT treatment (Sigma-Aldrich, Canada). The CORT was administered at the same time of day mixed with cookie crumbs, water and oil for consumption.



**Figure 8.** Time chart illustrating the order of manipulations and behavioural tests. (A) Exp. 1: MR and GR agonist treatments. Skilled reaching and open field activity performance were assessed prior to treatment (baseline). Animals were administered the MR agonist on day 8 to 10. Video recordings of skilled reaching and open field were taken on day 8 and 9. Animals were tested on skilled reaching from day 1 to 17. The GR agonist was administered from day 18 to 19. Video recordings of skilled reaching and open field were taken on day 18 and 19. Blood samples for glucose assessments were collected on day 7, 10 and 20. (B) Exp. 2: Restraint and CORT treatment with GR and MR antagonist treatment. Skilled reaching and open field activity were assessed from video recordings prior to (baseline) and at chronic time points. GR or MR antagonists were administered on day 1, 13 and 14 of restraint or CORT treatment. Blood samples for glucose measurements were collected at baseline and chronic testing time points.

*Oral GR and MR Antagonist and Agonist Drug Administration.* Animals received oral treatment of the GR antagonist, RU-486 (mifepristone; Sigma-Aldrich, Canada) 50 mg/kg 22 hours prior to testing (Krugers et al., 2006). The MR antagonist, RU-28318 (spironolactone; Sigma-Aldrich, Canada) was given at 100 mg/kg one hour prior to testing (Cole et al., 2000; Pace and Spencer, 2005). The MR agonist, aldosterone (Sigma-Aldrich, Canada) was administered at 500 µg/kg one hour prior to testing (Devenport and Thomas, 1990). The GR agonist, dexamethasone (Sigma-Aldrich, Canada) was given at 750 µg/kg one hour prior to testing (Ginsberg et al., 2006). All drug doses and times administered were used in rats previously (Krugers et al., 2006; Cole et al., 2000; Pace and Spencer, 2005; Devenport and Thomas, 1990; Ginsberg et al., 2006). In these studies, the reported doses led to reproducible physiological changes. The drugs were administered at the same time of day and mixed with cookie crumbs, water and oil for consumption.

## **Behavioural Testing and Analysis**

### *Skilled Reaching Task*

*Skilled Reaching Task Apparatus.* The reaching box was made of clear Plexiglas (40 x 45 cm and 13.1 cm wide). Animals were trained to extend a forelimb to reach for food pellets through a 1.3 cm wide vertical opening in the middle of the front wall (Figure 9A). The vertical opening extended from the floor to a height of 15 cm. To hold the food pellet, a 2 cm wide by 4 cm long shelf was positioned outside the front wall of the box. The shelf was mounted 4 cm above the floor. Food pellets (45 mg banana flavored Dustless Precision Pellets, Bioserv Inc., Frenchtown, NJ) were placed in one of

two small indentions on the shelf. The indentations, each 5 mm in diameter and 1.5 mm deep, were 2 cm away from the inside wall of the box and were centered on the edges of the slit through which the rats reached (Metz and Whishaw, 2000).

*Training and Testing.* Once rats began to reach for food, food pellets were placed individually in the indentation contralateral to the limb which the rat used for reaching. Between individual reaching movements, rats were required to leave the food aperture and walk to the rear of the box in order to reposition themselves prior to the next reach. Each training and testing session required the rats to reach for 20 food pellets. Reaching performance was scored by counting misses and successful reaches for each limb (Metz and Whishaw, 2000). An ‘attempt’ was defined as a forelimb movement towards the pellet. A ‘success’ was recorded if an animal grasped a food pellet on the first attempt and withdrew the paw with the pellet through the slit to consume the food (Metz and Whishaw, 2002a). A ‘miss’ was recorded if an animal touched and missed the pellet using more than one attempt to grasp it. A ‘drop’ was scored if the animal lost the pellet in the cage after grasping it. Percent reaching success was calculated by counting the number of successful reaches divided by the number of pellets given in each session (20) multiplied by 100.

*Video Taping and Analysis.* On the last day of baseline, and chronic drug treatment sessions, the animals’ performance was video recorded from a frontal view for qualitative movement analysis. The rating of the reaching movements was performed from the videotapes by frame-by-frame inspection. The first three successful reaches were scored. Movements were analyzed using a framework derived from the Eshkol-Wachman movement notation which allows analysis of the relations and changes of

relations between parts of the body and limbs (Eshkol & Wachmann, 1958). The following movement components of reaching were analyzed from a frontal view (Whishaw and Pellis, 1999; Metz and Whishaw, 2000; Whishaw, 2000): (1) *Orient*: the head is oriented towards the target and the snout is inserted through the slit to locate the pellet. (2) *Limb lift*: the mass of the body weight is shifted to the hind limbs, and the hind limbs are aligned with the body and parallel to each other. The forelimb is lifted so that the digits are aligned with the midline of the body. (3) *Digits close*: the palm is partially supinated and approaches the midline of the body; the digits are semi-flexed. (4) *Aim*: the elbow comes in to the body with a shoulder movement while the digits retain their position on the midline of the body. (5) *Advance*: the elbow is positioned in a narrow angle to the body; the forelimb moves forward and is directed to the target. The head and the upper body are raised and the weight is shifted to the front. This movement is accompanied by a moderate lateral body movement towards the reaching limb. (6) *Digits open*: the digits are opened by a discrete limb movement; the palm is not fully pronated. (7) *Pronation*: the elbow adducts and is pronated over the target in an arpeggio movement. (8) *Grasp*: the arm remains still, while the digits close and then the paw is lifted holding the food pellet. (9) *Supination I*: the elbow is adducted and the palm is supinated by approximately 90°. (10) *Supination II*: The palm is supinated to present the food pellet to the mouth. The head drops to the level of the paws and the rat sits back on the haunches. (11) *Release*: the food pellet is released into the mouth by opening the digits.

For each of the eleven movement components, a score of 0 was given when the movement was absent, a score of 0.5 was given if the movement was present but

abnormal, and a score of 1 was given if the movement was normal (Metz and Wishaw, 2000).

### *Open Field Task*

*Open Field Apparatus.* The open field box, measuring 100 x 100 x 18 cm, was made of opaque black Plexiglas. The bottom of the box was divided into 16 zones (22 x 22 cm) using white masking tape.

*Testing.* Each rat was individually placed in the middle of the open field box and video recorded for 5 min with a camera mounted above open field. After testing of each rat was completed, the floor of the box was cleaned with a disinfectant.

*Video Taping and Analysis.* Video recordings were scored for activity (number of fields entered), number of rears, novel fields entered and % time spent in the center and outside fields. Entered fields were scored when more than 50% of the animal's body crossed a subdivision of the open field.

### *Video Recording Procedures*

Videotaping in all tasks was performed using a Sony ZR70 portable digital video camera. The shutter speed was set at 1/1000s. Tapes were analyzed frame-by-frame on a Sony Mini DV player. The testing setup was illuminated by a white light source (Lowe Inc.). In addition, the skilled reaching apparatus was also illuminated by a two-arm cold light source (Zeiss KL 1500, Carl Zeiss Inc., Jena, Germany).

### *Blood Glucose Analysis*

Rats were anesthetized using 4% isoflurane. Anesthesia was maintained for 5 minutes in which 1.0 ml of blood was collected from the tail vein. An Ascensia Breeze Blood Glucose Meter (Bayer, Toronto, ON) was used to analyze glucose levels in animals.

### *Statistical Analysis*

The statistical analysis was performed using a SPSS software package 11.5 (SPSS Inc., IL, 2002). The results were subject to analysis of variance (ANOVA) for repeated measurements across testing sessions and for overall sex differences. Comparisons of means and variances between and among groups were performed using unpaired t-tests, and paired t-tests for within-subject comparison. In all statistical analyses, a p-value of less than or equal to 0.05 was considered significant. All data are presented as mean +/- standard error of the mean (SEM).

## **Results**

### *Skilled Reaching Success*

There was no significant difference between males and females in any of the seven groups. During baseline testing animals obtained approximately 9 out of 20 pellets, resulting in a  $46.6 \pm 2.51\%$  success rate.

*MR and GR Agonist.* There was no significant difference between animals over the testing days ( $F(1,10)=1.01$ ,  $p=0.490$ ). Animals had a significantly lower success rate on day 6 and 7 of MR agonist administration compared to the last day of baseline (Figure

9B;  $t(19)=10.667$ ,  $p<0.001$ ;  $t(19)=3.290$ ,  $p<0.001$ ). Animals treated with GR agonist had a significantly lower success rate on days 16 and 17 when compared to day 15 ( $t(19)=10.338$ ,  $p<0.001$ ;  $t(19)=16.884$ ,  $p<0.001$ , respectively).

*Stress and MR or GR Antagonist.* There was no overall difference between testing days for either the MR or GR antagonists ( $F(1,17)=25.56$ ,  $p=0.35$ ;  $F(1,12)=0.481$ ,  $p=0.872$ , respectively). Animals in both restraint groups had a 10% reduction in reaching success on day 1 of stress and GR or MR antagonist treatment when compared to the last day of baseline testing ( $t(22)=2.176$ ,  $p<0.05$ ;  $t(26)=3.144$ ,  $p<0.01$ , respectively). On stress day 2 animals administered the GR antagonist had a 20% lower success rate when compared to day 1 of stress and drug treatment ( $t(22)=7.780$ ,  $p<0.001$ ). On day 13 and 14 of chronic stress and GR or MR antagonist treatment animals had doubled their success rate when compared to day 12 of restraint stress ( $t(22)=-6.362$ ,  $p<0.001$ ;  $t(22)=-8.894$ ,  $p<0.001$ ;  $t(26)=-8.30$ ,  $p<0.001$ ;  $t(26)=9.15$ ,  $p<0.001$ , respectively). There was an overall significant difference between MR antagonist animals when compared to stress controls ( $F(1,45)=25.56$ ,  $p<0.001$ ). GR antagonist treated animals had a trend for an overall difference when compared stress controls ( $F(1,41)=3.260$ ,  $p=0.078$ ). On day 6, 18 and 19 of stress and MR or GR antagonist treatment animals had a significantly higher percent success rate when compared to stress controls (Figure 9C;  $t(40)=9.086$ ,  $p<0.001$ ;  $t(45)=6.935$ ,  $p<0.001$ ;  $t(40)=2.652$ ,  $p<0.05$ ;  $t(45)=3.551$ ,  $p<0.001$ ;  $t(40)=4.747$ ,  $p<0.001$ ;  $t(45)=5.615$ ,  $p<0.001$ , respectively).

*CORT Treatment and MR or GR Antagonist.* There was no overall difference between testing days for either the MR or GR antagonists ( $F(1,16)=0.405$ ,  $p=0.0914$ ;  $F(1,15)=2.404$ ,  $p=0.061$ , respectively). Animals had a significantly lower success rate on

day 1 of CORT and MR antagonist treatment when compared to baseline (data not shown;  $t(24)=2.125$ ,  $p<0.05$ ). Animals on day 1 of CORT and GR or MR antagonist treatment had significantly higher success rates when compared to day two of CORT treatment only ( $t(25)=5.997$ ,  $p<0.001$ ;  $t(24)=2.125$ ,  $p<0.05$ , respectively). There was an overall difference between stress and MR or GR antagonist when compared to CORT controls animals ( $F(1,32)=12.98$ ,  $p<0.01$ ;  $F(1,32)=29.78$ ,  $p<0.001$ , respectively). On day 1, 13 and 14 of CORT and MR or GR antagonist treatment animals had a significantly higher success rate when compared to CORT control animals (Figure 9D;  $t(32)=4.291$ ,  $p<0.001$ ;  $t(32)=4.866$ ,  $p<0.001$ ;  $t(32)=6.619$ ,  $p<0.001$ ;  $t(32)=6.461$ ,  $p<0.01$ ;  $t(32)=4.847$ ,  $p<0.001$ ;  $t(32)=3.829$ ,  $p<0.001$ , respectively).

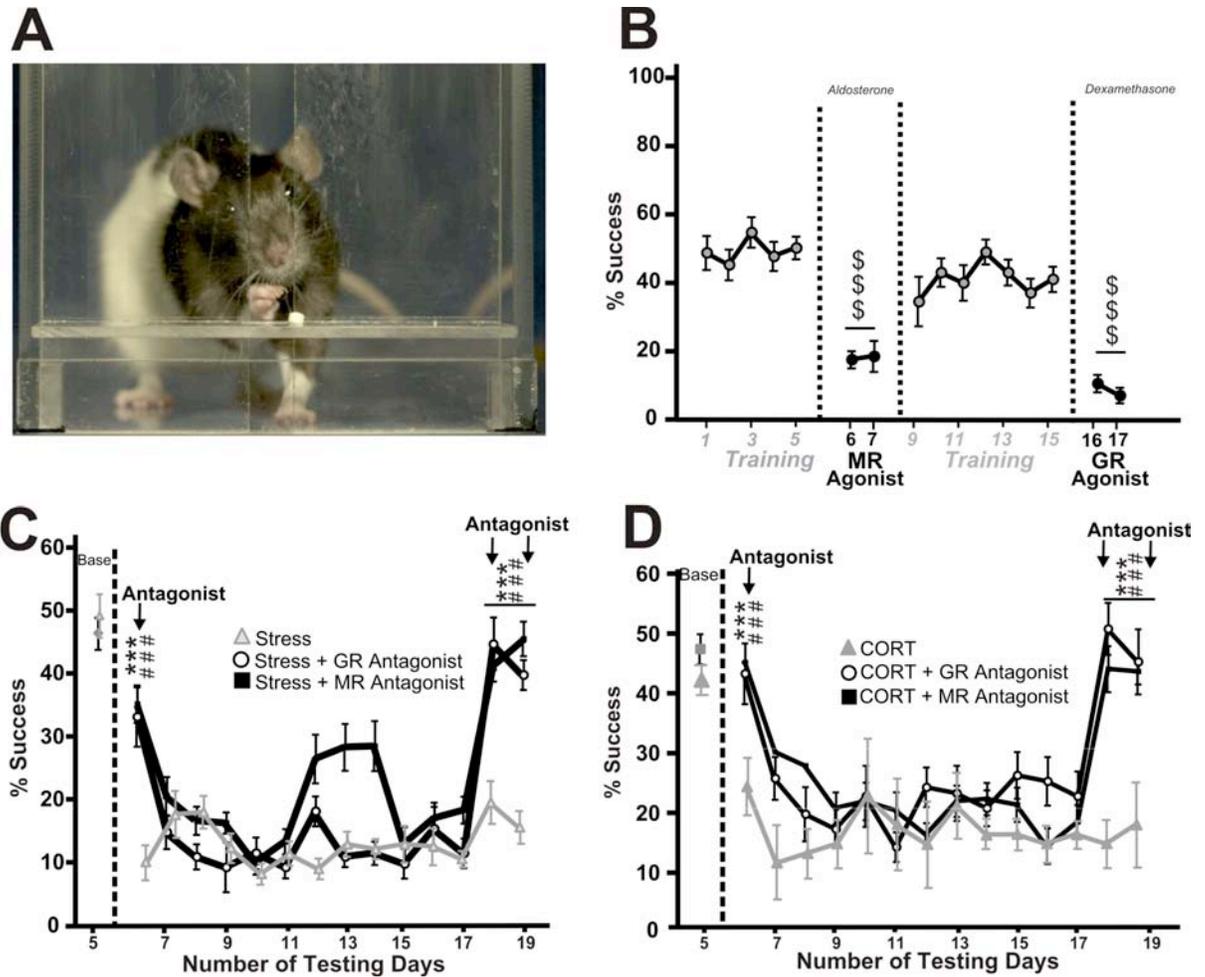
#### *Skilled Reaching Number of Attempts*

There was no significant statistical difference between males and females in any of the groups. Therefore, male and female animals were grouped together.

*MR and GR Agonist.* There was no overall difference between testing days ( $F(1,10)=0.657$ ,  $p=0.731$ ). Animals administered either MR or GR agonist made significantly more attempts to obtain pellets on the first day of drug administration when compared to baseline values (Figure 10A;  $t(19)=-11.816$ ,  $p<0.001$ ;  $t(19)=-4.865$ ,  $p<0.001$ , respectively)

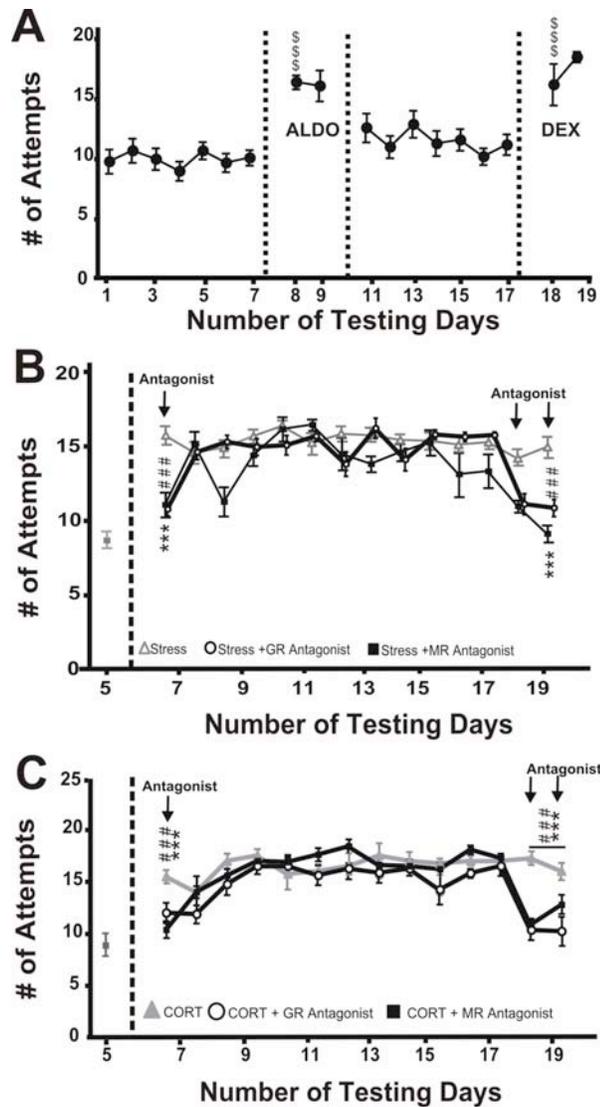
*Stress and MR or GR Antagonist.* There was no overall difference between testing days ( $F(1,17)=1.18$ ,  $p=0.37$ ;  $F(1,11)=0.263$ ,  $p=0.98$ , respectively). Animals administered either the GR or MR antagonist made significantly more attempts to obtain pellets on day 2 of stress when compared to day 1 of stress and GR antagonist (Figure

10A;  $t(22)=-6.794$ ,  $p<0.001$ ;  $t(26)=-3.138$ ,  $p<0.01$ , respectively). Animals made significantly less attempts on day 13 and day 14 of chronic stress and GR or MR antagonist treatment when compared to day 12 of stress ( $t(22)=6.471$ ,  $p<0.001$ ;  $t(22)=7.232$ ,  $p<0.001$ ;  $t(26)=6.499$ ,  $p<0.001$ ;  $t(26)=7.605$ ,  $p<0.001$ , respectively). Animals made significantly less attempts on day 14 of chronic stress and MR antagonist treatment when compared to animals on day 1 of acute stress and MR antagonist treatment ( $12 + 0.63$ ;  $t(26)=2.678$ ,  $p<0.05$ ). There was a trend for overall significant difference between animals administered MR antagonist animals compared to stress controls ( $F(1,41)=3.67$ ,  $p=0.062$ ). Animals administered GR antagonist had no overall difference when compared to controls ( $F(1,40)=0.882$ ,  $p=0.353$ ). On day 1,13 and 14 of stress and GR or MR antagonist treatment animals made significantly less attempts when compared to stress controls (Figure 10B;  $t(41)=-8.548$ ,  $p<0.001$ ;  $t(45)=5.745$ ,  $p<0.001$ ;  $t(41)=-2.093$ ,  $p<0.05$ ;  $t(45)=-3.326$ ,  $p<0.01$ ;  $t(41)=-4.192$ ,  $p<0.001$ ;  $t(45)=-5.535$ ,  $p<0.001$ , respectively).



**Figure 9.** Quantitative skilled reaching performance. (A) Photograph illustrating the skilled reaching task in which rats were required to grasp and retrieve individual food pellets. (B) Daily percent success of animals treated with MR and GR agonist. Note the significant reduction in percent success on Day 6 and 7 of MR agonist treatment and Day 16 and 17 of GR agonist treatment when compared to last day of training. Daily percent success of animals administered either MR or GR antagonist exposed to restraint stress (C) and oral CORT administration (D). Note the significant enhancement in percent success of GR or MR antagonist treated animals compared to controls on Day 1, 18 and 19. Dollar signs (\$) indicate significances between the last day of training and MR or GR agonist treatment days. Asterisks (\*) indicate significances between MR and control animals. Number signs (#) indicate significances between GR and control animals. \*  $p < 0.05$ ; \*\*\*  $p < 0.001$ , unpaired t-test comparison between animals, respectively.

*CORT Treatment and MR or GR Antagonist.* There was no overall difference between testing days for either the MR or GR antagonists ( $F(1,16)=0.389$ ,  $p=0.923$ ;  $F(1,15)=1.59$ ,  $p=0.202$ , respectively). Animals made significantly more attempts on day 1 of acute CORT treatment and MR antagonist when compared to day two of CORT treatment only ( $t(25)=-4.010$ ,  $p<0.001$ ;  $t(25)=-3.536$ ,  $p<0.01$ , respectively). Animals made significantly less attempts on day 13 and 14 of chronic CORT treatment and GR or MR antagonist when compared to day 12 of CORT treatment only ( $t(25)=8.092$ ,  $p<0.001$ ;  $t(25)=8.005$ ,  $p<0.001$ ;  $t(25)=8.411$ ,  $p<0.001$ ;  $t(25)=9.369$ ,  $p<0.001$ , respectively). There was an overall difference between animals administered MR or GR antagonists when compared to controls ( $F(1,32)=6.84$ ,  $p<0.05$ ;  $F(1,32)=8.25$ ,  $p<0.01$ , respectively). On day 1, 13 and 14 of CORT and MR or GR antagonist treatment animals made significantly less attempts when compared to CORT controls (Figure 10C;  $t(32)=-4.334$ ,  $p<0.001$ ;  $t(32)=-5.246$ ,  $p<0.001$ ;  $t(32)=-6.869$ ,  $p<0.001$ ;  $t(32)=-6.512$ ,  $p<0.001$ ;  $t(32)=-5.9038$ ,  $p<0.001$ ;  $t(32)=-4.130$ ,  $p<0.001$ , respectively).



**Figure 10.** Number of attempts to grasp a single food pellet. Number of attempts of animals administered MR (ALDO, aldosterone) and GR agonist (DEX, dexamethasone ;A). Note the significant increases in the number of attempts on days 8, 9, 18 and 19 of drug treatment when compared to training days. Number of attempts of animals administered MR and GR antagonist exposed to restraint stress (B) and oral CORT administration(C). Note the significant reductions in the number of attempts on days 1, 13 and 14 of drug treatment when compared to control animals. Dollar signs (\$) indicate significant differences between the last day of training and MR or GR agonist treatment days. Asterisks (\*) indicate significant differences between MR and control animals. Number signs (#) indicate significance in comparisons between GR and control animals. \* p<0.05; \*\*\* p<0.001, unpaired t-test comparison between drug administered animals or training days.

### *Skilled Reaching Movement Score*

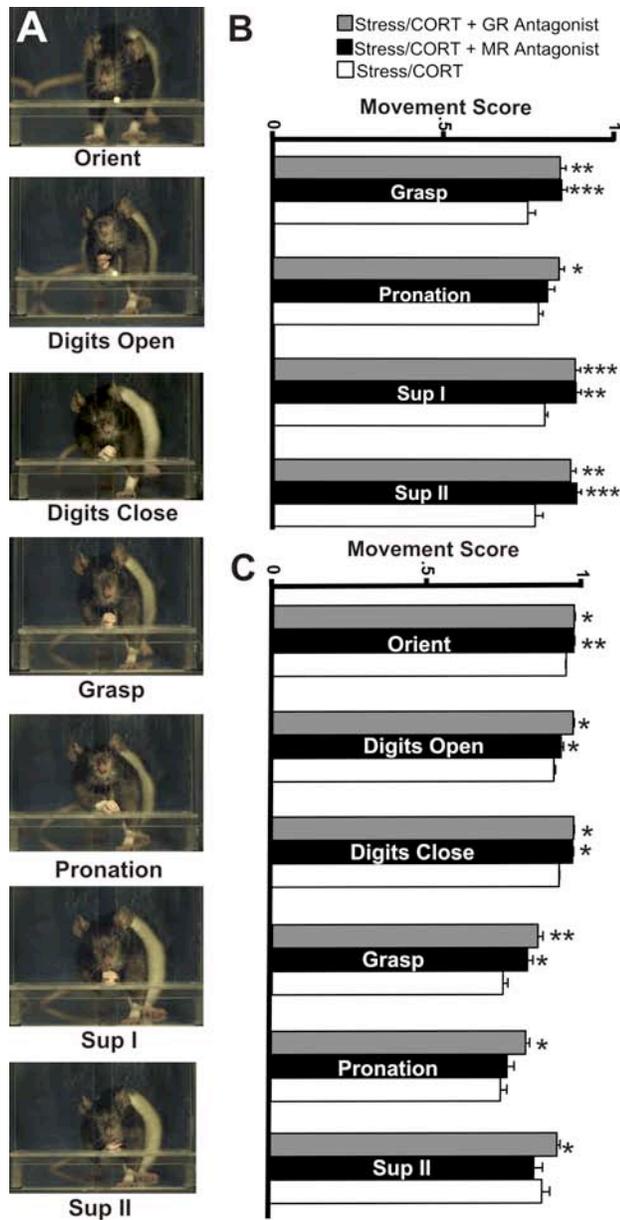
*MR and GR Agonist.* Animals administered the MR agonist had a significantly digits open movement score when compared to animals administered the GR agonist ( $t(17)=-2.170$ ,  $p<0.05$ ). In addition there was an overall sex difference during all three testing time points in the aim movement component of animals administered MR agonist. Females had a significantly lower score when compared to males ( $F(1,14) = 16.243$ ,  $p<0.01$ ).

*Stress and MR or GR Antagonist.* There was no overall difference between males and females administered the GR or MR antagonist, so male and female animals were grouped together. There was no difference between the GR antagonist and stress controls in the total movement score at the baseline and chronic time points. Animals administered the MR antagonist had a higher total movement score than stress controls ( $t(48)=5.036$ ,  $p<0.001$ ). Animals exposed to chronic stress and GR antagonist had significantly higher pronation movement score when compared to baseline ( $t(21)=-3.505$ ,  $p<0.01$ ). Animals administered the GR antagonist had a significantly higher baseline release movement score when compared to animals exposed to chronic restraint stress (Figure 11A;  $t(21)=2.625$ ,  $p<0.05$ ). Animals administered GR antagonist had a higher pronation movement score when compared to stress controls ( $t(40)=2.626$ ,  $p<0.05$ ). Animals administered GR or MR antagonist had a significantly higher grasp, supination I and supination II score when compared to stress controls (Figure 11B;  $t(39)=3.263$ ,  $p<0.01$ ;  $t(48)=4.305$ ,  $p<0.001$ ;  $t(40)=4.436$ ,  $p<0.001$ ;  $t(48)=3.565$ ,  $p<0.001$ ;  $t(48)=3.553$ ,  $p<0.01$ ;  $t(40)=4.436$ ,  $p<0.001$ , respectively).

*CORT Treatment and MR or GR Antagonist.* There was no difference in males and females, therefore they were grouped together. Animals administered the GR antagonist had a significantly higher movement score when compared to baseline ( $t(25)=-4.608$ ,  $p<0.001$ ). No difference was observed in MR antagonist-treated animals. Animals administered the GR antagonist had a significantly higher total movement score when compared to CORT controls ( $t(26)=3.569$ ,  $p<0.01$ ). Both GR or MR antagonist administration caused elevation in orient, digits close and grasp movement scores when compared to CORT controls (Figure 11C;  $t(26)=2.703$ ,  $p<0.05$ ;  $t(32)=2.768$ ,  $p<0.01$ ;  $t(26)=2.793$ ,  $p<0.05$ ;  $t(32)=0.049$ ,  $p<0.05$ ;  $t(26)=3.133$ ,  $p<0.01$ ;  $t(32)=2.131$ ,  $p<0.05$ , respectively). In contrast, only the GR antagonist led to elevation of digits open scores, while MR antagonist reduced these scores ( $t(26)=2.530$ ,  $p<0.05$ ;  $t(32)=2.063$ ,  $p<0.05$ ). Animals administered the GR antagonist had significantly higher pronation and supination II movement scores when compared to CORT controls ( $t(26)=2.725$ ,  $p<0.05$ ;  $t(26)=2.126$ ,  $p<0.05$ , respectively).

### *Open Field*

*MR and GR Agonist.* There was no significant sex difference in animals administered either GR or MR antagonist. Animals were significantly more active when administered either the MR or GR agonist compared to baseline testing ( $t(19)=-4.183$ ,  $p<0.01$ ;  $t(18)=-5.397$ ,  $p<0.001$ ; respectively). Animals administered the MR ( $30.4 \pm 1.04$ ) and GR ( $31.7 \pm 1.4$ ) agonist made significantly more rears when compared to baseline ( $21.2 \pm 1.8$ ;  $t(18)=-5.235$ ,  $p<0.001$ ;  $t(18)=-5.186$ ,  $p<0.001$ , respectively).

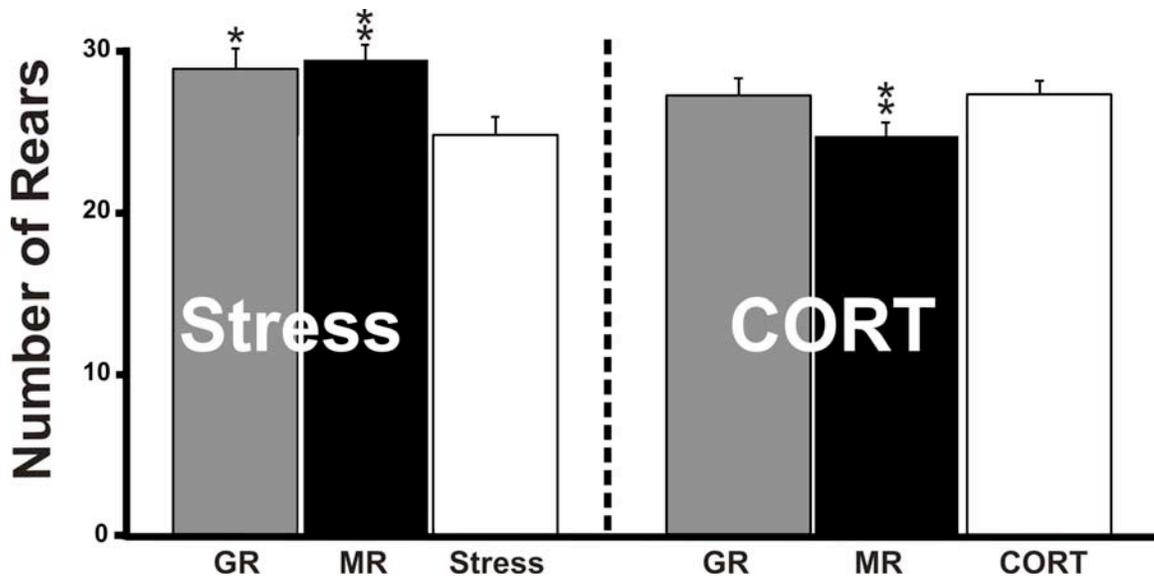


**Figure 11.** Qualitative skilled reaching performance. (A) Photographs of reaching movement components. Movement score for animals exposed to chronic restraint stress (B) and chronic CORT (C). Note that animals administered either GR or MR antagonist had a significantly higher movement score when compared to controls. Asterisks indicate significant differences between MR or GR and control animals. \*  $p < 0.05$ ; \*\*\*  $p < 0.001$ , unpaired t-test.

*Stress and MR or GR Antagonist.* There was an overall significant sex difference between the males and females in animals administered the GR antagonist ( $F(1,21)=11.536$ ,  $p<0.01$ ). There were no sex or group differences in activity or novel fields entered. Females reared significantly more after chronic restraint stress when compared to baseline (Figure 12;  $t(11)=-2.984$ ,  $p<0.05$ ). Males had a trend for rearing more often after chronic stress when compared to baseline ( $t(10)=-1.848$ ,  $p=0.094$ ). The MR group made significantly more rears when compared to baseline ( $t(25)=-2.937$ ,  $p<0.01$ ). Animals administered GR or MR antagonist made significantly more rears than stress control animals ( $t(41) = 2.286$ ,  $p<0.05$ ;  $t(44) = 3.164$ ,  $p<0.01$ , respectively).

Males spent less time in the center field during baseline when compared to the chronic drug and stress testing time point ( $t(10)=2.332$ ,  $p<0.05$ ). After chronic stress, the MR group spent significantly less time in the center fields when compared to baseline ( $t(25)=3.94$ ,  $p<0.01$ ).

Males spent significantly more time in the outside fields during the chronic restraint stress and GR antagonist testing time point when compared to baseline ( $t(10)=-2.332$ ,  $p<0.05$ ). There was no difference in the percent of time spent in the outside fields of females administered the GR antagonist during both testing sessions. Animals tested at the chronic restraint stress and MR antagonist time point spent significantly more time in the outside fields when compared to the baseline ( $t(25)=-3.944$ ,  $p<0.01$ ).



**Figure 12.** Number of rears after chronic restraint and CORT treatment in open field at chronic restraint and CORT treatment. Note that animals in both stress and GR or MR antagonist groups made significantly more rears when compared to restraint controls. Note that CORT and GR antagonist animals made significantly more rears when compared to CORT controls. \*  $p < 0.05$ ; \*\*  $p < 0.01$ , unpaired t-test comparison between chronic drug administered testing time point and control animals.

*CORT Treatment and MR or GR Antagonist.* There was no significant difference between males and females administered either the GR or MR antagonist. Animals were significantly less active after chronic GR antagonist and CORT treatment when compared to baseline ( $t(25)=2.170$ ,  $p<0.05$ ). Animals treated with MR antagonist made significantly less rears at the chronic CORT time point when compared to baseline (Figure 12;  $t(24)=-2.88$ ,  $p<0.05$ ). Animals administered the MR antagonist made significantly more rears when compared to CORT controls ( $t(31)=3.134$ ,  $p<0.01$ ).

#### *Glucose Measurements*

*MR and GR Agonist.* There was an overall significant sex difference ( $F(1, 18)=5.421$ ,  $p<0.05$ ). Females had significantly lower blood glucose concentration at the GR agonist collection time point when compared to baseline ( $t(9)=2.417$ ,  $p<0.05$ ).

*Stress and MR or GR Antagonist.* There was a significant sex difference in animals administered the GR antagonist ( $F(1,21)=5.507$ ,  $p<0.05$ ). Males had significantly higher blood glucose concentrations when compared to females during the chronic testing time point ( $t(21)=2.15$ ,  $p<0.05$ ). Animals administered the MR antagonist had a significantly higher blood glucose concentration at the chronic stress and drug administration time point when compared to baseline ( $t(26)=2.135$ ,  $p<0.05$ ).

*CORT Treatment and MR or GR Antagonist.* There was no sex difference or difference between time points for animals administered the GR antagonist. Animals had a significantly lower blood glucose concentration at MR antagonist and chronic CORT treatment when compared to baseline ( $t(25)=2.793$ ,  $p<0.05$ ).

## **Discussion**

The aim of this study was to investigate whether the GR or MR are involved in modulating skilled motor function and stress-induced motor impairments. Administration of the GR or MR antagonists after acute and chronic restraint stress or CORT administration significantly improved reaching success while at the same time decreasing the number of attempts made to obtain pellets. Increases in vertical activity were observed after either GR or MR antagonist administration. The open field data suggest that the GR and MR antagonists did not completely eliminate the behavioural response associated with exposure to restraint stress or elevated levels of CORT. Furthermore, animals administered either GR or MR agonist showed decreases in reaching success and increases in the number of attempts. MR and GR agonist-treated animals were also more active in the open field in terms of rearing. Thus, both GR and MR activation can mimic some of the effects of stress on skilled and non-skilled motor function. These findings indicate a role for both GR and MR in modulating motor function during HPA axis activation.

Previous work has suggested that the GR modulates the behavioural response to stress, since it binds to CORT when elevated, whereas the role of the MR in the stress response remains less clear (de Kloet et al., 1999; Spencer et al., 1998; Meldow et al., 2005). Recently Pace and Spencer (2005) and Derijk et al. (2006) have shown that the MR participates in behavioural changes in animals exposed to stress. Specifically, Pace and Spencer (2005) showed that blocking the MR prior to exposure to restraint stress facilitates increases in CORT release. Derijk et al. (2006) studied a polymorphism in the MR and found that human individuals with the MR180V variant had enhanced cortisol

release after experience of psychological stress. The present study found that animals administered the MR antagonist during acute and chronic stress and CORT treatment perform significantly better when compared to controls. This adds to the considerable amount of evidence suggesting that MRs participate in the stress response (Smythe et al., 1997; Cole et al., 2000; Pace and Spencer, 2005; Derijk et al., 2006).

The present study shows that activation of both GR and MR can cause skilled motor impairments. Animals administered either the MR or GR agonists showed a behavioural response comparable to that displayed by animals that underwent restraint stress-or CORT-treatment. GR and MR activation can mimic the decreased success rate and increased number of attempts in skilled reaching that was also found in stress or CORT treated animals (Metz et al., 2005). A study by Devenport et al. (1990) showed that GR and MR activation causes an increase in weight gain, similar to what is seen in animals with elevated CORT levels. The results of this study suggest that both the GR and MR are involved in modulating skilled motor function in response to restraint stress and elevated CORT levels.

Blocking the GR has been suggested to be an effective therapy for individuals affected by stress-related disorders (Belanoff et al., 2002; Metz et al., 2007). Blocking the GR has been proven beneficial for depression or to alleviate neurodegenerative events after brain lesion (Belanoff et al., 2001; Young et al., 2004 Simpson et al., 2005; Krugers et al., 2006). Specifically, treatment with RU-486 in individuals with psychotic depression can restore cognitive impairments (DeBattista et al., 2006). In Alzheimer's disease, administration of RU-486 can slow the cognitive decline (Belanoff et al., 2002). This study and previous studies have identified that the MR participates in the stress

response, and therefore it can be hypothesized that blocking both the GR and MR may prove to be more effective for stress-related disorders and neurodegenerative diseases (Fledman and Weidenfeld, 1999).

## **Conclusion**

In conclusion, the present study demonstrates that both the MR and GR are involved in the modulation of the motor function in response to stress. Thus, the present study revises the classic notion that the GR is the key player in mediating behavioural outcome after exposure to stress (de Kloet, 1999). The present data demonstrate that both the MR and GR play an equal role in modulating motor function in response to stress.

#### **4. Experiment 3**

### **Sex Differences in Skilled Movement in Response to Restraint Stress and During Recovery from Stress**

#### **Abstract**

Sex differences exist in both skilled movement and cognitive tasks. These variations have been thought to arise from fundamental differences between males and females to accomplish a goal. Stress has previously been shown to have a negative influence on skilled movement in rats. The purpose of this study was to investigate sex differences of skilled motor function in response to stress. Males and females rats were trained and then tested on the skilled reaching and skilled walking task. Both groups of animals were then exposed to a fourteen day-stress period. Daily testing continued for 21 days after exposure to stress. Open field analysis and blood sample collection took place before, during and after the stress period. Observations showed that females performed significantly better on the skilled reaching task than males during the stress period, however, no sex difference was observed in skilled walking. Further analysis during the post-stress testing time point revealed a sex difference in the skilled reaching. The results indicate sex differences in skilled reaching in response to stress, as well as the recovery period after stress. Skilled walking was not influenced.

#### **Introduction**

The behaviour in males and females can differ considerably. For example, sex differences have been found in motor performance, including limb and body movements (Field and Pellis, 1998). Previous studies have shown sex differences in hind limb use

during vertical exploration (Field et al., 2006) and sexually dimorphic postural adjustments during skilled reaching (Field and Whishaw, 2005). It has been hypothesized that these different postural strategies are related to differential exposure to gonadal hormones in the perinatal period (Field et al., 2000).

In addition to behavioural performance, males and females also show differences in their response to experience. For example, sex differences in sensitivity to stress have been documented in many animal species, including rats. Depending on the variables measured, male and female rats show divergent sensitivity to stress in cognitive function and open field locomotion (Faraday, 2002; Luine, 2002; Mashoodh et al., 2008). In general, male rats seem more prone to stress-induced disturbance of memory function (Luine, 2002). So far, sex differences in response to stress in motor function are limited to assessment of locomotion in an open field. Acute stress causes a decrease in locomotor activity in an open field in males, while females seem to be less sensitive (Haleem et al., 1988; Faraday, 2002). In addition, locomotor effects of stress depend on rat strain with Long-Evans rats being less sensitive than other strains (Faraday, 2002).

The sexually dimorphic behaviour during stress has been related to fundamental physiological differences between males and females. For example, a study in humans by Frankenhaeuser et al. (1976) found that males secreted higher levels of adrenaline when compared to females of the same age while exposed to the same stressor. In addition, estrogens enhance the glucocorticoid response to acute stress (Figueiredo et al., 2007). Sex differences in response to stress in rats have also been identified (Haleem et al., 1988; Campbell et al., 2003). Female rats are thought to present with generally higher basal levels of circulating corticosterone than males (Michaelis et al., 2001). Carey et al.

(1995) have shown that activity of the hypothalamic-pituitary-adrenal (HPA) axis in female rats depends upon the respective stage during estrous cycle. These physiological differences may result in differential responses when a stressor is presented. For instance, exposure to repeated restraint stress leads to decreases in body weight of male rats but not female rats (Faraday, 2002).

Sexual dimorphism in the physiological status might also play a role in behavioural responses to stress. Stress is a potent influence on normal performance of both skilled and unskilled movement (Metz et al., 2001, 2005; Metz, 2007). With regard to sex differences in both motor performance and the stress response, it is reasonable to expect that gender and stress represent interacting factors to affect motor system function (Haleem et al., 1998; Faraday, 2002; Figueiredo et al., 2002). This assumption is supported by previous research demonstrating sex differences in response to stress when performing cognitive tasks or during exploration of an open field (Haleem et al., 1988; Bowman et al., 2003).

The purpose of the present study was to expand previous research by exploring possible sex differences in the susceptibility of the motor system to the influence of stress in rat. The rats' performance was tested in skilled reaching and skilled walking tasks, which represent highly sensitive tasks to display slight aberrations in distal limb use and bodily adjustments (Field and Whishaw, 2005; Metz et al., 2005; Field et al., 2006). Recovery from acute stress was assessed by comparing reaching performance at 10 min versus 60 min after restraint stress, and recovery from chronic stress was tested after cessation of the chronic stress period. The observations revealed a moderate influence of acute and chronic stress on quantitative and qualitative aspects of reaching in male and

female rats. Most interestingly, the largest sex differences were found in the course of recovery after the cessation of stress.

## **Materials and Methods**

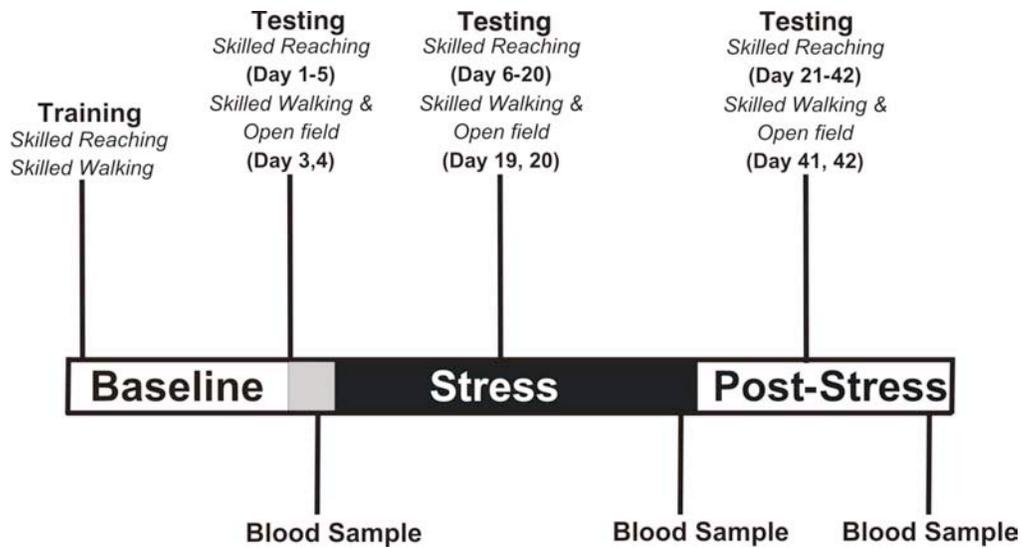
### *Subjects*

Subjects were 10 female and 10 male adult Long-Evans hooded rats, raised at the University of Lethbridge vivarium and weighing 250-600g at the beginning of the experiment. A male died in the middle of the experiment, his data was included in statistical analysis up to his death, as it did not differ significantly from other males. Animals were housed in groups of two or three in shoebox cages under a 12:12 h light/dark cycle with light starting at 07:30 h. The housing room was maintained at a room temperature of 20°C and 30% relative humidity.

Prior to the experiment, rats were food deprived to 95% of their initial body weight to encourage participation in the reaching task. To maintain body weight, supplementary food was given daily in their home cages five hours after behavioural testing was completed. Once animals acquired the reaching task, they were allowed to gain weight, which did not compromise their continued participation in this task. Animals were weighed daily before and during behavioural testing. Water was available *ad libitum*. The experiment was performed according to the standards set by the Canadian Council of Animal Care.

### *Experimental Design*

Testing and training of the animals was performed during the light phase of the cycle each day at the same time. Figure 13 illustrates the time course of manipulations and behavioural measurements. Rats were initially trained in the skilled reaching and ladder rung walking tasks. Twenty-one days later, baseline measurements for the skilled reaching task were taken from the last 5 days of training. Skilled reaching and ladder rung walking was video recorded and open field baseline measurements were taken the last two days prior to the onset of stress (Baseline). All animals were exposed to 14 days of daily restraint stress. To compare for post-stress intervals, half the animals were tested 10 minutes after stress and the other half, 60 min after stress. Chronic stress video recordings of reaching and ladder rung walking were taken and open field activity was measured at the end of this interval (Chronic Stress). Animals were then tested for 21 days after cessation of the stress period (Post-stress). Post-stress testing was terminated when stable asymptotic reaching success rates were observed. At the end of this interval animals were again video recorded in skilled reaching and ladder rung walking performance and open field activity was measured. Blood samples were collected to analyze for CORT concentration at 1) baseline, 2) day 15 of stress (Chronic Stress) and 3) day 21 of post-stress (Post-stress). In addition, blood glucose concentrations were taken at same time points.



**Figure 13.** Time chart illustrating the order of manipulations and behavioural tests. Training and testing included the pellet-reaching task. Skilled reaching, skilled walking and open field activity performance were assessed from video recordings collected before the stress treatment (baseline), at chronic stress and chronic post-stress time points. Blood samples for corticosterone and glucose analyses were collected at baseline, chronic stress and chronic post-stress time points as indicated.

### *Restraint Stress*

Animals were placed individually in custom-made transparent Plexiglas containers (5 cm inner diameter) for a period of 20 min each day (Garcia et al., 2000; Faraday, 2002; Mercier et al., 2003). The container had perforated ends to allow for ventilation. The container maintained the animals in a standing position with slight compression of the body.

## **Behavioural Testing and Analysis**

### *Skilled Reaching Task*

*Skilled Reaching Task Apparatus.* The reaching boxes were made of clear Plexiglas (40 x 45 cm and 13.1 cm wide). Animals extended their forelimbs to reach for food pellets through a 1.3 cm wide vertical opening in the middle of the front wall (Figure 15A). The vertical opening extended from the floor to a height of 15 cm. To hold the food pellet, a 2 cm wide by 4 cm long shelf was positioned outside the front wall of the box. The shelf was mounted 4 cm above the floor. Food pellets (45 mg banana flavored Dustless Precision Pellets, Bio-Serv Inc., Frenchtown, NJ) were placed in one of two small indentions on the shelf. The indentations, each 5 mm in diameter and 1.5 mm deep, were 2 cm away from the inside wall of the box and were centered on the edges of the slit through which the rats reached (Metz and Whishaw, 2000).

*Training and Testing.* Once rats began to reach for food, food was placed in the indentation contralateral to the limb that the rat used for reaching. Between individual reaching movements, rats were required to leave the food aperture and walk to the rear end of the box in order to reposition themselves prior to the next reach. Each training and

testing session required the rats to reach for 20 food pellets. Reaching performance was scored by counting misses and successful reaches for each limb (Metz and Whishaw, 2000). An ‘attempt’ was defined as a repeated forelimb movement towards the pellet and obtaining the pellet after more than one reach. A ‘success’ was recorded if an animal grasped a food pellet on the first attempt and withdrew the paw with the pellet through the slit to consume the food. A ‘miss’ was recorded if an animal touched and missed the pellet using more than one attempt to grasp it (Metz and Whishaw, 2002a). Additionally, if the animal lost the pellet in the cage after grasping it, a ‘drop’ was scored. Percent reaching success was calculated by counting the number of successful reaches divided by the number of pellets given in each session (20) multiplied by 100.

*Video Taping and Analysis.* On the last day of baseline, stress, and post-stress test sessions, the animals’ performance was video recorded from a frontal view for qualitative movement analysis. The rating of the reaching movements was performed from the videotapes by frame-by-frame inspection. The first three successful reaches were scored. Movements were analyzed using a framework derived from the Eshkol-Wachman movement notation which allows analysis of the relations and changes of relation between parts of the body and limbs (Eshkol and Wachmann, 1958). The following movement components of reaching were analyzed from a frontal view (Whishaw and Pellis, 1990; Metz and Whishaw, 2000; Whishaw, 2000): (1) *Orient*: the head is oriented towards the target and the snout is inserted through the slit to locate the pellet. (2) *Limb lift*: the mass of the body weight is shifted to the hind limbs, and the hind limbs are aligned with the body and parallel to each other. The forelimb is lifted so that the digits are aligned with the midline of the body. (3) *Digits close*: the palm is partially supinated

and approaches the midline of the body; the digits are semi-flexed. (4) *Aim*: the elbow comes in to the body with a shoulder movement while the digits retain their position on the midline of the body. (5) *Advance*: the elbow is positioned in a narrow angle to the body; the forelimb moves forward and is directed to the target. The head and the upper body are raised and the weight is shifted to the front. This movement is accompanied by a moderate lateral body movement towards the reaching limb. (6) *Digits open*: the digits are opened by a discrete limb movement; the palm is not fully pronated. (7) *Pronation*: the elbow adducts and is pronated over the target in an arpeggio movement. (8) *Grasp*: the arm remains still, while the digits close and then the paw is lifted holding the food pellet. (9) *Supination I*: the elbow is adducted and the palm is supinated by approximately 90°. (10) *Supination II*: The palm is supinated to present the food pellet to the mouth. The head drops to the level of the paws and the rat sits back on its haunches. (11) *Release*: the food pellet is released into the mouth by opening the digits. To enhance the resolution for correlation analysis, each of the individual subcomponents was also rated using a previously described 35-point scale (Metz and Whishaw, 2000)

For each of the eleven movement components, a score of 0 was given when the movement was absent, a score of 0.5 was given if the movement was present but abnormal, and a score of 1 was given if the movement was normal (Metz and Whishaw, 2000).

### *Skilled Walking Task*

*Ladder Rung Walking Task Apparatus*. The horizontal ladder was made of two side-walls (1 m long and 20 cm high) of clear Plexiglas with metal rungs (3 mm in

diameter), inserted at random distances ranging from 1 to 5 cm to create a floor (Figure 16A; Metz and Whishaw, 2002b). The irregular pattern was used to maintain the difficulty of the task in repeated test sessions. The ladder was elevated 30 cm above ground with a neutral start box and the animals' home cage at the end.

*Training and Testing.* Animals were trained in the ladder rung walking task in five trials. Each trial required the animal to cross the length of the ladder to reach the home cage placed at the end of the apparatus. One training session was administered, with the baseline test session on the following day. One test session was performed at baseline, pre-lesion and post-lesion time points. Each test session consisted of 3 trials during which the animals' performance was videotaped.

*Video Taping and Analysis.* The ladder rung walking performance was video-recorded from a lateral perspective (Metz and Whishaw, 2002b). The camera was positioned at a slight ventral angle, so that both sides and the paw positions could be recorded simultaneously from a ventral view. The tapes were analyzed frame-by-frame for quantitative and qualitative analysis. Quantitative analysis was based on the number of errors in each crossing. Based on the limb placement scoring system (see below), an error was defined as a limb placement that involved missing the rung or slipping off the rung (score of 0, 1 or 2 points according to the scale). The mean number of errors per step of each fore- and hind limb was calculated and averaged for three trials. The errors for the contralateral fore- and hind limbs were averaged and expressed as percent errors per total number of steps.

The qualitative analysis of forelimb and hind limb placements was performed using the foot fault scoring system developed earlier (Metz and Whishaw, 2002b).

Consecutive steps were analyzed, excluding the last step before a pause and the first step after a pause. The last stepping cycle at the end of the ladder rung apparatus was also excluded from scoring. Limb placement was scored by categorizing the placement of the limb on a rung and the limb protrusion between rungs when a miss occurred by using a 7-category scale (Metz and Whishaw, 2002b). (1) *Total miss*: the limb completely misses the rung (0 point). (2) *Deep slip*: the limb is initially placed on a rung, but then slips off when weight bearing and causes the limb to fall in-between rungs (1 point). (3) *Slight slip*: the limb is placed on a rung, but slips off when weight bearing without causing a fall that interrupts walking (2 points). (4) *Replacement*: the limb is placed on a rung, but withdraws before weight bearing and is placed on another rung (3 points). (5) *Correction*: the limb aiming for one rung is placed on another rung without touching the first one (4 points). Alternatively, the limb is repositioned on the same rung. (6) *Partial placement*: the limb is placed on the rung with either the wrist or digits of the forelimb or the heel and toes of the hind limb (5 points). (7) *Correct placement*: a correct placement with the mid-portion of the palm weight bearing (6 points) (Metz and Whishaw, 2002b).

An error was counted when an animal missed a rung or slipped off (error scores of 0,1 and 2). Percent error was calculated by dividing the number of errors by total number of steps. The number of foot placement errors was calculated as a percentage of the total number of steps made in a respective trial.

### *Open Field Task*

*Open Field Apparatus.* The open field box, measuring 100 x 100 x 18 cm, was made of opaque black Plexiglas. The bottom of the box was divided into 16 zones (22 x 22 cm) using white masking tape (Figure 17A).

*Testing.* Each rat was individually placed in the middle of the open field box and video recorded for 5 min. After testing of each rat was completed, the floor of the box was cleaned with soap.

*Video Taping and Analysis.* Video recordings were scored for activity (total number of fields entered), number of novel fields entered, and % time spent in the center and outside fields. Entered fields were scored when more than 50% of the animal's body crossed a subdivision of the open field.

### *Video Recording Procedures*

Videotaping in all tasks was performed using a Sony ZR70 portable digital video camera. The shutter speed was set at 1/500s. Tapes were analyzed frame-by-frame on a Sony Mini DV player. The testing setup was illuminated by a white light source. In addition, the skilled reaching apparatus was also illuminated by a one-arm cold light source (Schott, Jena, Germany).

### *Blood Sampling*

Rats were anesthetized using 4% isoflurane. Anesthesia was maintained for 5 minutes in which 1.0 ml of blood was collected from the tail vein. The blood was transferred to centrifuge tubes and plasma was obtained by centrifugation at 10,000 rpm

for 8 min. The samples were stored at -20°C. Plasma corticosterone levels were determined by a radioimmunoassay (RIA) kit for corticosterone (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA; Ishida et al., 2003) and by enzyme linked immunosorbent assay (ELISA; Kavushansky and Richter-Levin, 2006).

### *Glucose Measurements*

Glucose samples were collected after blood sample collection occurred. An Ascensia Breeze Blood Glucose Meter (Bayer, Toronto, ON) was used to collect blood and analyze glucose levels.

### *Statistical Analysis*

Statistical analysis was performed using a SPSS software package 11.5 (SPSS Inc., Chicago, IL, 2002). The results were subject to analysis of variance (ANOVA) for repeated measures across testing sessions. Comparisons of means and variances between groups were performed using unpaired t-tests, and paired t-tests were used for within-subject comparison. In all statistical analyses, a p-value of less than or equal to 0.05 was considered significant. All data are presented as mean +/- standard error of the mean (S.E.M.).

## **Results**

### *Skilled Reaching*

*Reaching Success.* During baseline reaching both males and females obtained 10 out of 20 pellets successfully, resulting in a  $55.61 \pm 1.61\%$  success rate (Figure 14B).

There was no significant difference in success rate between males and females at this time. Because there was no difference in reaching success between animals that were tested either 10 or 60 min after stress, these two groups were combined for further analysis.

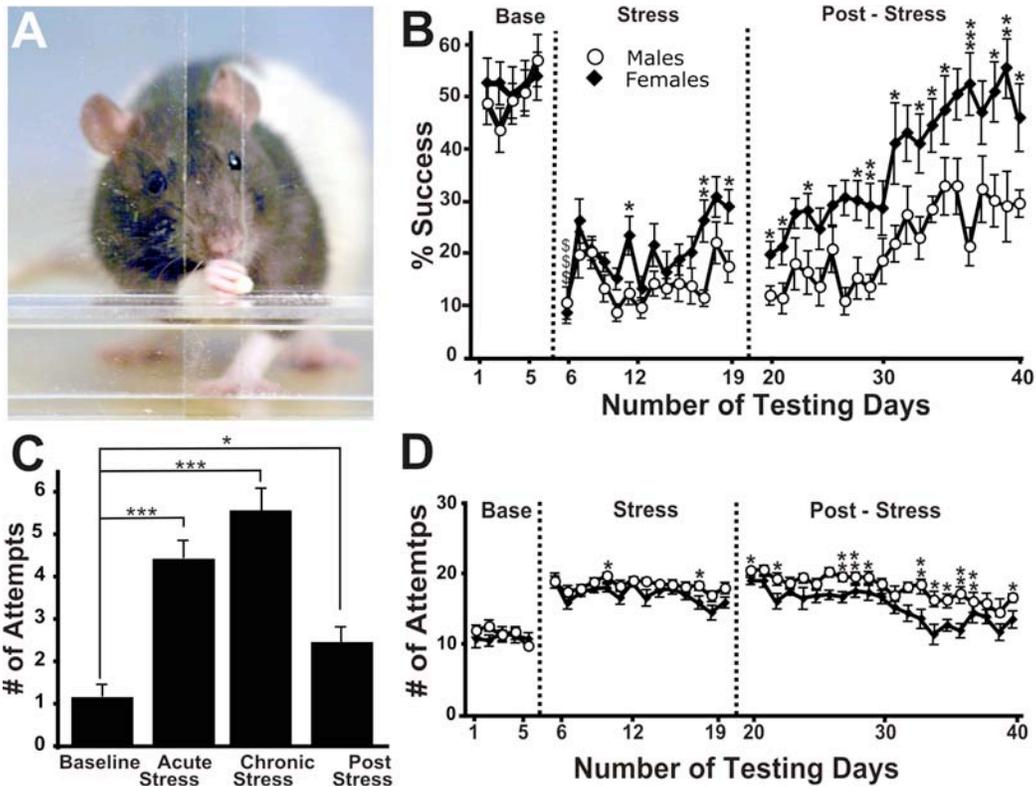
Both males and females had significantly lower reaching success on the first day of stress when compared to the last day of baseline ( $t(8)=6.63$ ,  $p<0.001$ ;  $t(9)=9.22$ ,  $p<0.01$ ). Throughout the 14-day stress period females ( $20.96\pm 1.79\%$ ) generally obtained significantly more pellets successfully when compared to males [ $14.14\pm 1.18\%$ ;  $F(1,17)=7.23$ ,  $p<0.05$ ]. On day 14 of restraint stress females had a significantly higher reaching success ( $30.0\pm 3.50\%$ ) than males [ $17.5\pm 3.24\%$ ;  $t(17)=6.48$ ,  $p<0.05$ ]. Furthermore, during the post-stress period females had an overall significantly higher reaching success ( $36.98\pm 2.47\%$ ) than males [ $21.10\pm 1.69\%$ ;  $F(1,17)=11.78$ ,  $p<0.01$ ]. In addition, both females ( $45.50\pm 6.56\%$ ) and males ( $29.89\pm 2.61\%$ ) had significantly lower reaching success on the last day of post-stress when compared to the last day of baseline testing ( $t(18)=4.34$ ,  $p<0.0001$ ). Testing was terminated when no further improvements were recorded for seven days on day 21 post-stress.

*Number of Attempts.* There was no overall sex difference in the number of attempts between female and male rats at the baseline testing session. Overall, animals made more attempts at acute and chronic stress time points when compared to baseline [ $t(18)=-6.45$ ,  $p<0.001$ ;  $t(18)=-8.32$ ,  $p<0.001$ , respectively; Figure 14C]. Furthermore, animals made less attempts during the post-stress interval when compared to the acute and chronic stress time points ( $t(18)=4.26$ ,  $p<0.001$ ;  $t(18)=4.11$ ,  $p<0.001$ , respectively;

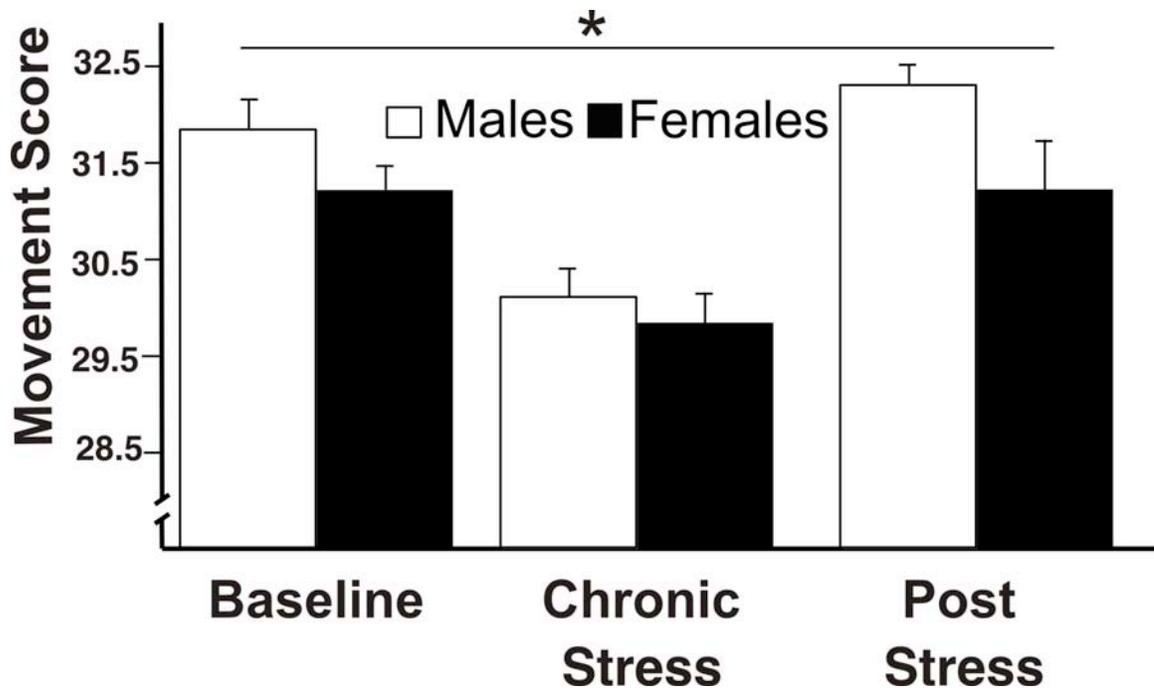
Figure 14C). However, overall animals still made significantly more attempts post-stress when compared to baseline ( $t(18) = -2.59$ ,  $p < 0.05$ ).

There was a significant sex difference during the restraint stress interval ( $F(1,18) = 4.71$ ,  $p < 0.05$ ). Specifically, females ( $14.1 \pm 0.93\%$ ) made fewer attempts when compared to males ( $16.5 \pm 0.50\%$ ) on day 6 and 12 of stress exposure ( $t(18) = -2.344$ ,  $p < 0.05$ ;  $t(18) = 2.26$ ,  $p < 0.05$ , respectively; Figure 14D). During the post-stress interval an overall significant sex difference was also present ( $F(1,18) = 14.26$ ,  $p < 0.01$ ). For instance, on the last day of post-stress testing females made significantly less attempts ( $10.5 \pm 1.22$ ) when compared to males ( $13 \pm 0.54$ ;  $t(18) = 2.44$ ,  $p < 0.05$ ; Figure 14D).

*Reaching Movement Score.* Animals had significantly lower movement scores at chronic testing when compared to baseline ( $t(19) = 6.46$ ,  $p < 0.001$ ). Males had an overall significantly higher qualitative score when compared to females ( $F(1,17) = 5.35$ ,  $p < 0.05$ ; Figure 15). There was no difference between males and females at specific time points or between time points, however.



**Figure 14.** Quantitative skilled reaching performance before, during and after restraint stress. (A) Photograph illustrating skilled reaching task in which rats were required to grasp and retrieve individual food pellets. (B) Time course of reaching success. There was a significant reduction in percent success from last day of baseline testing to first day of stress. Also note significant sex differences during stress and during the post-stress testing periods. (C) Overall number of attempts to grasp a single food pellet during stress in males and females combined. Note that acute (day 1) and chronic stress (day 14) caused a significant increase in the numbers of attempts when compared to baseline. (D) Time course of number of attempts in male versus female rats. Note significant sex differences throughout the stress and post-stress test time points. Asterisks indicate significance levels: \*  $p < 0.05$ ; \*\*\*  $p < 0.001$ , unpaired t-test comparison between males and females (B, D) and paired t-test comparison between animals at baseline and chronic stress testing time point (C). \$\$\$  $p < 0.001$ , paired t-test between last day of baseline testing and first day of stress (B).



**Figure 15.** Qualitative skilled reaching performance. Reaching movement score assessed by the detailed 35-point scale before (baseline) and after 14 days (chronic) of restraint stress. Note that males had a trend for higher overall movement score. Asterisks indicate significance levels: \*  $p < 0.05$ , ANOVA comparing male and female movement scores.

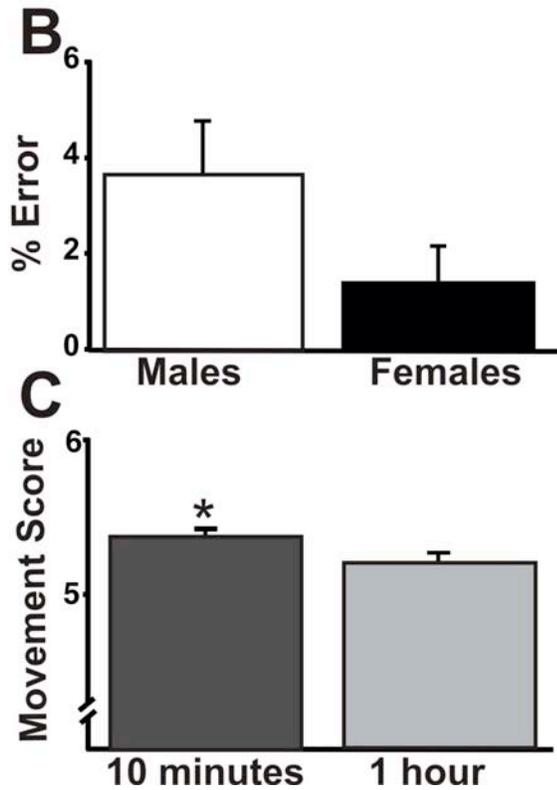
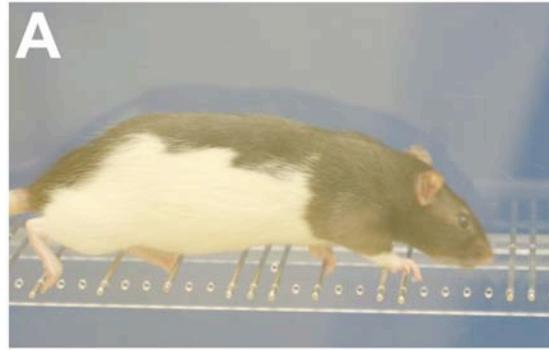
### *Skilled Walking*

*Number of Placement Errors.* During the baseline testing session males ( $3.72 \pm 1.08$ ) made more than twice as many errors in than females [ $1.49 \pm 0.71$ ;  $t(38) = 1.887$ ,  $p = 0.076$ ; Figure 16B]. This difference in percentage of errors, however, was not significant. This trend disappeared at both the chronic stress and post-stress testing time points.

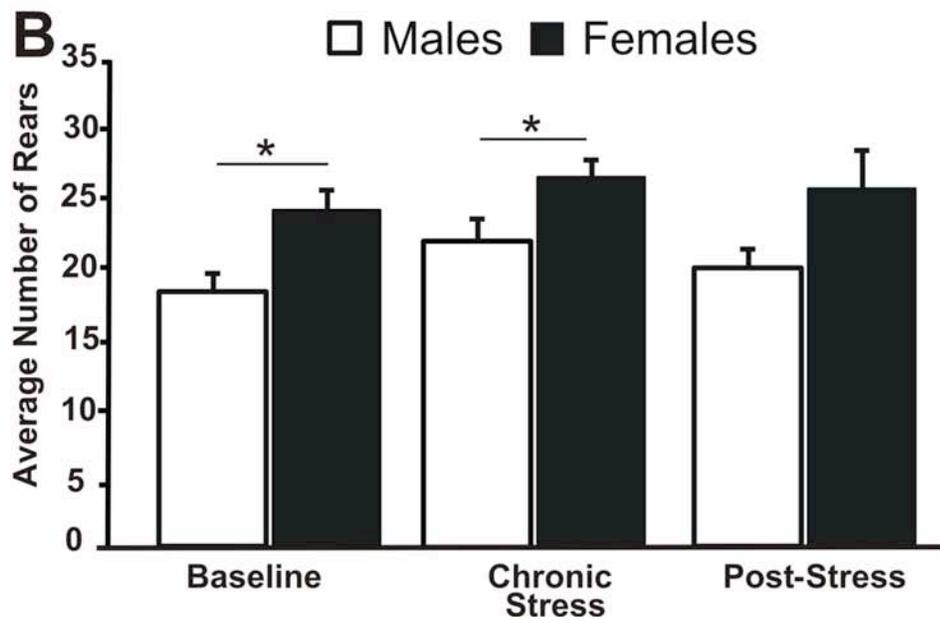
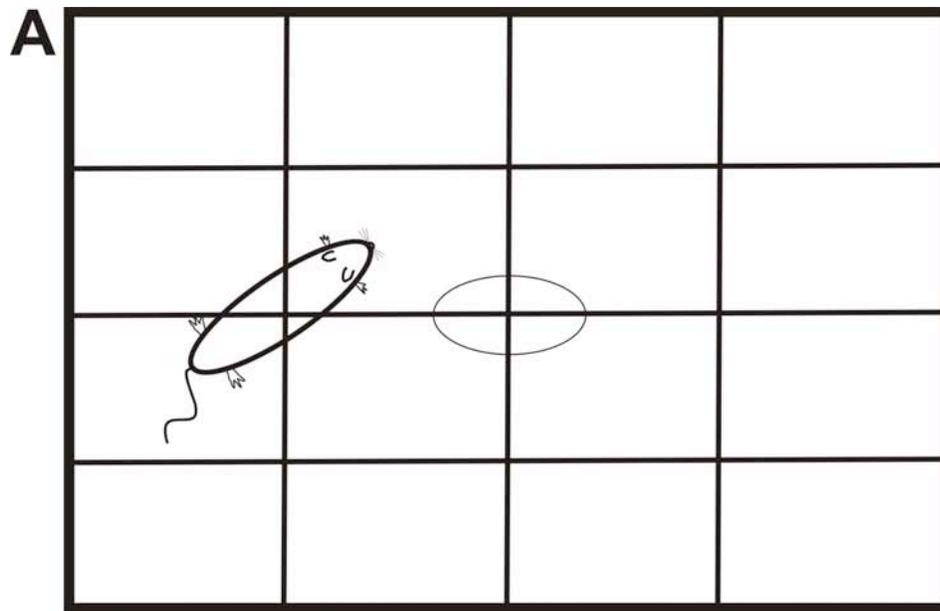
*Foot Fault Scoring.* There were no differences in movement scores between males and females at baseline, chronic stress or post-stress test sessions. There was, however, a significant difference in movement scores in animals tested 10 and 60 min after stress at the chronic stress time point ( $F(1,35) = 6.66$ ,  $p < 0.05$ ). Animals tested 10 minutes after restraint stress had significantly higher movement scores when compared to animals tested 60 minutes after stress ( $t(38) = -2.145$ ,  $p < 0.02$ ; Figure 16C).

### *Open Field Activity*

There was an overall difference between males and females during both baseline and chronic stress sessions [ $F(1,18) = 4.85$ ,  $p < 0.05$ ;  $F(1,18) = 7.13$ ,  $p < 0.05$ , respectively]. In these sessions, female rats showed greater vertical activity than males (baseline  $t(18) = -2.83$ ,  $p < 0.05$ ; chronic stress  $t(18) = -2.17$ ,  $p < 0.05$ ; Figure 17B).



**Figure 16.** Skilled walking task. (A) Photograph illustrating a rat crossing the ladder rung walking apparatus. (B) Number of errors at baseline. There was a trend of higher error numbers in male rats as compared to females. (C) Movement score at chronic stress time point. Note animals tested 10 minutes after stress have a significantly higher movement score than animals tested 60 min after stress. Asterisks indicate significance levels: \*  $p < 0.05$ , unpaired t-test comparison between 10 and 60 minute groups.



**Figure 17.** Open field activity measurement. (A) Picture illustrating the open field task. (B) Number of rears in an open field. Note the significantly increased number of rears in at baseline and after 14 days of restraint stress. Asterisks indicate significance level: \*  $p < 0.05$ , unpaired t-test comparison between males and females.

### *Corticosterone Levels*

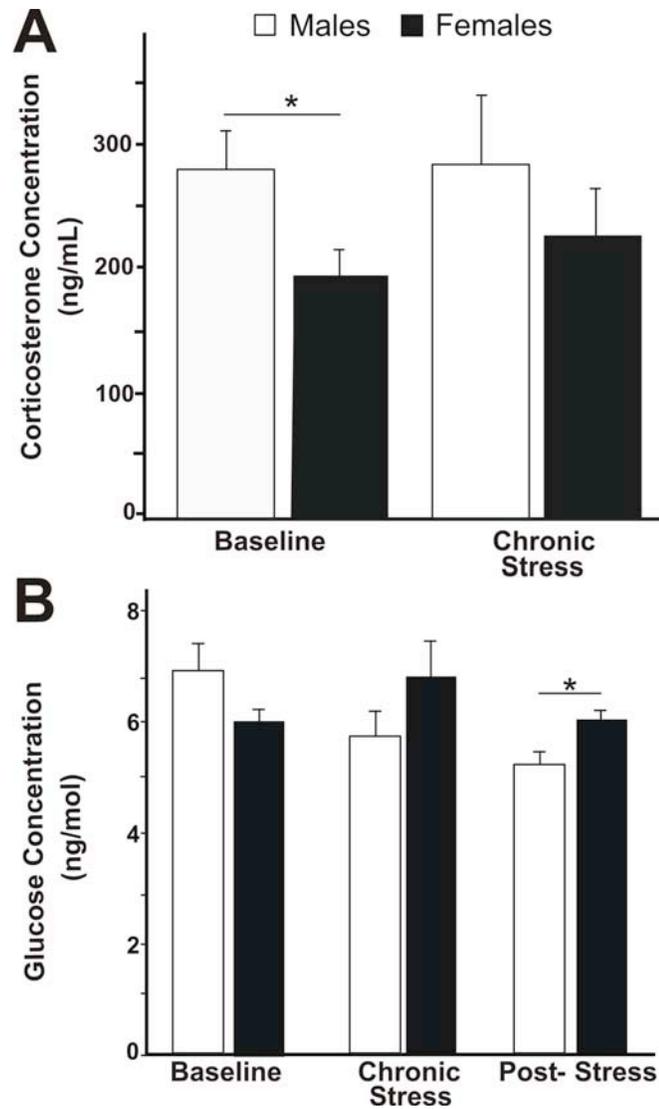
CORT levels in males and females were significantly different at baseline (Figure 18A). Males had significantly higher CORT concentrations than females ( $t(18)=2.21$ ,  $p<0.05$ ). At the chronic stress time point this difference had disappeared. Variance at the chronic stress time point increased when compared to baseline data, the increase in variance may have hid any difference between and within groups. Females had significantly higher CORT levels than males at the post-stress collection time point ( $t(16)=-3.458$ ,  $p<0.003$ ).

### *Glucose Measurements*

There was no sex difference in glucose measurements taken at baseline and chronic stress time points. The post-stress measurement revealed that males had higher glucose concentrations than females ( $t(17)=-2.48$ ,  $p<0.05$ ; Figure 18B).

### *Body Weight*

Male and female rats had similar body weights in baseline and during the chronic stress period. During the post-stress period, however, males and females were significantly different ( $F(1,17)= 5.96$ ,  $p<0.05$ ). Males had higher weights for two weeks during three-week post-stress testing.



**Figure 18.** (A) Plasma CORT (A) and glucose (B) concentrations prior to stress (baseline) and after 15 days of daily exposure to restraint stress. Glucose levels are also shown for 21 days post-stress. Note that males had significantly higher CORT. Asterisks indicate significant levels: \*  $p < 0.05$ , unpaired t-test at post-stress between males and females.

## **Discussion**

The objective of this study was to compare the effects of stress on motor function in male and female Long-Evans rats. The experiment utilized a standard stress model and a high-resolution behavioural test battery to obtain quantitative and qualitative measures of fine motor performance. The behavioural test battery revealed that males and females exposed to restraint stress for 14 days were impaired at both skilled and non-skilled motor performance. Sex differences in stress sensitivity were observed in skilled reaching and vertical exploration of an open field, but not in skilled walking. Interestingly, sex differences were observed in the recovery from stress-induced motor disturbances in skilled reaching. While males showed generally reduced reaching accuracy and needed more attempts to grasp a pellet, they also recovered more slowly from stress-induced impairments.

The present stress-induced alterations in skilled limb use resemble those described earlier (Metz et al., 2005; Metz, 2007). Acute and chronic restraint stress lead to reduction in reaching success along with an increase in number of attempts needed to grasp a pellet. These findings indicate reduced accuracy of skilled movements in animals exposed to stress. Previous work has shown that an increase in the number of attempts and a decrease in percent success are both sufficient indicators of behavioural impairment in response to restraint stress (Metz et al., 2005). The absence of a significant plasma corticosterone elevation during chronic restraint stress also is in line with previous work (Metz et al., 2005) suggesting that stress-induced motor impairments might be independent of corticosterone. Although the absence of upregulated hypothalamic-pituitary-adrenal (HPA) axis activity might be interpreted as habituation (Pitman et al.,

1998; Armario et al., 1990), stress-associated emotional responses, including anxiety and associated changes in serotonergic activity, might explain the present findings (Dalla et al., 2005; Metz et al., 2005).

Although there was a trend for female rats to acquire the reaching task faster than males, there was no sex difference in baseline overall reaching success and the number of attempts made. By contrast, the exposure to chronic restraint stress uncovered sex differences in these parameters. While on the first day of stress both sexes were equally impaired, as exposure to stress progressed females began to improve more steadily than males so that success rates and number of attempts started to diverge. These data are in contrast to reports of adult female Wistar rats showing greater locomotor effects and physiological susceptibility to chronic mild stress, such as restraint (Dalla et al., 2005). This discrepancy might derive from strain-dependent behavioural and physiological differences. Locomotor activity in Long-Evans females has been described as more stable than male activity (Faraday, 2002). Nevertheless, the present findings of impaired reaching success in both genders emphasize the sensitivity of the skilled reaching task to detect stress-induced motor disturbance. Furthermore, changes in stress-exposed rats might be task-specific. The narrow opening of the skilled pellet reaching apparatus restricts the degree of freedom in movements and so the use of compensatory adjustments is relatively limited. Thus, decreased success rates and increased reaching attempts might directly reflect deviations in limb trajectories or body position in front of the opening (Kirkland et al., 2008). Lastly, as indicated by baseline measures, the difference in stress-induced male performance is independent of elevated corticosterone levels. This finding is also supported by absent effects of the post-stress testing. In neither males nor females

did earlier testing after the stress session (i.e., 10 minutes instead of 60) cause a larger movement deficit. Males, however, might have experienced greater difficulty habituating to the disturbing effects of chronic stress and so did not develop successful compensatory movement strategies. This might be indicated by their rather preserved qualitative movement patterns compared to females. Females showed a lower reaching movement score than males, which might reflect deviations in the original movement pattern in order to develop adaptive compensatory strategies, which were generally more successful than male ones. This relationship might explain the observation that higher success rates in females were accompanied by lower movement scores.

Compared to skilled limb use, the stress regimen used in the present study had a less pronounced effect on gross movement and motor activity. Open field exploratory activity is thought to serve as indicator of stress-associated emotional reactivity (Levine et al., 1967; Crawley, 1985). There is a large variety in the effects of stress on locomotor activity described in the literature. While an acute stressor might produce hyperactivity, as reflected by elevated horizontal and vertical activity (Windle et al., 1997), it can at the same time also reduce exploration of male rats (Faraday, 2002). The present findings show that females perform significantly more rears than males during both baseline and after chronic stress. Thus, our findings are in accordance with the literature describing that chronic stress does not alter open field exploration in neither male nor female Long-Evans rats (Faraday, 2002). Recovery from stress, in turn, led to disappearance of this difference, although a trend still existed.

A particularly interesting finding of the present study is the observation of sex differences in the recovery from stress. Recovery from an acute stress session was usually

not affected by sex, however, the results in skilled walking indicate that restraint stress exerts its effects slowly i.e., 60 min after the stress session, at a time when the peak of corticosterone elevation passed. Again, this observation supports the notion that stress might affect motor system function independently of elevated corticosterone levels. The largest difference between males and females in recovery, however, was seen after the chronic stress period. Animals tested after being exposed to chronic restraint stress showed an asymptotic increase in success rates up to two weeks. Both males and females did not reach the baseline success rate, and males reached lower success rates than females. These findings are in line with observations that exposure to chronic restraint stress cannot be reversed. For example, a study by Vyas et al. (2004) found that animals exposed chronic restraint for three weeks still showed enhanced anxiety three weeks after cessation of stress. In contrast, stress-induced expression of polysialylated neural cell adhesion molecule is lost six weeks after chronic stress (Pham et al., 2003). Although many morphological changes persist after stress, some such as apical dendritic retraction may be reversed within three weeks after stress (Radley et al., 2005). The post-stress testing period revealed that skilled motor impairments can partially be reversed, however, motor performance did reach asymptotic limits.

The present data demonstrate that acute and chronic stress is associated with characteristic motor impairments depending on gender. It is generally believed that females react more robustly to stress, both behaviourally and physiologically (Faraday, 2002; Kandandrea et al., 2002; Mashoodh et al., 2008). HPA axis activity occurs as a function of ovarian cycle stage. For example, females have a stronger reaction to a stressor during the proestrus stage of the estrous cycle (Carey et al., 1995; Marcondes et

al., 1996; Young et al., 2001; Figueiredo et al., 2007). Many studies have concluded that estrogen is the key factor for sex differences in response to stress (Salicioni et al., 1993; Handa et al., 1994; Redei et al., 1994; Carey et al., 1995; Conrad et al., 2005). For example, a previous study found that estradiol treatment, the major female estrogen, in ovariectomized rats leads to increase in HPA axis response (Young et al., 2001) and enhanced CORT responses to restraint stress (Figueiredo et al., 2007). Interestingly, female rats show characteristic regional changes in glucocorticoid receptor densities after exposure to stress in various brain areas, including hippocampus. Therefore, females are able to regulate the severity of their stress responses (Kandandrea et al., 2000, 2002).

Sex differences like the ones described here have also been found in a number of other behavioural parameters. The literature suggests major sex differences in stress effects on cognition and emotion (Pare et al., 1999; Conrad et al., 2004; Renard et al., 2005). For example, acute stress has been shown to impair spatial memory in males but not females (Conrad et al., 2004). In fact, acute stress might even facilitate memory processes regardless of their stage in estrous cycle (Conrad et al., 2004). Interestingly, Pare et al. (1999) proposed that males are more susceptible to acute stress whereas females are more susceptible to chronic stress. Furthermore, under certain circumstances, males and females might even respond in opposite directions to similar experiences. For instance, in response to one acute stressful experience, male rats acquire an associative learning task faster (Shors et al., 1992; Shors, 2001), whereas female rats exposed to the same stimulus are learning impaired and show fewer conditioned responses (Wood and Shors, 1998; Shors et al., 1998; Wood et al., 2001).

## **Conclusion**

The present study is the first to extend previous studies to the motor system. Our findings show that movement performance in both male and female rats is disturbed by acute and chronic stress, although different aspects of movement are affected. The test battery allowed the separation of discrete movement alterations that are characteristic for either male or female rat. Movement patterns and recovery from stress indicate that males or females might be equally susceptible to stress, however, they use different strategies to overcome stress-induced motor disturbance. While male rats prefer to use original movement patterns, females tend to modify these patterns in order to increase reaching success. This strategy proves more effective in the recovery from stress in that females show faster improvements.

#### **4. Experiment 4**

### **Restraint Stress Impairs Skilled Motor Function by Disrupting Cell Signaling in Motor Circuits**

#### **Abstract**

Stress and stress hormones represent potent modulators of motor system function and disease. It can be hypothesized that stress affects motor function and recovery after lesion by interfering with intracellular signaling cascades. Previous studies have shown that the stress response influences regulation of neuronal cell signaling pathways, including neuronal growth and synaptic transmission by regulating the transcription factor proliferating cells nuclear antigen (PCNA), the protein kinase phosphatidyl inositol-3 kinase (PI3-K), mitogen activated protein kinase or extracellular regulated kinases (MEK1, MEK2), and protein kinase B (AKT). These pathways are vital for maintaining cellular integrity and cell survival. The objective of this study was to determine interactions of chronic stress with cell signaling pathways in the motor system. Groups of adult female rats were exposed to restraint stress daily for two weeks. Cerebellar, striatal and brain stem tissue from one hemisphere of stress-exposed and control animals were then analyzed for protein levels of PCNA, PI3-K, MEK1, MEK2, and AKT using immunoblot techniques. Samples were corrected for loading using Actin and films were analyzed by densitometry. The analysis revealed that in the cerebellum, restraint stress upregulated PI3-K and downregulated AKT and PCNA expression. In the striatum, PI3-K expression was downregulated and MEK1, MEK2 expression was upregulated after exposure to restraint stress. In the brain stem AKT, MEK1, MEK2 and PCNA expression

were upregulated after exposure to restraint stress. Upregulation of these proteins might represent a neuroprotective response to excessive levels of glucocorticoids, whereas downregulation might compromise neuronal function and survival. These observations suggest that chronic stress changes the profile of protein expression patterns and pathway function in the motor system, which parallels with disrupted movement performance.

## **Introduction**

The stress response involves the activation of the hypothalamic-pituitary-adrenal (HPA) axis, which then causes the release of glucocorticoids (GCs), such as corticosterone. Stress hormones readily cross the blood brain barrier and bind to receptors in the cytoplasm of neurons in the central nervous system. The receptor ligand complex then moves to the nucleus of the cell, where it affects transcription and translation (Cole et al., 2000; Bale and Vale, 2004; Rogerson et al., 2004). Exposure to stress can modulate these processes by changing the function of neuronal signaling pathways (Shen et al., 2004; Suenaga et al., 2004; Yang et al., 2004; Sabban et al., 2006). Furthermore, stress and GCs have been suggested to regulate cell survival (Joels et al., 2004; Sandi, 2004), most likely via regulating intracellular signaling pathways (Shen et al., 2004; Imbe et al., 2005; Herbert et al., 2005). Some of the key proteins involved in cell survival are the protein kinase phosphatidyl inositol-3 (PI3-K), protein kinase B (AKT), mitogen extracellular kinases (MEK1, MEK2), and proliferating cells nuclear antigen (PCNA). All these proteins have been previously shown to be a part of an interconnected pathway that is crucial for neuronal survival (Silasi et al., 2004).

PI3-K and AKT have been shown to play a role in neuronal growth, maintenance, synaptic plasticity and memory function (Zundel and Giaccia, 1998; Sweatt, 2001; Frebel and Wisnes, 2006; Mayford, 2007). PI3-K is activated by a number of signals, including growth factors, and then in turn it activates AKT (Philpott et al., 1997; Zundel and Giaccia, 1998). PI3-K and AKT have been linked to anti-apoptotic effects. For example, a study by Philpott et al. (1997) showed that overexpression of both PI3-K and AKT block cell death. Activation of AKT has also been shown to promote cell survival by inactivating apoptotic proteins (Chen et al., 1998).

MEK1, MEK2 are members of the mitogen activated protein kinase (MAPK) family and are controlled by second messengers in the central nervous system (Grewal et al., 1999). These proteins are involved in regulating the activity of transcription factors, synaptic plasticity, learning, cell proliferation, differentiation and apoptosis (Grewal et al., 1999; Li et al., 2003; Pan et al., 2006; Zebisch et al., 2007). The MAPK family is also involved in regulating transcription factors that control immediate early genes, one of which is c-fos (Li et al., 2003). Levels of c-fos in neurons are disrupted in animals exposed to stress (Melia et al., 1994; Stamp and Herbert, 2001; Skorzewska et al., 2006). PCNA, on the other hand, is involved in cell cycle progression, deoxyribonucleic acid (DNA) replication and repair (Xu and Morris, 1999). PCNA is activated by DNA damage and cooperates with protein 53 (p53), another cell signaling protein, to inhibit DNA replication while DNA repair proceeds (Xu and Morris, 1999; Karuppayil et al., 1998). Previous studies assessing the effects of stress on cell signaling have revealed alterations in the above-mentioned cell signaling proteins (Philpott et al., 1997; Zundel and Giaccia, 1998; Sweatt, 2001; Yong Lee et al., 2006). For example, Yong Lee et al. (2006) showed

that exposure to restraint stress causes a 13-fold increase in MEK1, MEK2 and a 7-fold increase in AKT. Changes in expression of PCNA have been found in animals exposed to restraint stress (Tsuchiya and Hori, 1999). Restraint stress serves as a common model of mild to moderate stress in rodents (Metz et al., 2005).

Previous studies have shown that restraint stress leads to disrupted motor control (Metz et al., 2005) and exaggerated cell death in areas of the motor system (Smith et al., 2008). The mechanism of this phenomenon is unclear, however, the present study seeks to provide new insight to the intracellular changes in the motor system in response to stress. The purpose of this study was to determine the interaction of chronic restraint stress with neuronal cell signaling in the motor system. Expression of PI3-K, AKT, MEK1, MEK2, and PCNA proteins in the cerebellum, striatum and brain stem were assessed in rats exposed to chronic restraint stress. The results indicate that restraint stress causes changes in cell signaling pathways in the motor system.

## **Materials and Method**

### *Subjects*

Subjects were 16 female adult Long-Evans Hooded rats, raised at the University of Lethbridge vivarium and weighing 250-350 g at the beginning of the experiments. We have previously demonstrated motor impairments in female rats after exposure to restraint stress (Metz et al., 2005). The animals were housed in groups of two in standard polycarbonate shoebox cages (45.5 x 25.5 x 20 cm) on corn cob bedding (Bed O Cobs 1/8'). The light cycle was 12:12h with lights on at 07:30 h. The housing room was maintained at a temperature of 20°C and 30% relative humidity. Food and water was

available *ad libitum*. The animal experiment was approved by the University of Lethbridge Animals Welfare Committee.

### *Experimental Design*

Animals were divided into two groups, restraint stress (n=8) and non-stress controls (n=8). The stress-inducing procedures were performed daily at the same time of day over a period of 14 days. Blood samples and glucose were collected at the of the stress period (Figure 20).

### *Stress Procedure*

Animals were placed in a transparent Plexiglas container (5 cm inner diameter) for a period of 20 min each day (Garcia et al., 2000; Faraday, 2002; Mercier et al., 2003). The container had perforated ends to allow for ventilation. The container maintained the animals in a standing position without compression of the body.

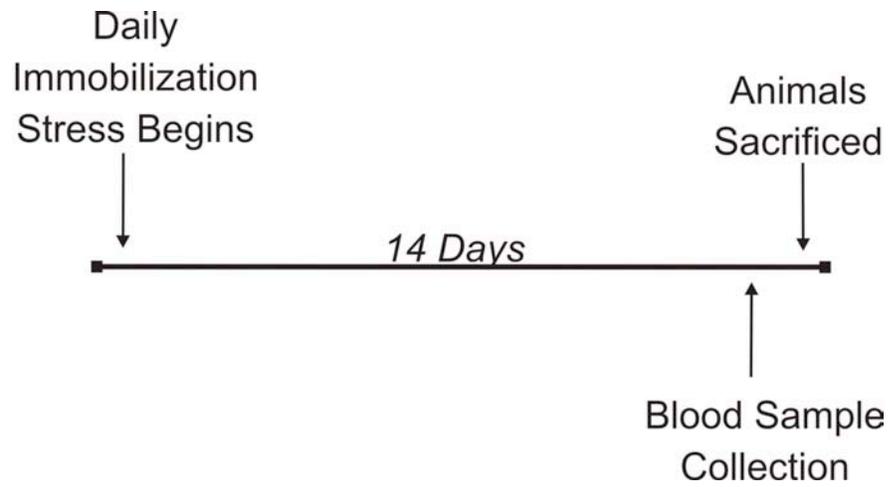
### *Blood Sampling*

Blood samples were collected at the chronic stress time point. The rats were anesthetized using 4% isoflurane. Anesthesia was maintained for 5 minutes in which 1.0ml of blood was collected from the tail vein. An Ascensia Breeze Blood Glucose Meter (Bayer, Toronto, ON) was used to collect blood and analyze glucose levels in animals. The remaining blood was transferred to centrifuge tubes and plasma was obtained by centrifugation at 10,000 rpm for 8 min. The samples were stored at -20°C.

Plasma corticosterone levels were determined by enzyme linked immunosorbent assay (ELISA; Kavushansky and Richter-Levin, 2006).

#### *Western Immunoblot*

Western blotting for P13, AKT, MEK1-2 and PCNA was conducted on tissue obtained from the cerebellum, striatum and brain stem. Tissue samples from left hemispheres were sonicated in a 500 or 1000 $\mu$ L mixture of 50 mM Tris, 150 mM NaCl, protease inhibitor cocktail and sodium dodecyl sulfate (SDS), boiled for 5 minutes and then centrifuged for 10 minutes. Small aliquots (25  $\mu$ L) were used for protein quantification using Bradford assay reagents from (Bio-Rad, Hercules, CA). Equal amounts of proteins (22  $\mu$ g) were separated by SDS-polyacrylamide electrophoresis (PAGE) in slab gels of 10% polyacrylamide. Gels were stained for Coomassie to confirm successful movement of proteins through the gel and were transferred to the nitrocellulose membranes. Membranes were incubated with antibodies against PCNA (1:700, Santa Cruz Biotechnologies, Santa Cruz, CA), Anti-P13 Kinase p8 (1:2000, Upstate Cell Signaling Solutions, Temecula, CA), MEK1, MEK2 (1:1000, Cell Signaling Technology, Danvers, MA) and AKT (1:750, Cell Signaling Technology, Danvers, MA).



**Figure 20.** Time chart illustrating the order of experimental manipulations. Blood samples for corticosterone analysis and blood glucose were collected from control and stress animals on day 14 of stress (chronic stress).

Antibody binding was revealed by incubation with secondary peroxidase-conjugated antibody (1:5000, Jackson ImmunoResearch, West Grove, PA) and ECL Plus immunoblotting detection system (Amersham, Baie d'Urfé, QC). Chemiluminescence was detected by Biomax MR film (Kodak, Rochester, NY). Signals were quantified by NIH Image analysis software (Version 1.63, National Institutes of Health, Bethesda, MA) using density measurement. Band analysis was corrected for background and normalized to beta-Actin (1:1000, Cell Signaling, Cambridge, Technology, Danvers, MA).

### *Statistical Analysis*

The statistical analysis was performed using SPSS software package 11.5 (SPSS Inc., IL, 2002). The results were subject to analysis of comparisons of means. Variances between and among groups were performed using unpaired t-tests. In all statistical analyses, a p-value of less than or equal to 0.05 was considered significant. All data are presented as mean +/- standard error of the mean (SEM).

## **Results**

### *Blood Sample Analysis*

Animals in the restraint stress group ( $1096.2 \pm 326.3$  ng/mL) showed a trend for increased plasma circulating CORT concentration when compared to controls ( $1075.2 \pm 177.1$  ng/mL).

### ***Glucose Analysis***

Animals in the restraint stress group ( $4.88 \pm 0.34$  ng/mol) had no changes in blood glucose concentrations (ng/mol) when compared to controls ( $4.51 \pm 0.16$  ng/mol).

### ***Western Immunoblot Analysis***

*Phosphatidyl inositol-3-kinase (PI3-K)*. There was a trend for upregulation of PI3-K protein expression in the cerebellum of animals exposed to restraint stress when compared to controls ( $t(10)=-1.21$ ,  $p=0.25$ ; Figure 21A). In the striatum PI3-K expression showed a trend for decreased expression after stress when compared to controls ( $t(12)=0.86$ ,  $p=0.41$ ). PI3-K expression in the brain stem was not altered by restraint stress ( $t(14)=-0.12$ ,  $p=0.91$ ).

*Protein Kinase B (AKT)*. AKT expression in animals exposed to stress was showed a trend for downregulation by one-third when compared to controls within the cerebellum ( $t(9)=0.71$ ,  $p=0.49$ ; Figure 21B). A trend for downregulation of AKT expression was observed in the striatum ( $t(12)=0.11$ ,  $p=0.91$ ). AKT expression in the brain stem was upregulated by three quarters in restraint stressed animals when compared to non-stress controls ( $t(10)=-1.60$ ,  $p=0.14$ ; Figure 21C).

*Mitogen activated protein kinase (MEK1, MEK2)*. A trend was observed for down-regulation of MEK1, MEK2 in the cerebellum and upregulation in the striatum and brain stem ( $t(13)=0.13$ ,  $p=0.9$ ;  $t(10)=-0.48$ ,  $p=0.65$ ;  $t(14)=-0.3$ ,  $p=0.78$ ).

*Proliferating cells nuclear antigen (PCNA)*. There was a trend for downregulation of PCNA in the cerebellum ( $t(10)=0.27$ ,  $p=0.79$ ) and upregulation in the brain stem ( $t(10)=-0.45$ ,  $p=0.67$ ).

## **Discussion**

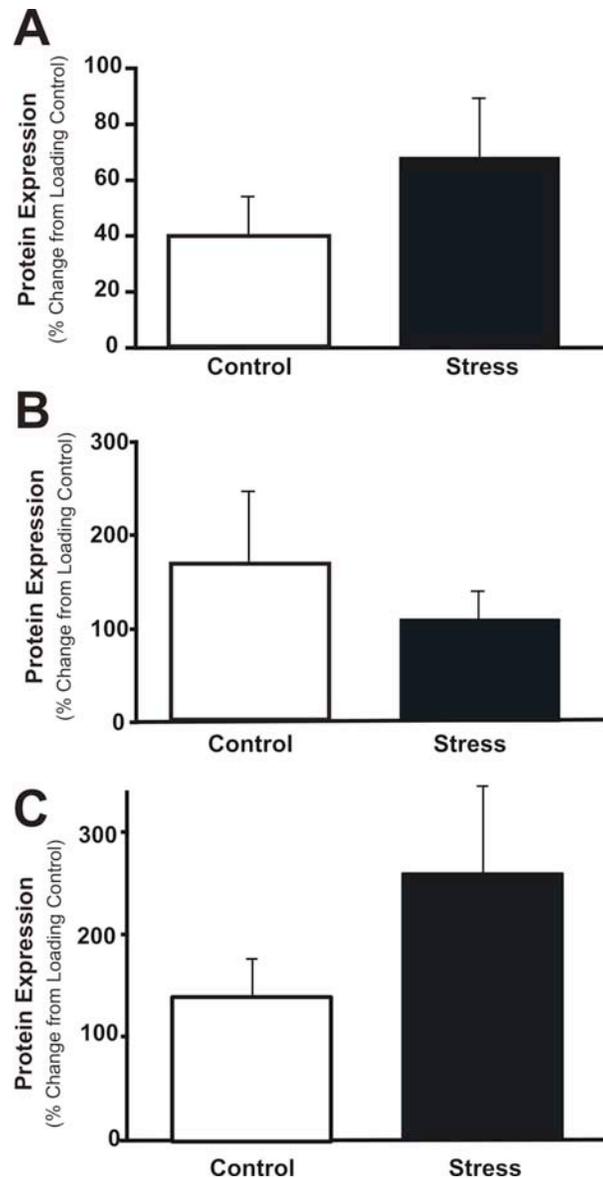
The aim of this study was to investigate the neuronal cell signaling pathways in the motor system of rats exposed to chronic restraint stress. Proteins evaluated included PI3-K, AKT, MEK1, MEK2 and PCNA. PI3-K protein expression was upregulated in the cerebellum, whereas it was downregulated in the striatum. AKT protein expression was upregulated in both the cerebellum and brain stem. No changes in MEK1, MEK2 and PCNA were observed. No difference in CORT concentration or blood glucose was observed.

Repeated exposure to restraint stress can lead to habituation and learned helplessness (Kant et al., 1985; Pitman et al., 1988; Armario et al., 1990; Wood et al., 2008). Habituation might be responsible for the lack of elevated CORT. Pitman et al. (1988) showed that 60 minutes of restraint stress for more than 7 days caused a decrease in circulating plasma CORT, however, habituation was not observed prior to 7 days of the stress treatment. Furthermore, habituation to chronic restraint stress was evident in this study by absence of elevated blood glucose in stress animals. A previous study by Volchegorskii et al. (2003) showed that only four exposures to restraint stress caused significant decreases in blood glucose. Even unpredictable stress such as chronic variable stress causes significant decreases in blood glucose concentrations (Tauchi et al., 2007), while stress usually is thought to result in elevated blood glucose concentrations. Habituation to the daily exposure to stress may explain why no changes in MEK1, MEK2 and PCNA protein expression were observed. Aside from these findings chronic restraint has been shown to affect motor system function (Metz et al. 2001, 2005). Thus, although

physiological habituation might occur, the motor system remains chronically affected by stress.

The response to stress depends on its duration and severity (Sapolsky, 1992). Exposure to chronic stress has been shown to cause cell death in numerous regions of the brain (Hebert et al., 2005; Kavushanky and Richter-Levin, 2006; Kwon et al., 2006). The data from this experiment provides evidence that changes in cell signaling patterns may occur in motor areas after chronic exposure to restraint stress, which could be the cause for behavioural impairments such as disturbed skilled limb use (Metz et al., 2005). In addition, the data from this experiment may also help explain the stress-induced exaggeration of cell death observed in motor areas stressing the rat model of Parkinson's disease (Smith et al., 2008). Furthermore, Yang et al. (2004) has shown that injections of MEK1, MEK 2 inhibitors reversed the effects of stress within the hippocampus. At the present it remains unknown which targets of PI3-K, AKT, MEK1, MEK 2 and PCNA mediate stress effects, however, previous work has helped piece together the puzzle of how all the proteins interact (Yang et al., 2004; New et al., 2007).

The present data showed upregulation of PI3-K expression in the cerebellum. PI3-K is activated by growth factors, such as brain derived neurotrophic factor (BDNF; Yang et al., 2004). BDNF expression was shown to increase significantly in animals exposed to stress for a possible compensatory response to preserve homeostasis or as a form of neuronal plasticity in response to stressful stimuli (Yang et al., 2004; Hetman et al., 1999, Yuan et al., 1997; Lin and Thiele, 2007). Enhanced upregulation of PI3-K expression therefore may indicate that protective mechanisms are increased in response



**Figure 21.** Protein expression of PI3-K in cerebellum and AKT in cerebellum and brain stem. (A) PI3-K protein expression of control and restraint stress animals in the cerebellum. Representative bands from each group shown below the x-axis. Note the increase in PI3-K protein expression of stress-treated animals. (B) AKT protein expression in the cerebellum of control and stress animals. Representative bands from each group shown below the x-axis. Note the decrease in AKT protein expression of stress treated animals. (C) AKT protein expression of control and restraint stress animals in the brain stem of control and stress animals. Representative bands from each group shown below the x-axis. Note the increase in AKT protein expression in stress treated animals.

to stress. AKT and PI3-K are closely linked, however, a parallel upregulation of AKT expression within the cerebellum was not observed. A possible explanation for this discrepancy is that there maybe another signaling pathway, in addition to PI3-K, that regulates AKT protein expression (New et al., 2007; Vauzour et al., 2007). AKT has been shown to be integral to the PI3-K pathway, however activation of AKT is accomplished by a number of extracellular signals, such as G-protein coupled receptors and integrin and growth factor receptor/tyrosine kinase super families (New et al., 2007). Lastly, dominant negative forms of AKT have been shown to trigger apoptosis (New et al., 2007). Previous studies investigating cell signaling changes in response to stress have shown significant alterations in MEK1, MEK2 protein expression. For example, Shen et al. (2004) showed enhanced phosphorylation of the MAPK family, which includes MEK1 and MEK2, in animals exposed to swim stress. In turn, blocking MEK1 or MEK2 activation completely impedes the effects of stress (Shen et al., 2004). Our present study did not show any changes in MEK1, MEK2 in the motor system.

The limitation of this study was its focus on total protein expression. It is possible that assessment of the phosphorylated stage of these proteins might have revealed a different pattern of expression. Phosphorylated proteins represent the activated form of the signaling cascade components (Barford et al., 1998) and as such might have been more informative to gain insights into motor system function. Previous studies have shown that stress causes significant changes in phosphorylated protein expression, specifically of MEK1,2 and cyclic- responsive element-binding protein (CREB; Cai et

al., 2007; Luo et al., 2005; Shen et al., 2004). Investigation of the phosphorylated stage of these proteins therefore remains an interesting task for future studies.

## **Conclusion**

The present study is the first to describe changes in intracellular signaling pathways in the motor system of rats exposed to stress. The evaluation of cell signaling proteins revealed that chronic restraint stress induces differential protein expression. Upregulation of proteins may lead to protective mechanisms, however, downregulation may inhibit normal cellular function leading to cell death. The observations from this study describe intracellular changes, which may underlie the previously described motor impairments.

## **6. Discussion**

### **6.1. Summary of Results**

This thesis described the route of action of stress and stress hormones on the motor system in a rat model. The main findings and conclusions of the four experiments described in this thesis are as follows. Chapter 2 outlined the correlation relationship between circulating plasma CORT concentrations from three physiological manipulations (swim stress, restraint stress and oral CORT administration) and performance on skilled reaching and walking. The findings from chapter 2 suggest that CORT is not the main factor modulating motor function in response to stress.

In Chapter 3 investigated the role of MR and GR in modulating motor behaviour in response to acute and chronic restraint stress and oral CORT treatment. The experiment revealed that both receptors are involved in modulating the skilled motor impairment observed previously in animals exposed to stress and treated with oral CORT.

In Chapter 4, the effects of 14 days of restraint stress and 21 days of recovery from stress were assessed in male and female rats. We observed significant sexual dimorphisms in the skilled reaching task during stress and post-stress time points that males and females performed significantly different to restraint Chapter 5 systematically investigated and described changes in cell signaling pathways within the motor system after chronic exposure to stress. We found that exposure to chronic restraint stress affects neuronal functioning in the cerebellum, striatum and brain stem.

## 6.2. Review of Stress

Stress is one of the most critical influences on behaviour (Seyle, 1976). A substantial amount of research has investigated the effects of stress on limbic system functions, for example cognition (Holscher, 1999). Recent findings have also shown that stress is a modulator of motor function, both non-skilled and skilled (Metz et al., 2001, 2005; Metz, 2007; Kirkland et al., 2008). Although these behavioural effects of stress on motor function have been thoroughly investigated, the mechanism causing the behavioural impairments had not been determined yet. The results from this thesis present the first evidence of direct interaction of stress and motor system function.

Within the motor system, specifically the striatum, cerebellum, basal ganglia and motor cortex, there is a high density of GR (Ahima and Harlan, 1990). The increased density of these receptors suggests that the motor system is susceptible to the effects of GCs (Metz, 2007). Previous studies have shown that acute stress causes hyperactivity, whereas chronic stress exposure causes decreases in activity levels of animals in the open field task (Roth and Katz, 1979). Recently, our laboratory has shown that stress causes impairments in skilled motor function. For example, animals exposed to restraint stress obtain significantly fewer pellets successfully when compared to baseline performance (Metz et al., 2005). Furthermore, animals exposed to restraint stress tested on the skilled walking task make significantly more errors when compared to non-stress controls (Metz et al., 2005). The mechanism through which stress modulates these motor behaviours remains unknown. The experiments in this thesis focused on investigating how stress and stress hormones may influence motor function.

#### **6.4. Significance of Results**

One of the hallmarks of the stress response is an increase in CORT (McEwen, 2000; Pedersen et al., 2001; Lucas et al., 2007). Therefore, CORT has been suggested to modulate motor function in response to stress (Metz et al., 2001). However, through correlation studies in Chapter 2, we have shown that CORT does not directly modulate motor function in response to stress. Furthermore, previous data have suggested that other hormones or neurotransmitters may potentially modulate the motor response to stress (Kawahara et al., 1999; Lucas et al., 2007). For example, Lucas et al. (2007) showed that repeated restraint stress causes increases in the dopamine transporter and dopamine D2 receptor in striatal subregions. Dopamine is involved in motor function and could therefore lead to disruptions in dopamine after exposure to stress (Dunn and File, 1983; Abercombie et al., 1989; Moghaddam, 1993). There are two receptors in the brain that bind to the stress hormone, both of these receptors have differential affinities and are therefore proposed to bind to different levels of corticosterone or cortisol (de Kloet et al., 1999, 2005).

The MR has been described previously to bind during phases of basal levels of CORT (de Kloet et al., 1999). Recent evidence, however, has suggested that MR participates in the stress response (Smythe et al., 1997; Spencer et al., 1998; Cole et al., 2000; Pace and Spencer, 2005; DeJrik et al., 2006). In Chapter 3, we have showed that both GR and MR are involved in modulating the motor response to stress. The results from this thesis in addition to previous studies support the theory that both the MR and the GR participate in the stress response (Smythe et al., 1997; Spencer et al., 1998; Cole et al., 2000; Pace and Spencer, 2005; Derijk et al., 2006). This novel theory requires

further investigation to determine details of the pathway how the MR is involved in the stress response. Pace and Spencer (2005) suggests that the receptor is involved in both mediating and turning off the stress response, whereas Derijk et al. (2006) suggested that the MR modulates the cognitive process when GCs are increased. Modulation of apoptosis and neurogenesis by administering drugs that interfere with MR and GR binding may represent an effective therapy for limiting the negative consequences associated with chronic stress (Lucassen et al., 2006).

### **6.5. Clinical Relevance and Future Direction**

Exposure to chronic stress has been suggested to increase an individual's vulnerability to diseases (Selye, 1976). Gaining further understanding of how stress modulates behaviour, specifically motor function, will help identifying effective therapies for stress-related motor disorders. For example, a recent study from our laboratory has shown that stress accelerates motor impairments in rodents in the animal model of Parkinson's disease, which involves unilateral injections of 6-hydroxydopamine (Smith et al., 2008). Another example by Kirkland et al. (2008) shows that stress prior to a motor cortex lesion diminishes the ability for animals to compensate in the skilled reaching task after the lesion. In addition post-lesion restraint stress caused a larger lesion volume in the brain. The increase in lesion volume could be due to increased levels of glutamate release associated with stress (Madrigal et al., 2003; Kirkland et al., 2008). Blocking the receptors involved in modulating motor function after exposure to stress could potentially serve as an effective therapy for reducing acceleration of either Parkinson's disease and

stroke-related symptoms and then be translated to humans affected with the disease (Smith et al., 2002; Kirkland et al., 2008; Smith et al., 2008).

Currently, glucocorticoid receptor blockers are being used to help treat individuals with depression (Belanoff et al 2001; DeBattista et al., 2006). A study by Belanoff et al. (2002) describes a study that administered a glucocorticoid receptor antagonist to individuals suffering from Alzheimer's disease and found that the drug slowed the progression of cognitive impairments seen in affected individuals. The cell signaling changes described in chapter 5 within motor areas can lead to the development of drugs to reverse the cellular changes that are induced by stress.

Preventing chronic exposure to stress is important in order to increase health in individuals (Keegan, 2003). A study by Underdown et al. (2006) showed that massage in babies six months or younger caused a beneficial impact on the number of hormones controlling stress. A similar result was seen in a study by Bost and Wallis (2006), where 15 min weekly massages were found to reduce psychological stress levels in nurses. Taking steps to prevent stress may help decrease the risk of negative effects that have been outlined in this thesis and previous studies.

## **6.6. Thesis Summary**

Recent findings from our laboratory have shown that stress represents a major modulator of motor function in the intact and damaged brain. The purpose of this thesis was to investigate mechanisms by which stress and stress hormones affect motor system function. The results of the experiments indicate that corticosterone may not control the impairments observed in motor function, however, both the MR and GR do modulate the motor response. The general conclusion from the four

experiments described in this thesis is that stress acts via a number of different pathways and activates a number of hormones, which impair motor function. There are also implications from the experiments that stress works through different mechanisms in males and females. Lastly, the behavioural impairments described in this thesis are possibly the result of the changes in cell signaling pathway function within the motor system.

## References

- Abercrombie, E. D., Keefe, K. A., DiFrischia, D. S., and Zigmond, M. J. (1989). Differential effect of stress on in vivo dopamine release in striatum, nucleus accumbens, and medial frontal cortex. *J Neurochem*, 52(5), 1655-1658.
- Ahima, R., and Harlan, R. (1990). Charting of type II glucocorticoid receptor-like immunoreactivity in the rat central nervous system. *Neurosci*, 39(3), 579-604.
- Ahima, R., Krozowski, Z., and Harlan, R. (1991). Type I corticosteroid receptor-like immunoreactivity in the rat CNS: distribution and regulation by corticosteroids. *J Comp Neurol*, 313(3), 522-538.
- Akana, S. F., Scribner, K. A., Bradbury, M. J., Strack, A. M., Walker, C. D., and Dallman, M. F. (1992). Feedback sensitivity of the rat hypothalamo-pituitary-adrenal axis and its capacity to adjust to exogenous corticosterone. *Endocrinol*, 131(2), 585-594.
- Akil, H., Cullinan, W., Lechan, R., Toni, R., Watson, S., and Moore, R. (1999). Neuroendocrine Systems I: Overview - Thyroid and Adrenal Axes. In M. Zigmond, F. Bloom, S. Landis, J. Roberts & L. Squire (Eds.), *Fundamental Neuroscience* (pp. 1127-1150). Toronto: Academic Press.
- Albeck, D. S., McKittrick, C. R., Blanchard, D. C., Blanchard, R. J., Nikulina, J., McEwen, B. S., and Sakai, R.R. (1997). Chronic social stress alters levels of corticotropin-releasing factor and arginine vasopressin mRNA in rat brain. *J Neurosci*, 17(12), 4895-4903.
- Arakawa, H., Kodama, H., Matsuoka, N., and Yamaguchi, I. (1997). Stress increases plasma enzyme activity in rats: differential effects of adrenergic and cholinergic blockades. *J Pharmacol Exp Ther*, 280(3), 1296-1303.
- Armario, A., Garcia-Marquez, C., and Jolin, T. (1987). The effects of chronic intermittent stress on basal and acute stress levels of TSH and GH, and their response to hypothalamic regulatory factors in the rat. *Psychoneuroendocrinology*, 12(5), 399-406.
- Armario, A., Marti, J., and Gil, M. (1990). The serum glucose response to acute stress is sensitive to the intensity of the stressor and to habituation. *Psychoneuroendocrinology*, 15(5-6), 341-347.
- Avital, A., Segal, M. and Richter-Levin, G .2006. Contrasting role of corticosteroid receptors in hippocampal plasticity. *J Neuro*, 26 (36), 30-34.

- Baccan, G., Oliveira, R., and Mantovani, B. (2004). Stress and immunological phagocytosis: possible nongenomic action of corticosterone. *Life Sciences*, 75(11), 1357-1368.
- Bachman, C., Lintorst, A., Hosboer, F., and Reul, J. (2003). Effect of chronic administration of selective of GR anataognist on the rat HPA axis. *Neuropsychopharmacology*, 28(6), 1056-1067.
- Bale, T. L., and Vale, W. W. (2004). CRF and CRF receptors: role in stress responsivity and other behaviours. *Annu Rev Pharmacol Toxicol*, 44, 525-557.
- Bamberger, C., Schulte, H., and Chrousos, G. (1996). Molecular determinants of GR function and tissue sensivity to glucocorticoids. *Endocrine Reviews*, 17(3), 245-260.
- Barford, D., Das, A. K., & Egloff, M. P. (1998). The structure and mechanism of protein phosphatases: insights into catalysis and regulation. *Annu Rev Biophys Biomol Struct*, 27, 133-164.
- Bates, H. E., Kiraly, M. A., Yue, J. T., Goche Montes, D., Elliott, M. E., Riddell, M. C., et al. (2007). Recurrent intermittent restraint delays fed and fasting hyperglycemia and improves glucose return to baseline levels during glucose tolerance tests in the Zucker diabetic fatty rat--role of food intake and corticosterone. *Metabolism*, 56(8), 1065-1075.
- Beck, C., Estes, P., Bona, B., Muro-Cacho, C., Nordeen, S., and Edwards, D. (1993). The steroid antagonist exerts different effect on glucocorticoid and progesterone receptors. *Endocrinol*, 133(2), 728-740.
- Belanoff, J. K., Flores, B. H., Kalezhan, M., Sund, B., and Schatzberg, A. F. (2001). Rapid reversal of psychotic depression using mifepristone. *J Clin Psychopharmacol*, 21(5), 516-521.
- Belanoff, J. K., Jurik, J., Schatzberg, L. D., DeBattista, C., and Schatzberg, A. F. (2002). Slowing the progression of cognitive decline in Alzheimer's disease using mifepristone. *J Mol Neurosci*, 19(1-2), 201-206.
- Benabid, N., Mesfioui, A., and Ouichou, A. (2008). Effects of photoperiod regimen on emotional behaviour in two tests for anxiolytic activity in Wistar rat. *Brain Res Bull*, 75(1), 53-59.
- Berridge, C. W., & Dunn, A. J. (1989). CRF and restraint-stress decrease exploratory behaviour in hypophysectomized mice. *Pharmacol Biochem Behav*, 34(3), 517-519.
- Bogolepov, N. N., Koplik, E. V., Krivitskaya, G. N., Popova, E. N., & Sudakov, K. V. (2001). Structural and functional characteristics of neurons in the sensorimotor

- cortex of rats with different resistance to emotional stress. *Bull Exp Biol Med*, 132(2), 715-718.
- Bost, N., and Wallis, M. (2006). The effectiveness of a 15 minute weekly massage in reducing physical and psychological stress in nurses. *Aust J Adv Nurs*, 23(4), 28-33.
- Bowman, R., Maclusky, N., Diz, S., Zrull, M., and Luine, V. (2006). Aged rats: sex difference and responses to chronic stress. *Brain Research*, 1126(1), 156-166.
- Cai, Q., Zhu, Z., Huang, S., Li, H., Fan, X., Jia, N., et al. (2007). Sex and region difference of the expression of ERK in prenatal stress offspring hippocampus. *Int J Dev Neurosci*, 25(4), 207-213.
- Campbell, T., Lin, S., DeVires, C., and Lambert, K. (2003). Coping strategies in male and female rats exposed to multiple stressors. *Physiol and Behav*, 78(3), 495-504.
- Carey, M., Deterd, C., Koning, J. D., Helmerhorst, F., and de Kloet, E.R. (1995). The influence of ovarian steroids on the hypothalamic-pituitary-adrenal regulation in the female rat. *J Endocrino* (144), 311-321.
- Cenci, M., Whishaw, I., and Schallert, T. (2002). Animal models of neurological deficits: how relevant is the rat? *Nat Rev Neurosci.*, 3(7), 574-590.
- Chadda, R., & Devaud, L. L. (2005). Differential effects of mild repeated restraint stress on behaviors and GABA(A) receptors in male and female rats. *Pharmacol Biochem Behav*, 81(4), 854-863.
- Chen, R. H., Su, Y. H., Chuang, R. L., and Chang, T. Y. (1998). Suppression of transforming growth factor-beta-induced apoptosis through a phosphatidylinositol 3-kinase/Akt-dependent pathway. *Oncogene*, 17(15), 1959-1968.
- Cole, M., Kalman, B., Pace, T., Topczewski, F., Lowrey, M., & and Spencer, R. (2000). Selective blockade of the MR impairs hypothalamic-pituitary-adrenal axis expression of habituation. *J Neuroendocrinol*, 12(10), 1034-1042.
- Conrad, C., Jackson, J., Wiczorek, L., Baran, S., Harman, J., Wright, R., and Korol, D.L. (2004). Acute stress impairs spatial memory in male but not female rats: influence of estrous cycle. *Pharmacol Biochem Behav*, 78(3), 569-579.
- Crawley, J. N. (1985). Exploratory behaviour models of anxiety in mice. *Neuroscience Biobehavioural Reviews*, 9(1), 37-44.
- de Kloet, E. R., Oitzl, M. S., and Joels, M. (1999). Stress and cognition: are corticosteroids good or bad guys? *Trends Neurosci*, 22(10), 422-426.

- de Kloet, E. R., Joels, M., and Holsboer, F. (2005). Stress and the brain: from adaptation to disease. *Nat Rev Neurosci*, 6(6), 463-475.
- Dalla, C., Antoniou, K., Drossopolou, G., Xagoratis, M., Kokras, N., Sfikakis, A., and Papadopoulou-Daifoti, Z. (2005). Chronic Mild Stress Impact: Are females More Vulnerable? *Neurosci*, 135(3), 703-714.
- Dayas, C. V., Buller, K. M., Crane, J. W., Xu, Y., and Day, T. A. (2001). Stressor categorization: acute physical and psychological stressors elicit distinctive recruitment patterns in the amygdala and in medullary noradrenergic cell groups. *Eur J Neurosci*, 14(7), 1143-1152.
- DeBattista, C., Belanoff, J., Glass, S., Khan, A., Horne, R. L., Blasey, C., Carpenter, L.L., and Alva, G. (2006). Mifepristone versus placebo in the treatment of psychosis in patients with psychotic major depression. *Biol Psychiatry*, 60(12), 1343-1349.
- Derijk, R. H., Wust, S., Meijer, O. C., Zennaro, M. C., Federenko, I. S., Hellhammer, D. H., Giacchetti, G., Vreugdenhil, E., Zitman, F.G., and de Kloet, E.R. (2006). A common polymorphism in the mineralocorticoid receptor modulates stress responsiveness. *J Clin Endocrinol Metab*, 91(12), 5083-5089.
- Devenport, L., Thomas, T., Knehans, A., and Sundstrom, A. (1990). Acute, chronic, and interactive effects of type I and II corticosteroid receptor stimulation on feeding and weight gain. *Physiol Behav*, 47(6), 1221-1228.
- Doherty, M., & Gratton, A. (2007). Differential involvement of ventral tegmental GABA(A) and GABA(B) receptors in the regulation of the nucleus accumbens dopamine response to stress. *Brain Res*, 1150, 62-68.
- Dronjak, S., Gavrilovic, L., Filipovic, D., and Radojcic, M. B. (2004). Immobilization and cold stress affect sympatho-adrenomedullary system and pituitary-adrenocortical axis of rats exposed to long-term isolation and crowding. *Physiol Behav*, 81(3), 409-415.
- Dunn, A. J., and File, S. E. (1983). Cold restraint alters dopamine metabolism in frontal cortex, nucleus accumbens and neostriatum. *Physiol Behav*, 31(4), 511-513.
- Eshkol, N., and Wachmann, A. (1958). *Movement Notation*. London, UK: Weidenfeld and Nicolson.
- Faraday, M. (2002). Rat sex and brain differences in responses to stress. *Physiol Behav*, 75 (4), 507-522.
- Feldman, S., and Weidenfeld, J. (1999). GR antagonists in the hippocampus modify the negative feedback following neural stimuli. *Brain Research*, 821(1), 33-37.

- Field, E.F., Whishaw I.Q., and Pellis, S.M. (1996) A kinematic analysis of evasive dodging movements used during food protection in the rat (*Rattus norvegicus*): evidence for sex differences in movement. *J Comp Psychol*, 110 (3), 298-306.
- Field, E., and Pellis, S. (1998). Sex difference in the organization of behaviour patterns: endpoint measures do not tell the whole story. In L. Ellis & L. Ebertz (Eds.), *Males, Females, and Behaviour*. London: Praeger.
- Field, E.F., Whishaw, I.Q., and Pellis, S.M. (2000) Sex differences in catalepsy: evidence for hormone-dependent postural mechanisms in haloperidol-treated rats. *Behav Brain Res*. 109(2):207-12.
- Field, E., and Whishaw, I. (2005). Sexually dimorphic postural adjustments are used in a skilled reaching task in the rats. *Behavioural Brain Research*, 163(2), 237-245.
- Figueiredo, H., Dolgas, C., and Herman, J. (2002). Stress activation of cortex and hippocampus is modulated by sex and stage of estrus. *Endocrinology*, 143(7), 2534-2540.
- Figueiredo, H. F., Ulrich-Lai, Y. M., Choi, D. C., and Herman, J. P. (2007). Estrogen potentiates adrenocortical responses to stress in female rats. *American Journal of Physiology, Endocrinology and Metabolism*, 292(4), E1173-1182.
- Frankenhaeuser, M., Dunne, E., and Lundberg, U. (1976). Sex differences in sympathetic adrenal medullary reactins induced by different stressors. *Psychopharmacology*, 47 (1), 1-5.
- Frebel, K., and Wiese, S. (2006). Signaling molecules essential for neuronal survival and differentiation. *Biochem Soc Trans*, 34(Pt 6), 1287-1290.
- Froc, D. J., and Christie, B. R. (2005). Corticotrophin-releasing hormone decreases synaptic transmission in rat sensorimotor cortex in vivo. *Neurosci*, 134(3), 965-973.
- Garcia, A., Marti, O., Valles, A., Dal-Zotto, S., and Armario, A. (2000). Recovery of the hypothalamic-pituitary-adrenal response to stress. Effect of stress intensity, stress duration and previous stress exposure. *Neuroendocrinology*, 72(2), 114-125.
- Garcia-Bueno, B., Caso, J. R., Perez-Nievas, B. G., Lorenzo, P., & Leza, J. C. (2007). Effects of peroxisome proliferator-activated receptor gamma agonists on brain glucose and glutamate transporters after stress in rats. *Neuropsychopharmacology*, 32(6), 1251-1260.
- Ghez, C, and J Krakauer. "The Organization of Movement." Principals of Neural Science. Eds. ER Kandel, JH Schwartz and TM Jessel. 4th ed. Toronto: McGraw Hill, 2000.

- Ginsberg, A. B., Frank, M. G., Francis, A. B., Rubin, B. A., O'Connor, K. A., and Spencer, R. L. (2006). Specific and time-dependent effects of glucocorticoid receptor agonist RU28362 on stress-induced pro-opiomelanocortin hnRNA, c-fos mRNA and zif268 mRNA in the pituitary. *J Neuroendocrinol*, 18(2), 129-138.
- Greenwood, B. N., & Fleshner, M. (2008). Exercise, Learned Helplessness, and the Stress-Resistant Brain. *Neuromolecular Med.* Epub ahead of print.
- Grewal, S. S., York, R. D., and Stork, P. J. (1999). Extra cellular-signal-regulated kinase signalling in neurons. *Curr Opin Neurobiol*, 9(5), 544-553.
- Gunnar, M., & Quevedo, K. (2007). The neurobiology of stress and development. *Annu Rev Psychol*, 58, 145-173.
- Haleen, D., Kennett, G., and Curzon, G. (1988). Adaptation of female rats to stress: shift to male pattern by inhibition of corticosterone synthesis. *Brain Research*, 458(2), 339-347.
- Handa, R., Nunley, K., Lorena, S., Louie, J., McGivern, R., and Bollnow, M. (1994). Androgen regulation of adrenocorticotropin and corticosterone secretion in the male rat following novelty and foot shock stressors. *Physiology and Behaviour*, 55(1), 117-124.
- Harkin, A., Connor, T. J., O'Donnell, J. M., and Kelly, J. P. (2002). Physiological and behavioural responses to stress: what does a rat find stressful? *Lab Anim*, 31(4), 42-50.
- Harris, R. B., Gu, H., Mitchell, T. D., Endale, L., Russo, M., & Ryan, D. H. (2004). Increased glucocorticoid response to a novel stress in rats that have been restrained. *Physiol Behav*, 81(4), 557-568.
- Hebert, M. A., Serova, L. I., & Sabban, E. L. (2005). Single and repeated immobilization stress differentially trigger induction and phosphorylation of several transcription factors and mitogen-activated protein kinases in the rat locus coeruleus. *J Neurochem*, 95(2), 484-498.
- Hellal-Levy, C., Fagart, J., Souque, A., & Rafestin-Oblin, M. E. (2000). Mechanistic aspects of mineralocorticoid receptor activation. *Kidney Int*, 57(4), 1250-1255.
- Henn, F. A., & Vollmayr, B. (2005). Stress models of depression: forming genetically vulnerable strains. *Neurosci Biobehav Rev*, 29(4-5), 799-804.
- Hetman, M., Kanning, K., Cavanaugh, J. E., & Xia, Z. (1999). Neuroprotection by brain-derived neurotrophic factor is mediated by extra cellular signal-regulated kinase and phosphatidylinositol 3-kinase. *J Biol Chem*, 274(32), 22569-22580.

- Holscher, C. (1999). Stress impairs performance in spatial water maze learning tasks. *Behavioural Brain Research*, 100(1-2), 225-235.
- Imbe, H., Murakami, S., Okamoto, K., Iwai-Liao, Y., and Senba, E. (2004). The effects of acute and chronic restraint stress on activation of ERK in the rostral ventromedial medulla and locus coeruleus. *Pain*, 112(3), 361-371.
- Ishida, H., Mitsui, K., Nukaya, H., Matsumoto, K., and Tsuji, K. (2003). Study of active substances involved in skin dysfunction induced by crowding stress. I. Effect of crowding and isolation on some physiological variables, skin function and skin blood perfusion in hairless mice. *Biol & Pharm Bull*, 26(2), 170-181.
- Joels, M., Karst, H., Alfarez, D., Heine, V. M., Qin, Y., van Riel, E., Verkuyl, M., Lucassen, P.J., and Krugers, H.J. (2004). Effects of chronic stress on structure and cell function in rat hippocampus and hypothalamus. *Stress*, 7(4), 221-231.
- Kaas, J. H. (1991). Plasticity of sensory and motor maps in adult mammals. *Annu Rev Neurosci*, 14, 137-167.
- Kandandrea, D., Kittas, C., and Kitraki, E. (2002). Forced swimming differentially affects male and female brain corticosteroid receptors. *Neuroendocrinology*, 75(4), 217-226.
- Karuppayil, S. M., Moran, E., and Das, G. M. (1998). Differential regulation of p53-dependent and -independent proliferating cell nuclear antigen gene transcription by 12 O<sup>6</sup>EB1A oncoprotein requires CBP. *J Biol Chem*, 273(28), 17303-17306.
- Kavushansky, A., and Richter-Levin, G. (2006). Effects of stress and corticosterone on activity and plasticity in the amygdala. *J Neurosci Res.*, 84(7), 1580-7.
- Kawahara, Y., Kawahara, H., and Westerink, B. H. (1999). Comparison of effects of hypotension and handling stress on the release of noradrenaline and dopamine in the locus coeruleus and medial prefrontal cortex of the rat. *Naunyn Schmiedeberg's Arch Pharmacol*, 360(1), 42-49.
- Kirkland, S., Coma, A., Colwell, K., and Metz, G. (2008). Delayed recovery and exaggerated infarct size by post-lesion stress in a rat model of focal cerebral stroke. *Brain Research*, 1201(27), 151-60.
- Kolb, B., and Whishaw, I. Q. (1998). Brain plasticity and behaviour. *Annu Rev Psychol*, 49, 43-64.
- Kolb, B. (2005). Neurological Models. In I. Whishaw & B. Kolb (Eds.), *The Behaviour of the Laboratory Rat: a handbook with tests*. Toronto: Oxford University Press.

- Korte, S. M., de Boer, S. F., de Kloet, E. R., and Bohus, B. (1995). Anxiolytic-like effects of selective mineralocorticoid and glucocorticoid antagonists on fear-enhanced behaviour in the elevated plus-maze. *Psychoneuroendocrinology*, *20*(4), 385-394.
- Kuhse, H., & Singer, P. (1998). *A Companion to Bioethics*. Boston: Blackwell Publishing.
- Kurgers, H., Goltstein, P., Linden, S. v. d., and Joels, M. (2006). Blockage of glucocorticoid receptors rapidly restores hippocampal CA1 synaptic plasticity after exposure to chronic stress. *European Journal of Neuroscience*, *23*(11), 3052-3055.
- Kwon, M. S., Seo, Y. J., Shim, E. J., Choi, S. S., Lee, J. Y., and Suh, H. W. (2006). The effect of single or repeated restraint stress on several signal molecules in paraventricular nucleus, arcuate nucleus and locus coeruleus. *Neuroscience*, *142*(4), 1281-1292.
- Lee, S. Y., Kang, J. S., Song, G. Y., and Myung, C. S. (2006). Stress induces the expression of heterotrimeric G protein beta subunits and the phosphorylation of PKB/Akt and ERK1/2 in rat brain. *Neurosci Res*, *56*(2), 180-192.
- Li, S. P., Junttila, M. R., Han, J., Kahari, V. M., and Westermarck, J. (2003). p38 Mitogen-activated protein kinase pathway suppresses cell survival by inducing dephosphorylation of mitogen-activated protein/extracellular signal-regulated kinase1, 2. *Cancer Res*, *63*(13), 3473-3477.
- Liss, B., and Roeper, J. (2007). Individual dopamine midbrain neurons: Functional diversity and flexibility in health and disease. *Brain Res Rev*.
- Levine, S., Glick, D., and Nakane, P. K. (1967). Adrenal and plasma corticosterone and vitamin A in rat adrenal glands during postnatal development. *Endocrinology*, *80*(5), 910-914.
- Lopez, J. F., Akil, H., and Watson, S. J. (1999). Neural circuits mediating stress. *Biol Psychiatry*, *46*(11), 1461-1471.
- Luine, V. (2002). Sex differences in chronic stress effects on memory in rats. *Stress: The International Journal on the Biology of Stress*, *5*(3), 205-216.
- Luo, C., Xu, H., & Li, X. M. (2005). Quetiapine reverses the suppression of hippocampal neurogenesis caused by repeated restraint stress. *Brain Res*, *1063*(1), 32-39.
- Lupien, S., and McEwen, B. (1997). The acute effects of corticosteroids on cognition: integration of animal and human model studies. *Brain Research Reviews*, *24*, 1-27.

- Lucas, L. R., Wang, C. J., McCall, T. J., and McEwen, B. S. (2007). Effects of immobilization stress on neurochemical markers in the motivational system of the male rat. *Brain Res*, 1155, 108-115.
- Lucassen, P. J., Heine, V. M., Muller, M. B., van der Beek, E. M., Wiegant, V. M., De Kloet, E. R., Joels, M., Fuchs, E., Swaab, D.F., and Czeh, B. (2006). Stress, depression and hippocampal apoptosis. *CNS Neurol Disord Drug Targets*, 5(5), 531-546.
- McEwen, B., and Sapolsky, R. (1995). Stress and cognitive function. *Current Opinion in Neurobiology*, 5(2), 205-216.
- McEwen, B. S. (2000). Effects of adverse experiences for brain structure and function. *Biol Psychiatry*, 48(8), 721-731.
- McEwen, B. S. (2000). The neurobiology of stress: from serendipity to clinical relevance. *Brain Res*, 886(1-2), 172-189.
- Madrigal, J.L., Caso, J.R., de Cristóbal, J., Cárdenas, A., Leza, J.C., Lizasoain, I., Lorenzo, P., Moro, M.A. (2003). Effect of subacute and chronic immobilisation stress on the outcome of permanent focal cerebral ischaemia in rats. *Brain Res.*, 979(1-2), 137-45.
- Marcondes, F., Lanza, V., and Spadari-Bartfisch, R. (1996). Stress-induced subsensitivity to catecholamine depends on the estrous cycle. *Canadian Journal of Physiology Pharmacology*, 74(6), 663-669.
- Marlier, L. N., Csikos, T., Rebaudengo, N., Borboni, P., Patacchioli, F. R., Angelucci, L., Privat, A., and Lauro, R. (1995). Distribution of glucocorticoid receptor mRNA in the rat spinal cord. *Neuroreport*, 6(16), 2245-2249.
- Mashoodh, R., Wright, L. D., Hebert, K., and Perrot-Sinal, T. S. (2008). Investigation of sex differences in behavioural, endocrine, and neural measures following repeated psychological stressor exposure. *Behav Brain Res*, 188(2), 368-379.
- Mayford, M. (2007). Protein kinase signaling in synaptic plasticity and memory. *Curr Opin Neurobiol*, 17(3), 313-317.
- Meaney, M. J., Diorio, J., Francis, D., Widdowson, J., LaPlante, P., Caldji, C., Sharma, S., Seckl, J.R., and Plotsky, P.M. (1996). Early environmental regulation of forebrain glucocorticoid receptor gene expression: implications for adrenocortical responses to stress. *Dev Neurosci*, 18(1-2), 49-72.
- Mercier, S., Frederic, Canini, Buguet, A., Cespuglio, R., Martin, S., and Bourdon, L. (2003). Behavioural changes after an acute stress: stressor and test types influences. *Behav Brain Res*, 139(1-2), 167-175.

- Melia, K. R., Ryabinin, A. E., Schroeder, R., Bloom, F. E., and Wilson, M. C. (1994). Induction and habituation of immediate early gene expression in rat brain by acute and repeated restraint stress. *J Neurosci*, *14*(10), 5929-5938.
- Metz, G. A., and Whishaw, I. Q. (2000). Skilled reaching an action pattern: stability in rat (*Rattus norvegicus*) grasping movements as a function of changing food pellet size. *Behavioural Brain Research*, *116*(2), 111-122.
- Metz, G.A., Schwab, M., and Welzl, H. (2001). The effects of acute and chronic stress on motor and sensory performance in male Lewis rats. *Physiology and Behavior*, *72*(1-2), 29-35.
- Metz, G. A., and Whishaw, I. Q. (2002a). Drug-induced rotation intensity in unilateral dopamine-depleted rats is not correlated with end point or qualitative measures of forelimb or hindlimb motor performance. *Neuroscience*, *111*(2), 325-336.
- Metz, G. A., and Whishaw, I. Q. (2002b). Cortical and subcortical lesions impair skilled walking in the ladder rung walking test: a new task to evaluate fore- and hindlimb stepping, placing, and co-ordination. *J Neurosci Methods*, *115*(2), 169-179.
- Metz, G.A., Jadavji, N., and Smith, L. (2005). Modulation of motor function by stress: a novel concept of the effects of stress and corticosterone on behaviour. *European Journal of Neuroscience*, *22*(5), 1190-1200.
- Metz, G. A. (2007). Stress as a modulator of motor system function and pathology. *Rev Neurosci*, *18*(3-4), 209-222.
- Meyer, A. S., and Schmidt, T. J. (1997). Differential effects of agonist and antagonists on autoregulation of glucocorticoid receptors in a rat colonic adenocarcinoma cell line. *J Steroid Biochem Mol Biol*, *62*(1), 97-105.
- Michaelis, T., Biurrun, G. d., and Watanabe, T. (2001). Gender differences in acute and chronic stress in Wistar Kyoto (WKY) rats. *J Psychiatr Res.*, *35*(4), 231-237.
- Moghaddam, B. (1993). Stress preferentially increases extraneuronal levels of excitatory amino acids in the prefrontal cortex: comparison to hippocampus and basal ganglia. *J Neurochem*, *60*(5), 1650-1657.
- Moldow, R., Beck, K., Weaver, S., and Servatius, R. (2005). Blockage of GR, but not mineralcorticoid receptors prevents the persistent increase in circulating basal corticosterone concentrations following stress in the rat. *Neurosci Lett*, *374* (1), 25-28.

- Morimoto, M., Morita, N., Ozawa, H., Yokoyama, K., and Kawata, M. (1996). Distribution of glucocorticoid receptor immunoreactivity and mRNA in the rat brain: an immunohistochemical and in situ hybridization study. *Neurosci Res*, 26, 235-269.
- New, D. C., Wu, K., Kwok, A. W., and Wong, Y. H. (2007). G protein-coupled receptor-induced Akt activity in cellular proliferation and apoptosis. *FEBS J*, 274(23), 6025-6036.
- Oomen, C. A., Mayer, J. L., de Kloet, E. R., Joels, M., and Lucassen, P. J. (2007). Brief treatment with the glucocorticoid receptor antagonist mifepristone normalizes the reduction in neurogenesis after chronic stress. *Eur J Neurosci*, 26(12), 3395-3401.
- Pan, Y., Siregar, E., and Carr, K. D. (2006). Striatal cell signaling in chronically food-restricted rats under basal conditions and in response to brief handling. *Neurosci Lett*, 393(2-3), 243-248.
- Pace, T., and Spencer, R. (2005). Disruption of mineralcorticoid receptor function increases corticosterone responding to mild, but not moderate psychological stressor. *J Physiol Endocrinol Metab*, 288, E1082-1088.
- Pare, W., Blair, G., Kluczynski, J., and Tejani-Butt, S. (1999). Gender differences in acute and chronic stress in Wistar Kyoto rats. *Integr Physiol Behav Sci*, 34(4), 227-241.
- Pedersen, W. A., Wan, R., and Mattson, M. P. (2001). Impact of aging on stress-responsive neuroendocrine systems. *Mech Ageing Dev*, 122(9), 963-983.
- Pham, K., Nacher, J., Hof, P. R., and McEwen, B. S. (2003). Repeated restraint stress suppresses neurogenesis and induces biphasic PSA-NCAM expression in the adult rat dentate gyrus. *Eur J of Neurosci*, 17(4), 879-886.
- Pham, T. M., Winblad, B., Granholm, A. C., and Mohammed, A. H. (2002). Environmental influences on brain neurotrophins in rats. *Pharmacol Biochem Behav*, 73(1), 167-175.
- Philpott, K. L., McCarthy, M. J., Klippel, A., and Rubin, L. L. (1997). Activated phosphatidylinositol 3-kinase and Akt kinase promote survival of superior cervical neurons. *J Cell Biol*, 139(3), 809-815.
- Pitman, D. L., Ottenweller, J. E., and Natelson, B. H. (1988). Plasma corticosterone levels during repeated presentation of two intensities of restraint stress: chronic stress and habituation. *Physiol Behav*, 43(1), 47-55.
- Quinn, S. J., and Williams, G. H. (1988). Regulation of aldosterone secretion. *Annu Rev Physiol*, 50, 409-426.

- Radley, J. J., and Morrison, J. H. (2005). Repeated stress and structural plasticity in the brain. *Ageing Res Rev*, 4(2), 271-287.
- Renard, G. M., Suarez, M. M., Levin, G. M., and Rivarola, M. A. (2005). Sex differences in rats: effects of chronic stress on sympathetic system and anxiety. *Physiol Behav*, 85(3), 363-369.
- Riva MA (Ghez and Kraksauer). The role of neurotrophic factor in the stress response. In T. Stecker, NH Kalin, JMHM Reul. Handbook of stress and the brain Part 1: the neurobiology of stress: Vol. 15. (pp.665-678). New York: NY: Elsevier.
- Rodgers, R. J., Haller, J., Holmes, A., Halasz, J., Walton, T. J., and Brain, P. F. (1999). Corticosterone response to the plus-maze: high correlation with risk assessment in rats and mice. *Physiol Behav*, 68(1-2), 47-53.
- Rogerson, F. M., and Fuller, P. J. (2000). Mineralocorticoid action. *Steroids*, 65(2), 61-73.
- Roth, K. A., and Katz, R. J. (1979). Stress, behavioural arousal, and open field activity--a reexamination of emotionality in the rat. *Neurosci Biobehav Rev*, 3(4), 247-263.
- Sabban, E. L., Liu, X., Serova, L., Gueorguiev, V., and Kvetnansky, R. (2006). Stress triggered changes in gene expression in adrenal medulla: transcriptional responses to acute and chronic stress. *Cell Mol Neurobiol*, 26(4-6), 845-856.
- Salicioni, A. M., Caronm, R. W., and R.P.Deis. (1993). Adrenal progesterone facilitates the negative feedback of estrogen on LH release in ovariectomized rats. *J Endocrino*, 139(2), 253-258.
- Sapolsky, R. (1992). Neuroendocrinology of the Stress-Response. In J. Becker, S. Breedlove & D. Crews (Eds.), *Behavioural Endocrinology* (Vol. 2nd Edition). Cambridge: MIT Press.
- Sapolsky, R. (2000). Stress Hormones: Good and Bad. *Neurobiol Dis*, 7(5), 540-542.
- Sapolsky, R., Romero, L. M., and Munck, A. (2000). How do Glucocorticoids Influence Stress Responses? Integrating Permissive, Suppressive, Stimulatory and Preparative Actions. *Endocrine Reviews*, 21(1), 55-89.
- Sandi, C. (2004). Stress, cognitive impairment and cell adhesion molecules. *Nat Rev Neurosci*, 5(12), 917-930.
- Selye, H. (1976). Stress and Distress. *Frontiers in Medicine*, 1(8), 9-13.

- Shen, C. P., Tsimberg, Y., Salvadore, C., and Meller, E. (2004). Activation of Erk and JNK MAPK pathways by acute swim stress in rat brain regions. *BMC Neurosci*, 5(1), 36.
- Shors, T. J., Weiss, C., and Thompson, R. F. (1992). Stress-induced facilitation of classical conditioning. *Science*, 257(5069), 537-539.
- Shors, T. J., Lewczyk, C., Pacynski, M., Mathew, P. R., and Pickett, J. (1998). Stages of estrous mediate the stress-induced impairment of associative learning in the female rat. *Neuroreport*, 9(3), 419-423.
- Shors, T., Chua, C., and Falduto, J. (2001). Sex differences and opposite effects on stress on dendritic spine density in the male versus female hippocampus. *J Neurosci*, 21(16), 6292-6297.
- Shors, T. J. (2004). Learning during stressful times. *Learn Mem*, 11(2), 137-144.
- Simpson, G. M., El Sheshai, A., Loza, N., Kingsbury, S. J., Fayek, M., Rady, A., and Fawzy, W.. (2005). An 8-week open-label trial of a 6-day course of mifepristone for the treatment of psychotic depression. *J Clin Psychiatry*, 66(5), 598-602.
- Skilbeck, K. J., Hinton, T., & Johnston, G. A. (2008). Sex-differences and stress: Effects on regional high and low affinity [(3)H]GABA binding. *Neurochem Int*, 52(6), 1212-1219.
- Skorzewska, A., Bidzinski, A., Lehner, M., Turzynska, D., Wislowska-Stanek, A., Sobolewska, A., Szyndler, J., Maciejak, P., Taracha, E., and Płaźnik, A. (2006). The effects of acute and chronic administration of corticosterone on rat behaviour in two models of fear responses, plasma corticosterone concentration, and c-Fos expression in the brain structures. *Pharmacol Biochem Behav*, 85(3), 522-534.
- Smagin, G., Heinrichs, S., and Dunn, A. (2001). The role of CRH in behavioural responses to stress. *Peptides*, 22(5), 713-724
- Smith, A. D., Castro, S. L., and Zigmond, M. J. (2002). Stress-induced Parkinson's disease: a working hypothesis. *Physiol Behav*, 77(4-5), 527-531.
- Smith, L. K., Jadavji, N. M., Colwell, K. L., Pehudoff, S. K., and Metz, G. A. (2008). Stress and glucocorticoid accelerate dopaminergic apoptosis and exaggerate motor symptoms in a rat model of Parkinson's disease. *Eur J Neurosci*, *In Press*.
- Smythe, J., Murphy, D., Timothy, C., and Costall, B. (1997). Hippocampal mineralocorticoid, but not glucocorticoid receptors modulate anxiety like behavior in rats. *Pharmacol Biochem Behav*, 56(3), 507-513.

- Sousa, N., Almeida, O. F., and Wotjak, C. T. (2006). A hitchhiker's guide to behavioural analysis in laboratory rodents. *Genes Brain Behav*, 5 (Suppl 2), 5-24.
- Spencer, R., Kim, P., Klamann, B., and Cole, M. (1998). Evidence for MR Facilitation of GR dependent regulation of HPA axis activity. *Endocrinol*, 139(6), 2718-2726.
- Stamp, J., and Herbert, J. (2001). Corticosterone modulates autonomic responses and adaptation of central immediate-early gene expression to repeated restraint stress. *Neurosci*, 107(3), 465-479.
- Steckler T (2005). The neurophysiology of stress. In T. Stecker, NH Kalin, JMHM. Reul. Handbook of stress and the brain Part 1: the neurobiology of stress: Vol. 15. (pp.25-42). New York: NY: Elsevier.
- Suenaga, T., Morinobu, S., Kawano, K., Sawada, T., and Yamawaki, S. (2004). Influence of immobilization stress on the levels of CaMKII and phospho-CaMKII in the rat hippocampus. *Int J Neuropsychopharmacol*, 7(3), 299-309.
- Sunanda, Rao, B. S., and Raju, T. R. (2000). Restraint stress-induced alterations in the levels of biogenic amines, amino acids, and AChE activity in the hippocampus. *Neurochem Res*, 25(12), 1547-1552.
- Suchecki, D., Duarte Palma, B., and Tufik, S. (2000). Pituitary-adrenal axis and behavioural responses of maternally deprived juvenile rats to the open field. *Behav Brain Res*, 111(1-2), 99-106.
- Sweatt, J. D. (2001). Protooncogenes subserve memory formation in the adult CNS. *Neuron*, 31(5), 671-674.
- Tamashiro, K. L., Nguyen, M. M., and Sakai, R. R. (2005). Social stress: from rodents to primates. *Front Neuroendocrinol*, 26(1), 27-40.
- Thach, W. (1999). Fundamentals of Motor Systems. In M. Zigmond, F. Bloom, S. Landis, J. Roberts & L. Squire (Eds.), *Fundamental Neuroscience* (pp. 855-888). Toronto: Academic Press.
- Thome, J., Pesold, B., Baader, M., Hu, M., Gewirtz, J. C., Duman, R. S., and Henn, F.A. (2001). Stress differentially regulates synaptophysin and synaptotagmin expression in hippocampus. *Biol Psychiatry*, 50(10), 809-812.
- Tsuchiya, T., and Horii, I. (1996). Epidermal cell proliferative activity assessed by proliferating cell nuclear antigen (PCNA) decreases following immobilization-induced stress in male Syrian hamsters. *Psychoneuroendocrinology*, 21(1), 111-117.

- Underdown, A., Barlow, J., Chung, V., and Stewart-Brown, S. (2006). Massage intervention for promoting mental and physical health in infants aged under six months. *Cochrane Database Syst Rev*(4), CD005038.
- Volchegorskii, I. A., Tseilikman, V. E., Ship, S. A., Bubnov, N. V., and Sinitskii, A. I. (2003). The effects of anxiogenic stress on glucocorticoid sensitivity, glucose tolerance, and alloxan resistance in rats. *Neurosci Behav Physiol*, 33(6), 595-599.
- Vyas, A., and Chattarji, S. (2004). Modulation of different states of anxiety-like behaviour by chronic stress. *Behav Neurosci*, 118(6), 1450-1454.
- Warren, S., and Juraska, J. (2000). Sex differences and estropausal phase effects on water maze performance in aged rats. *Neurobiology of Learning and Memory*, 74(3), 229-240.
- Weiller, C., and Rijntjes, M. (1999). Learning, plasticity, and recovery in the central nervous system. *Exp Brain Res*, 128(1-2), 134-138.
- Whishaw, I. Q., and Pellis, S. M. (1990). The structure of skilled forelimb reaching in the rat: a proximally driven movement with a single distal rotatory component. *Behavioural Brain Research*, 41(1), 49-59.
- Whishaw, I. Q., Dringenberg, H. C., and Pellis, S. M. (1992a). Spontaneous forelimb grasping in free feeding by rats: motor cortex aids limb and digit positioning. *Behav Brain Res*, 48(2), 113-125.
- Whishaw, I. Q., Pellis, S. M., and Gorny, B. P. (1992b). Medial frontal cortex lesions impair the aiming component of rat reaching. *Behav Brain Res*, 50(1-2), 93-104.
- Whishaw, I. Q. (1996). An endpoint, descriptive, and kinematic comparison of skilled reaching in mice (*Mus musculus*) with rats (*Rattus norvegicus*). *Behav Brain Res*, 78(2), 101-111.
- Whishaw, I. Q. (2000). Loss of the innate cortical engram for action patterns used in skilled reaching and the development of behavioural compensation following motor cortex lesions in the rat. *Neuropharmacology*, 39(5), 788-805.
- Whishaw, I. Q., and Metz, G. A. (2002). Absence of impairments or recovery mediated by the uncrossed pyramidal tract in the rat versus enduring deficits produced by the crossed pyramidal tract. *Behav Brain Res*, 134(1-2), 323-336.
- Windle, R. J., Shanks, N., Lightman, S. L., and Ingram, C. D. (1997). Central oxytocin administration reduces stress-induced corticosterone release and anxiety behaviour in rats. *Endocrinology*, 138(7), 2829-2834.

- Wood, G. E., and Shors, T. J. (1998). Stress facilitates classical conditioning in males, but impairs classical conditioning in females through activational effects of ovarian hormones. *Proc Natl Acad Sci U S A*, 95(7), 4066-4071.
- Wood, G. E., Beylin, A. V., and Shors, T. J. (2001). The contribution of adrenal and reproductive hormones to the opposing effects of stress on trace conditioning in males versus females. *Behav Neurosci*, 115(1), 175-187.
- Wood, G. E., Norris, E. H., Waters, E., Stoldt, J. T., & McEwen, B. S. (2008). Chronic immobilization stress alters aspects of emotionality and associative learning in the rat. *Behav Neurosci*, 122(2), 282-292.
- Xu, J., and Morris, G. F. (1999). p53-mediated regulation of proliferating cell nuclear antigen expression in cells exposed to ionizing radiation. *Mol Cell Biol*, 19(1), 12-20.
- Yang, C. H., Huang, C. C., and Hsu, K. S. (2004). Behavioural stress modifies hippocampal synaptic plasticity through corticosterone-induced sustained extracellular signal-regulated kinase/mitogen-activated protein kinase activation. *J Neurosci*, 24(49), 11029-11034.
- Young, E., Altemus, M., Parkison, V., and Shastry, S. (2001). Effects of estrogen antagonists and agonists on the ACTH response to restraint stress in female rats. *Neuropsychopharmacology*, 25(6), 881-891.
- Young, A. H., Gallagher, P., Watson, S., Del-Estal, D., Owen, B. M., and Ferrier, I. N. (2004). Improvements in neurocognitive function and mood following adjunctive treatment with mifepristone (RU-486) in bipolar disorder. *Neuropsychopharmacology*, 29(8), 1538-1545.
- Yuan, Z. M., Utsugisawa, T., Huang, Y., Ishiko, T., Nakada, S., Kharbanda, S., Weichselbaum, R., Kufe, D. (1997). Inhibition of phosphatidylinositol 3-kinase by c-Abl in the genotoxic stress response. *J Biol Chem*, 272(38), 23485-23488.
- Zebisch, A., Czernilofsky, A. P., Keri, G., Smigelskaite, J., Sill, H., and Troppmair, J. (2007). Signaling through RAS-RAF-MEK-ERK: from basics to bedside. *Curr Med Chem*, 14(5), 601-623.
- Zheng, G., Zhang, X., Chen, Y., Zhang, Y., Luo, W., & Chen, J. (2007). Evidence for a role of GABAA receptor in the acute restraint stress-induced enhancement of spatial memory. *Brain Res*, 1181, 61-73.
- Zhao, H., Xu, H., Xu, X., & Young, D. (2007). Predatory stress induces hippocampal cell death by apoptosis in rats. *Neurosci Lett*, 421(2), 115-120.
- Zundel, W., and Giaccia, A. (1998). Inhibition of the anti-apoptotic PI(3)K/Akt/Bad pathway by stress. *Genes Dev*, 12(13), 1941-1946.