

**BEHAVIORAL ASSESSMENT OF DEPRESSIVE-LIKE  
SYMPTOMS IN A RODENT MODEL OF SPINAL CORD INJURY**

An Honors Fellows Thesis

by

KELSEY LYNN LUEDTKE

Submitted to the Honors Programs Office  
Texas A&M University  
in partial fulfillment of the requirements for the designation as

**HONORS UNDERGRADUATE RESEARCH FELLOW**

April 2011

Major: Psychology

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Approved by:

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## ABSTRACT

Behavioral Assessment of Depressive-like Symptoms in a  
Rodent Model of Spinal Cord Injury. (April 2011)

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Spinal cord injury (SCI) currently affects over 250,000 people in the United States alone with approximately 11,000 new cases occurring each year. In addition to its debilitating physical consequences, spinal cord injury significantly impacts emotional and psychological well-being. This leads to an increased risk for depression in SCI patients. Previous studies have found the rate of major depressive disorder (MDD) among patients with SCI to be as high as 24%, compared with 8.95% in the general population. However, despite the prevalence of depression in human patients with SCI, there is no spinally injured animal model of depression. To address this issue, we used a battery of established behavioral tests (proposed to be indicative of depression-like behavior in rats) to assess depression following a moderate contusion injury. The proposed ethogram consisted of the sucrose preference test (SPT) and the forced swim test (FST), both common rodent paradigms for assessing depressive-like behavior. The battery also included open field activity, social exploration, and burrowing tasks. Subjects were

acclimated on the tasks prior to injury and baseline scores were obtained on the two days immediately preceding injury. Testing was then conducted on days 1 and 2, 9 and 10, and 19-21. BBB scores of recovery of motor function, weight gain, and appetite were assessed for 21 days following injury. Results from a hierarchical cluster analysis indicated that the ethogram could be divided into four main behavioral categories: loss of interest/pleasure and motivation, psychomotor retardation, locomotor recovery, and general health. Depressed subjects displayed a greater loss of interest/pleasure and motivation in the sucrose preference test, social exploration test, and the forced swim test, when compared to non-depressed subjects. Depressed subjects also displayed greater psychomotor retardation in the burrowing task and open field test. Depression did not have an effect on recovery of locomotor function or weight gain. Future studies should examine other symptomatic behaviors including cognitive impairments and sleep disturbances. Future directions will be to examine the use of anti-depressants to reverse behaviors observed in depressed subjects. Additional studies should also consider the effects of anti-depressants on locomotor recovery and weight gain.

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## NOMENCLATURE

|                  |   |
|------------------|---|
| BBB              | Basso, Beattie, and Bresnahan   |
| CMS              | Chronic Mild Stress   |
| <i>DSM-IV-TR</i> | The American Psychiatric Association Diagnostic and<br>Statistical Manual Fourth Edition, Revised |
| FST              | Forced Swim Test  |
| NOR              | Novel Object Recognition  |
| OFA              | Open Field Activity   |
| SCI              | Spinal Cord Injury  |
| SP               | Sucrose Preference  |
| SSRI             | Selective Serotonin Reuptake Inhibitor  |
| WKY              | Wistar-Kyoto  |

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# CHAPTER I

## INTRODUCTION

### **Spinal cord injury**

Spinal cord injury (SCI) currently affects over 250,000 people in the United States alone with approximately 11,000 new cases occurring year (NSCISC, 2010). Over 80% of the injuries occur in males and the average age at the time of injury is 40.2 years (NSCISC, 2010). The debilitating physical consequences of spinal cord injury include loss of locomotor, sensory, bowel, bladder and sexual function, as well as the development of spasticity and neuropathic pain (Anderson, 2004). These problems result from damage to ascending and descending fiber tracks in the spinal cord, which are responsible for relaying messages between the brain and the periphery.

The physical symptoms expressed after SCI depend on both the severity and the vertebral level of the spinal cord injury. With a complete transection of the spinal cord, no messages can be relayed and motor and sensory functioning is completely lost. An incomplete contusion injury allows the patient to retain some motor and sensory function, depending on the spinal segment that is damaged. A high level injury in the cervical cord results in decreased motor and sensory functioning in all limbs. Lower lesions in the thoracic, lumbar, or sacral regions result in decreased functioning of the

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This thesis follows the style of the *Journal of Neurotrauma*.

lower limbs only. Thirty eight percent of SCI patients suffer from incomplete tetraplegia, while twenty two percent suffer from incomplete paraplegia (NINDS, 2010).

In addition to loss of physical function, the cost of living with a spinal cord injury places an enormous financial strain on the patient and caregivers. This cost is estimated at more than \$250,000 a year (NINDS, 2007). As many spinal cord injuries occur when a person is in his/her twenties, the lifetime cost of SCI is substantial. The estimated lifetime costs range from \$500,000 to more than \$3,000,000 (NINDS, 2010). The government spends an estimated ten billion each year to provide medical and rehabilitative care to SCI patients (NINDS, 2007). However, SCI patients who are insured by Medicaid receive the fewest benefits compared to those insured by Workman's Compensation and third-party insurers. They receive little or no compensation for lost wages and report the greatest physical and social handicaps (Tate et al., 1994a). Patients who receive fewer benefits were less likely to return to school/work after injury (Tate et al., 1994b). In addition, over 75% of SCI patients, regardless of insurer type, are forced to pay for home accessibility features without the help of insurance (Rice and LaPlante, 1992). SCI patients who cannot afford home accessibility features and assistive technology are less likely to achieve functional independence and more likely to experience a decreased quality of life.

The stress associated with financial demands and the traumatic loss of mobility is reflected in significant decreases in the quality of life perceived by patients with SCI.

Not surprisingly, SCI patients report significantly lower 'quality of life' compared with traumatic brain injury patients and the non-disabled community (Kreuter et al., 1998). With these significant decreases in the quality of life experienced by spinal cord injury patients, it is imperative that SCI research continues to move forward toward finding a cure and improving the quality of life for those who are currently suffering.

### **Spinal cord injury and depression**

The effects of SCI on psychological and emotional well-being are underscored by the increased risk for depression. Previous studies have found that the rate of major depressive disorder (MDD) among patients with SCI ranges from 11% to 24% (Kishi et al., 1994, Judd et al., 1989, Frank et al., 1992, Krause et al., 2000), compared with approximately 8.95% in the general population (Kessler et al. 2005). Other studies reported significant clinical symptoms of depression in SCI patients, who do not meet criteria for MDD, and found that these rates range from 16% to 37% (Dryden et al., 2005, Judd et al., 1989, Scivoletto et al., 1997, Migliorini et al., 2009, Krause et al., 2000, Elliot and Frank, 1996).

According to the American Psychiatric Association (*DSM-IV-TR*), major depressive disorder is diagnosed when at least five of the following symptoms are met for at least two-week period (2000). At least one of the symptoms must be a depressed mood or a loss of interest or pleasure.

Symptoms of major depressive disorder (*DSM-IV-TR*, 2000):

1. Depressed mood most of the day, nearly every day
2. Markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day
3. Significant weight loss when not dieting or weight gain (e.g., a change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day
4. Insomnia or hypersomnia nearly every day
5. Psychomotor agitation or retardation nearly every day
6. Fatigue or loss of energy nearly every day
7. Feelings of worthlessness or excessive or inappropriate guilt
8. Diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others)
9. Recurrent thoughts of death, recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide

The SCI patients who are either diagnosed with MDD or experience symptoms of depression are not currently receiving the treatment they need. Previous research has shown that within the clinically depressed SCI population, only 29% of patients receive any form of antidepressants. Only 13% of depressed SCI patients receive the current guideline levels of drug dose and duration (Fann et al., 2011). Psychotherapy is the other common form of treatment for depression following SCI. However, only 11% of patients report receiving psychotherapy in the previous 3 months (Fann et al., 2011).

Despite the prevalence of depression in the SCI population, research in this area has been largely ignored. Research is needed to determine how current recommended antidepressant drugs affect recovery of function following SCI. Further research is also needed to develop more effective therapies for SCI patients who suffer from depression, and increase understanding of the psychological and molecular variables influencing the development of depression.

### **Animal models of depression**

Animal models are important for investigating new targets for anti-depressants and for understanding the molecular and cellular changes that lead to depression. In 1969, McKinney and Bunney proposed the following requirements that any animal model of depression must meet:

1. The symptoms of the depression so induced should be reasonably analogous to those seen in human depression.
2. There should be observable behavioral changes, which can be objectively evaluated.
3. Independent observers should agree on objective criteria for drawing conclusions about the subjective state.
4. The treatment modalities effective in reversing depression in humans should reverse the changes seen in animals.
5. The system should be reproducible by other investigators.



Methods for behaviorally assessing comorbid depression have been proposed for rodent models of many different disease or injury states, including epilepsy (Jones et al., 2008), traumatic brain injury (Fromm et al., 2004), Parkinson's (Itier et al., 2003), cancer (Lamkin et al., 2010; Pyter et al., 2009), cholestasis (Swain and Le, 1998), diabetes (Hilakivi-Clarke et al., 1990) and post-stroke (Wang et al., 2009). Assessment of depression in these animal models of varied disease and injury states depends on behavioral paradigms that focus on 'helplessness,' loss of interest in pleasurable activities, and decreases in activity or changes in appetite and sleep patterns. The behaviors measured in these paradigms are analogous to some, though not all, of the core symptoms of major depressive disorder (See Table 1 for a list of symptoms of depression and analogous behavioral measures in rats).

Table 1. Symptoms of Major Depressive Disorder and Corresponding Measure for Symptom-like Behavior in Rats

| Depression Symptom in the <i>DSM-IV-TR</i>  | Measure of Symptom-Like Behavior in Rats                | References   |
|---|---|--|
| Depressed mood most of the day, nearly every day  | Not applicable  |  |
| Markedly diminished interest or pleasure  | Sucrose Preference Test                                 | Jones et al., 2008; Lamkin et al., 2010; Wang et al., 2008; Pyter et al., 2009 |
|   | Social Exploration                                      | Overstreet et al., 2010<br>Swain and Le, 1998                                  |
| Significant weight loss when not dieting or weight gain or decrease or increase in appetite | Monitor subject weight and food consumption             | Itier et al., 2003<br>Lamkin et al., 2010                                      |
| Insomnia or hypersomnia   | Electroencephalography (EEG) and electromyography (EMG) | Dugovic et al., 2000   |

Table 1 (continued)

| Depression Symptom in the<br><i>DSM-IV-TR</i>   | Measure of Symptom-Like<br>Behavior in Rats | References   |
|---|---|--|
| Psychomotor agitation or retardation  | Open Field Test                             | Fromm et al., 2004; Jones et al., 2007; Wang et al., 2009; Itier et al., 2003; Lamkin et al., 2011 |
| Fatigue or loss of energy   | Burrowing                                   | Deacon, 2006   |
| Feelings of worthlessness or excessive or inappropriate guilt   | Not applicable                              |  |
| Diminished ability to think or concentrate, or indecisiveness   | Novel Object Recognition                    | Brennan et al., 1990<br>McGregor et al., 2003  |
| Recurrent thoughts of death, recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan | Forced Swim Test                            | Pyter et al., 2009<br>Hilakivi et al., 1990  |

### *Sucrose preference test*

The measures outlined in Table 1 allow researchers to observe behavioral changes and objectively evaluate them, thus meeting the second requirement proposed by McKinney and Bunney (1969). The first of these measures is the sucrose preference test.

Anhedonia, or a loss of interest in pleasurable activities, is a core symptom of major depressive disorder. Rats exhibiting anhedonia display a decrease in preference for sucrose solution over water when compared to baseline measures taken before the induction of injury or disease state. This model of depression has been used to study depressive-like behavior in various disease states including cancer (Pyter et al., 2009; Lamkin et al., 2011) and epilepsy (Jones et al., 2008). In a model of post-stroke depression, decreased sucrose preference was reversed by administration of the selective serotonin reuptake inhibitor (SSRI) anti-depressant drug citalopram (Wang et al., 2009).

### *Social interaction*

A second method for assessing markedly diminished interest or pleasure in rats is the social interaction task. According to a study of a rat model of cholestasis, rats are highly sociable creatures. However, bile duct resection (BDR) cholestasis induced rats display significantly less social interaction with a juvenile rat when placed together in an open field apparatus (Swain and Le, 1998). Acute administration of the tricyclic antidepressant desipramine has been shown to increase social interaction in the Flinders Sensitive Line (FSL) rat, a genetic animal model of depression (Overstreet and Greibel, 2004). Chronic administration of the SSRI fluoxetine has also been shown to increase social interaction behavior in rats (Bristow, et al., 2000).

### *Significant weight gain/weight loss or changes in appetite*

Despite being a simple and easy to obtain measure of a depressive-like symptom, few studies have reported on weight gain or weight loss in depressed animal subjects. In a study of an animal model of tumor induced depression, tumor bearing subjects that consumed less sucrose solution than controls also had gained significantly less weight than controls subjects at the end of the experiment (Lamkin et al., 2011). In the Parkinsonian model of depression, Parkin mutant mice have significantly less body weight than wild type mice (Itier et al., 2003). The use of antidepressants to reverse weight loss in models of depression has not been studied.

### *Insomnia or hypersomnia*

The Wistar-Kyoto rat displays many behaviors that are analogous to symptoms in depression in humans. WKY rats display increased immobility in the forced swim test

and decreased activity in the open-field test (Will et al., 2003) leading researchers to accept the strain as a genetic model of depression. Dugovic et al., (2000) examined the sleep patterns of WKY rats. The study found that WKY rats exhibit an increase in total REM sleep time during the light cycle and increased sleep fragmentation during the light and dark cycles. The use of antidepressants to reverse sleep disturbances in models of depression has not been studied.

#### *Open field test*

The open field test is a simple measure for depressive-like symptoms of psychomotor agitation or retardation. When placed in a large open field environment, rats who enter fewer squares display a lack of exploratory behavior. Studies have shown that the number of squares entered during the task is an accurate measure of depression in rats (Fromm et. al, 2004). Specifically, Fromm (2004) measured depression-like behavior using the open field test in a rat model of traumatic brain injury, another common form of neurological injury. The open field test has also been used to assess depression in rodent models of epilepsy (Jones et al., 2008), post-stroke (Wang et al., 2009), Parkinson's disease (Itier et al., 2003), and cancer (Lamkin et al., 2011).

#### *Burrowing*

The burrowing task is a behavioral assay recently developed by Deacon et al. (2006) that is primarily used to screen for prion disease in mice and IL-1 $\beta$ -expressing replication-deficient adenovirus in rats. In the burrowing task, a plastic tube containing an earth-like substrate is placed in the subject's home cage. Rats demonstrate vigorous burrowing activity, which suggests that it is a rewarding activity (Deacon, 2006). It has been

suggested that burrowing may be a useful measure for depressive-like symptoms of diminished pleasure or loss of energy (Deacon, 2006). However, the antidepressant fluoxetine has been shown to decrease burrowing behavior, though chronic administration of the drug has not been studied.

#### *Forced swim test*

The forced swim test (FST) is a widely used paradigm for modeling depression in rodents. Roger Porsolt developed the FST in 1977 as a model of depression to screen for new drug therapies. In this model, also referred to as the behavioral despair test, animals are placed in a cylinder filled with water from which they cannot escape. Duration of immobility is interpreted as a measure of behavioral despair. Hopelessness has been found to positively correlate with the seriousness of suicide ideation (Minkoff et al., 1973) and thoughts of suicide is one of the criteria for depression. Thus the forced swim test may model a similar sense of despair or hopelessness that is often present with suicide ideation. The forced swim test has been used to measure depressive-like behavior in studies of cancer (Pyter et al., 2009) and diabetes (Hilakivi-Clarke et al., 1990). Anti-depressant drug administration decreases immobility and increases escape behavior. Acute (3 days) and chronic (14 days) administration of reboxetine and moclobemide and chronic administration of fluoxetine have been shown to decrease immobility in the forced swim test (Cryan et al., 2005).

#### *Novel object recognition*

The novel object recognition task is used to assess cognitive behaviors analogous to the depression symptom of a diminished ability to think or concentrate. In the novel object

recognition task, subjects are expected to spend more time investigating a novel object than investigating an object they have been previously exposed to. Equal or greater time investigating the familiar object indicates a lack of memory for the familiar substance (Bevins and Besheer, 2006). The novel object recognition task has been used to study the effects of MDMA (ecstasy) on depressive-mood and behavior (McGregor et al., 2003). The use of antidepressants to reverse effects in the novel object recognition task has not been studied.

#### *Animal model controversies*

While many tests of depression have been proposed, there are criticisms of modeling human affective disorders in rodents. Treit and Menard (1998) cite problems with the *DSM* classification of depression as a source of complication in modeling the disorder in animals. For example, some behavioral symptoms of anxiety disorder are similar to, or the same as, symptoms of depression (i.e., insomnia and agitation). This makes the process of distinctly modeling these disorders in rodents difficult. Other issues arise because several symptoms require verbal information and cannot be measured in rodents, including the second core symptom – depressed mood most of the day, everyday. In addition, humans are capable of displaying more complex emotional behavior such as lowered self-esteem or excessive or inappropriate guilt, which cannot be modeled in rodents (Cryan and Mombereau, 2004).

Despite these issues, it should be recognized that no model will be behaviorally and biologically identical to the human disorder. Moreover, animal models do achieve high

levels of reliability by fulfilling the criteria outlined by McKinny and Bunny (1969). An animal model of depression following spinal cord injury, that fulfills these criteria, will be important in understanding the biological mechanisms that underlie the relationship between physical and affective disorders, and invaluable in the development of safe and efficacious treatments aimed at improving 'quality of life' in patients after SCI.

### **Clinical implications of an animal model of depression following SCI**

Understanding the psychophysiological factors that underlie depression following SCI is essential in order to improve the treatment options available to patients. Antidepressant prescription drug therapy is commonly prescribed for patients with SCI (Paralyzed Veterans of America, 1998). However, no systematic research has been done to determine the side effects of antidepressants after SCI (Elliot and Kennedy, 2004). These side effects could include attenuation of functional recovery, or potentiation of the symptoms of depression. An animal model will allow researchers to better determine the side effects and effectiveness of existing, as well as new pharmacological therapies for treating depression following SCI. (Pyter et al., 2009). However, no such model for depression following spinal cord injury has yet been developed.

## CHAPTER II

### METHODS

#### Subjects

The subjects were male Sprague-Dawley rats (Harlan, Houston, TX), between 90-110 days old. They were housed in pairs in Plexiglas bins (45.7 (length) x 23.5 (width) x 20.3 (height) cm) prior to surgery, and housed singly following surgery with *ad libitum* food and water. Food consumption and subject weight were recorded daily. Until subjects regained full bladder control, they were manually expressed in the morning (8-9:30 a.m.), and in the evening (6-7:30 p.m.). They were maintained on a 12 hour light/dark cycle and all behavioral testing was conducted during the light cycle. All of the experiments were reviewed and approved by the institutional animal care committee at Texas A&M University and all NIH guidelines for the care and use of animal subjects were followed.

#### Surgery

Subjects received a moderate contusion injury using a MASCIS device as described by Hook et al. (2009). Briefly, subjects were anesthetized with isoflurane (5%, gas), and after a stable level of anesthesia was reached, the concentration of isoflurane was lowered to 2-3%. Next, the subject's back was shaved and disinfected with iodine and a 7.0 cm incision was made over the spinal cord. Two incisions were then made on either side of the vertebral column, extending approximately 3 cm rostral and caudal to the



T12-T13 segment. The dorsal spinous processes at T12-T13 were removed (laminectomy), and the spinal tissue exposed. The vertebral column was fixed within the MASCIS device and the 10-g impactor was dropped 12.5 mm to produce a moderate injury. The wound was closed using Michel clips. To help prevent infection, subjects were treated with 0.3mL gentocin administered subcutaneously immediately after surgery and again for five days following surgery. To compensate for fluid loss, subjects also received a 2.5 ml injection of saline after surgery. In addition, subjects received a 0.3 mL subcutaneous injection of gentocin and a 5 mL subcutaneous injection of saline daily for 5 days following injury. For the first 24 hours following surgery, subjects were placed in a recovery room maintained at 26.6°C.

### **Locomotor recovery**

The recovery of hindlimb stepping ability was scored using the Basso, Beattie and Bresnahan (BBB) scale (Basso et al., 1995). The subject were placed in an open-field enclosure (99 cm diameter, 23 cm deep) and allowed to move freely. Because rats often freeze when first introduced to a new environment, they were acclimated to the open field test area for 5 minutes on 3 consecutive days prior to surgery. After injury, the locomotor capacity (BBB) of subjects was observed for 5 minutes and scored by a trained observer on days 1-7, 9, 11, 13, 15, 18, and 21.

**Behavioral test battery**

Subjects were acclimated to the behavioral tests for depression beginning seven days prior to injury. Subjects were acclimated to the open field/social interaction environment and the sucrose preference test setup on the fifth and seventh day prior to injury.

Subjects were acclimated to the burrowing tubes on the fourth and sixth day prior to injury. Baseline measurements for each test were taken on the three consecutive days immediately preceding injury. After injury, sucrose preference and activity in a novel environment were tested on days 1, 9 and 19. Burrowing and social exploration tests were conducted on days 2, 10, and 20. The forced swim test was conducted on day 21. Sensory functioning was assessed on day 4 and again on day 22+. All testing took place during the light cycle. On days in which more than one test was conducted, subjects were returned to their home cages after the first test for a minimum of two hours before beginning the second test.

**Sucrose preference test**

Sucrose preference tests were conducted in the subjects home cage. The home cage water bottle was removed during testing and replaced at the end of the test period. For testing, one pre-weighed water bottle filled with approximately 200 mL of 1% sucrose solution and pre-weighed bottle filled with an equal amount of filtered water were placed on either side of the subject's cage, and left for 60 minutes. The placement of the sucrose and the water solutions on either the left or right sides was counterbalanced between subjects. The position of the bottle (left/right) was reversed halfway through 60 minute

period, to prevent any positional biases from confounding results. At the end of the 60 minute period, the change in the weight of each bottle was determined. The absolute sucrose intake per gram of rat body weight and sucrose preference (SP) was then calculated using the following formula:  $SP = \text{sucrose solution intake (mL)} / [\text{sucrose solution intake (g)} + \text{water intake (g)}]$  (Wang et al., 2009). Baseline sucrose preference levels were assessed three days prior to injury. Following injury, sucrose preference was assessed on days 1, 9, and 19. Subjects were assigned a set of bottles during the acclimation period and used the same set for each of the testing periods.

### **Open field activity**

The open-field environment was a black plywood box [100 (length) x 50 (height) x 100 (width) cm]. The floor was partitioned into 100 squares [10 (length) x 10 (width) cm] marked with silver marker. A layer of clear Plexiglas was used to cover the top of the box. Subjects were acclimated to the testing room for 10 minutes prior to testing. The testing room was dark and the open-field environment was illuminated from above by a 60 W white light. The subject was placed in the center of the box to begin a 5-minute test session. The test was video recorded from above. The number of squares that the subject moved into, operationalized as having at least the front two paws in the square, was later scored by two independent observers. Between each trial, the open-field environment was wiped down with the disinfectant Nolvasan to eliminate any olfactory cues. Baseline activity was measured 3 days prior to injury. Open field activity was

assessed 1, 9, and 19 days post injury. Decreased activity in the open field as compared to baseline was interpreted as an indication of depression-like behavior.

### **Burrowing**

The burrowing apparatus was a polyvinyl chloride (PVC) tube [45 (length) x 15 (diameter) cm] closed on one end. Each subject was assigned a separate apparatus and testing was conducted in the subjects home cage. To begin testing, pre-weighed burrowing tubes were filled with approximately 500g of pine wood chips and placed in the subject's cage. After 2 hours the woodchips burrowed out of the tube were weighed. The burrowing apparatus was not disinfected between trials of the same subject in order to prevent the burrow from appearing foreign to the subject. Woodchips that were burrowed out during the testing period were returned to the subjects tube for future trials. Baseline burrowing scores were measured 2 days prior to injury. After injury, burrowing was assessed on days 2, 10, and 20 days post injury. Decreased burrowing as compared to baseline was interpreted as an indication of depression-like behavior.

### **Social exploration**

Social exploration was assessed in an open-field environment identical to the open-field apparatus used to measure activity. A subject was placed in the center of the open-field and allowed to explore for 5 minutes. A rat (<250 g weight) not exposed to any experimental treatment was placed in the open field as far from the test subject as possible. The subject and the social exploration rat were videotaped for 5 minutes. Time

spent performing social (move toward, anogenital sniffing, close following) and nonsocial behavior (resting, moving away, self-grooming, exploration of the open field) was scored by two independent observers (Swain & Le, 1998).

### **Forced swim test**

Subjects were allowed to acclimate to the testing room for 10 minutes. The testing room was maintained at 27.2°C. The subject was then placed in a cylinder [15 (diameter) x 40 (height) cm] filled with water maintained at  $23 \pm 1^\circ\text{C}$  from which the subject could not escape. The forced swim test is traditionally conducted using a 10-minute acclimation period and a 5-minute test period 24 hours later. However, Abel & Bilitzke (1990) found that immobility measured in 10-minute acclimation period was highly correlated with immobility measured in a 5-minute test period conducted 24 hours later. Therefore, acclimation was not performed for this test and the testing period consisted of the first ten minute exposure to the inescapable water environment. The test was video recorded from above and time spent immobile was scored by two independent observers. Care was taken to ensure high inter-rater reliability. Immobility was characterized as a lack of any movement except those required to keep the head above water (Porsolt et al., 1977).

### **Ethogram for measuring depression in rodents following spinal cord injury**

The proposed ethogram for depressive-like symptoms studied in this experiment consisted of the behaviors summarized in Table 2.

Table 2. Summary of the behavioral tests, outcomes of the tests interpreted to be indicative of depression and the proposed analogous depression symptom

| Behavioral test         | Behavior outcome indicative of depression   | Analogous Depression Symptom   |
|-------------------------|---|--|
| Sucrose Preference Test | Decreased sucrose consumption               | Loss of interest or pleasure   |
| Social Exploration      | Decreased time spent exploring juvenile rat | Loss of interest of pleasure   |
| Open Field Test         | Decreased number of squares entered         | Psychomotor agitation or retardation                                   |
| Burrowing               | Decreased amount of woodchips burrowed out  | Fatigue or loss of energy  |
| Forced Swim Test        | Decreased time spent swimming               | Despair, hopelessness<br>(Positively correlated with suicide ideation) |

### Statistical analysis

A hierarchical cluster analysis was performed to determine how to group tests in the ethogram for depression. Variables were first standardized by  $z$  scores. The cluster analysis was performed using Ward's method. Ward's method uses an agglomerative algorithm. It begins with each test as a single separate entity. In the first iteration, the algorithm forms a cluster by comparing the proportion of variation explained by a particular clustering of two entities to the proportion of variation explained by every other possible clustering of two entities.

The proportion of variance explained by a cluster is calculated as  $r^2$ , where

$$r^2 = \frac{\text{Total Sum of Squares} - \text{Error Sum of Squares}}{\text{Total Sum of Squares}}$$

The Total Sum of Squares is a measure of the variance between the variable or entity mean and the population mean. Error Sum of Squares (ESS) is a measure of the variance between the variable or entity mean and the mean in the potential cluster. If the ESS is small, then the data from the variable or entity in question is similar to the cluster mean and the potential cluster would contain like entities. Ward's method computes the ESS and the  $r^2$  value for each potential cluster and the cluster that yields the smallest ESS (or the largest  $r^2$ ) value is formed. In other words, the particular cluster that explains the greatest proportion of variance will be formed as a new entity and the algorithm will begin the next iteration.

In each subsequent iteration, one new cluster is formed between two entities. The new cluster may consist of previously unclustered entities or previously clustered entities. The algorithm continues its iterations until all of the variables have been clustered into one single entity. The Ward's method algorithm produces a dendrogram, which visually displays the clustering and the similarity distances between clusters. This information can be used to determine the appropriate number of clusters for the data set, based on clusters that contain similar variables, but are distant from other clusters.

A second hierarchical cluster analysis was performed using Ward's method to determine how to group subjects based on their depressive-state. In this cluster analysis, clusters were formed between subjects rather than between behavioral tests. The algorithm

compared subjects' means on each of the behavioral tests to identify clusters of subjects with similar depressive-states.

A series of independent-samples *t* tests were carried out to identify which behavioral tests conducted at different points in the recovery phase showed significant differences between depressed and non-depressed subjects and should therefore be most appropriate in an ethogram for depression following spinal cord injury. A repeated-measures ANOVA was performed to compare the locomotor and sensory recovery of depressed and non-depressed subjects.



## CHAPTER III

### RESULTS

#### **Cluster analysis**

The hierarchical cluster analysis across tasks indicated that the tests in the ethogram should be divided into four clusters (Fig. 1). The first cluster was labeled “Weight gain” and consisted of weight and food consumption on both days 9 and 19. The second cluster, labeled “Locomotor recovery,” consisted of BBB scores on days 11 and 21. The third cluster was labeled “Loss of interest/pleasure or motivation” and consisted of tests for social exploration, sucrose preference, and the forced swim test. The fourth cluster consisted of burrowing and open field activity tests and was labeled “Psychomotor retardation/fatigue.”

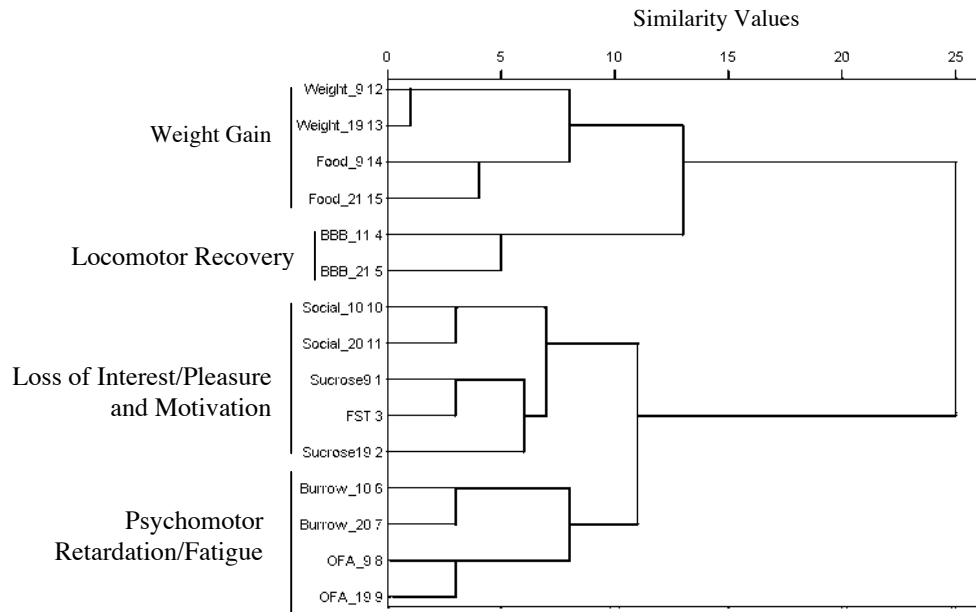


Fig. 1. Clusters of behavioral tests utilized in the ethogram for depression. Tests grouped in a cluster are underlined. The four groups produced by the cluster analysis were “Weight gain,” “Locomotor recovery,” “Loss of interest/pleasure and motivation,” and “Psychomotor retardation/fatigue.”

Based on the results from the four test clusters, a second cluster analysis by subjects produced the dendrogram depicted in Fig. 2. This graph indicates that subjects should be divided into two clusters. Independent-samples *t* tests were used to characterize these clusters. Based on the results of the *t* tests (summarized in Table 3) clusters 1 and 2 were labeled “Depressed” ( $n = 9$ ) and “Not Depressed” ( $n = 7$ ) respectively.

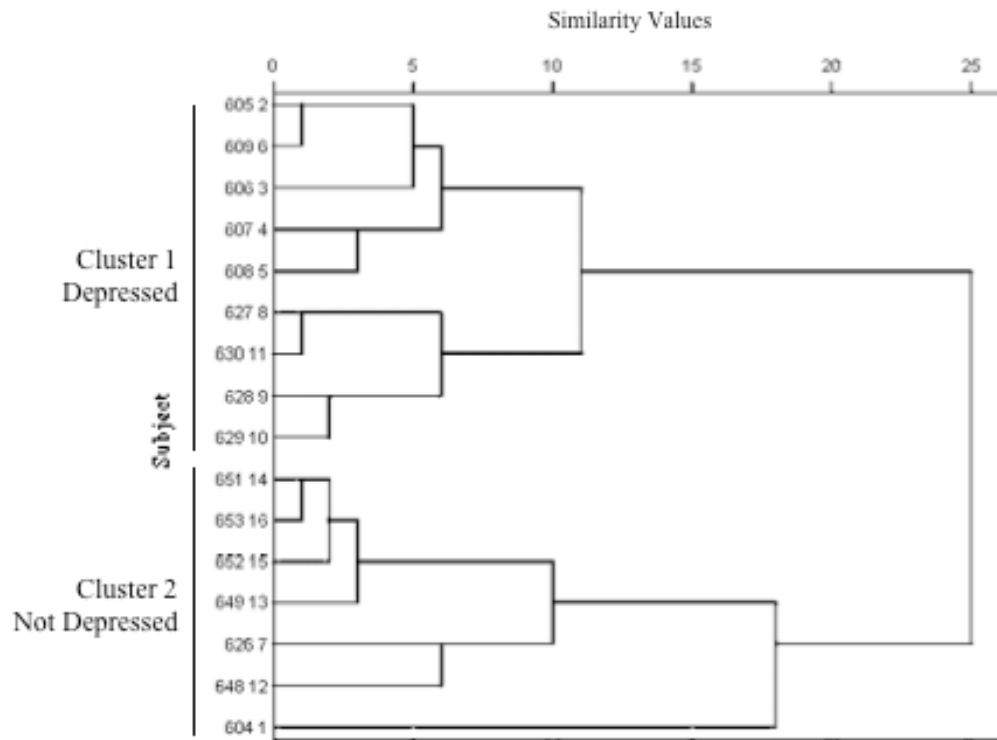


Fig. 2. Dendrogram of cluster analysis by cases. Subjects grouped in a cluster are underlined.

### Classification of subject clusters

First, focusing on the tasks indicative of a loss of interest or pleasure, an independent-samples  $t$  test showed that “non-depressed” subjects displayed a significantly higher preference for sucrose on day 9 compared with “depressed” subjects ( $t(15) = 3.96, p < .05$ , Figure 3). Non-depressed subjects also displayed a higher sucrose preference than depressed subjects on day 19, but this comparison was not significant ( $t(15) = 3.96, p = .187$ , Figure 3).

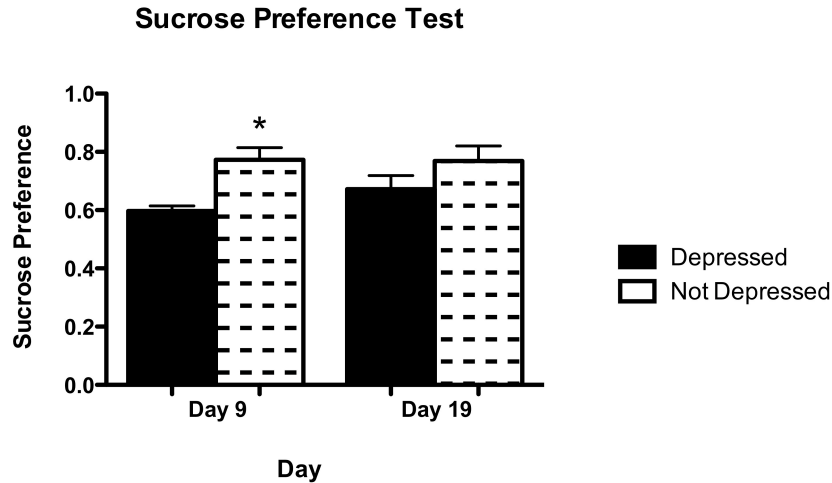


Fig. 3. Comparison of the average sucrose preference (Mean  $\pm$  SEM) displayed by the depressed and non-depressed clusters. Depressed subjects displayed a significantly lower average preference for sucrose solution than non-depressed subjects on day 9.  $*p < .05$

Non-depressed subjects also spent significantly more time swimming in the forced swim test than depressed subjects ( $t(15) = 2.24, p < .05$ , Figure 4). Similarly, the non-depressed subjects spent significantly more time interacting with a juvenile rat on day 10 than depressed subjects ( $t(15) = 2.77, p < .05$ ). While there was a tendency for a similar effect on day 20, with non-depressed subjects engaging in more social interaction, this was not significant ( $t(15) = 1.59, p = .134$ . See Figure 5).

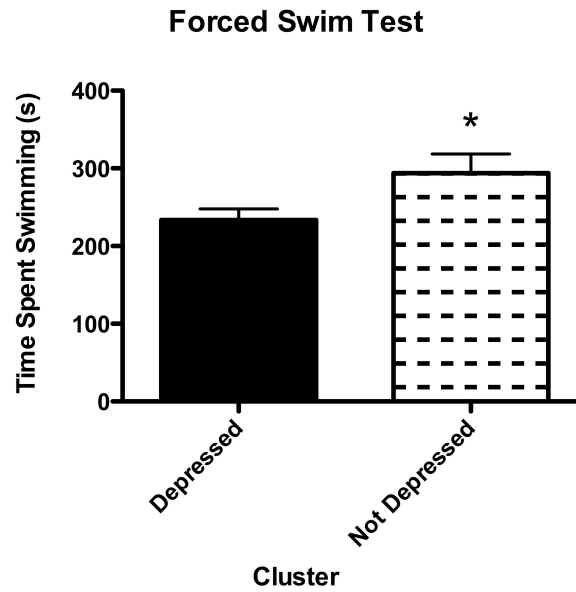


Fig. 4. Comparison of the average time spent swimming (Mean  $\pm$  SEM) displayed by the depressed and non-depressed clusters in the forced swim test. Depressed subjects spent significantly less time swimming than non-depressed subjects. \* $p < .05$

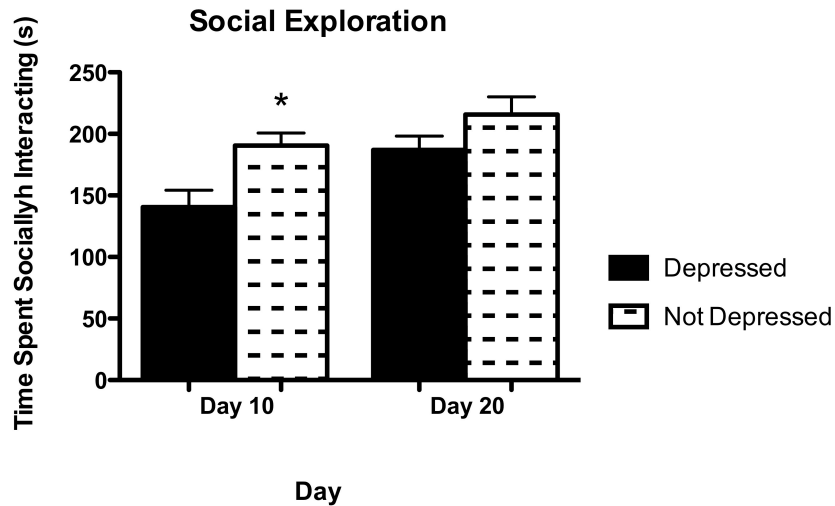


Fig. 5. Comparison of the average time spent socially interacting with a juvenile rat (Mean  $\pm$  SEM) displayed by the depressed and non-depressed clusters. Depressed subjects spent time socially interacting with a juvenile rat than non-depressed subjects on day 9. \* $p < .05$

For the measures of psychomotor agitation/retardation, non-depressed subjects burrowed significantly more woodchips out of the burrowing tubes than depressed subjects on both days 10 ( $t(15) = 3.02, p < .05$ ) and 20 ( $t(15) = 3.85, p < .05$ , see Figure 6). Although not significant on day 9 ( $t(15) = 1.30, p = .21$ ), non-depressed subjects also entered significantly more squares on day 19 than depressed subjects ( $t(15) = 2.34, p < .05$ . See Figure 7) in the open field activity task.

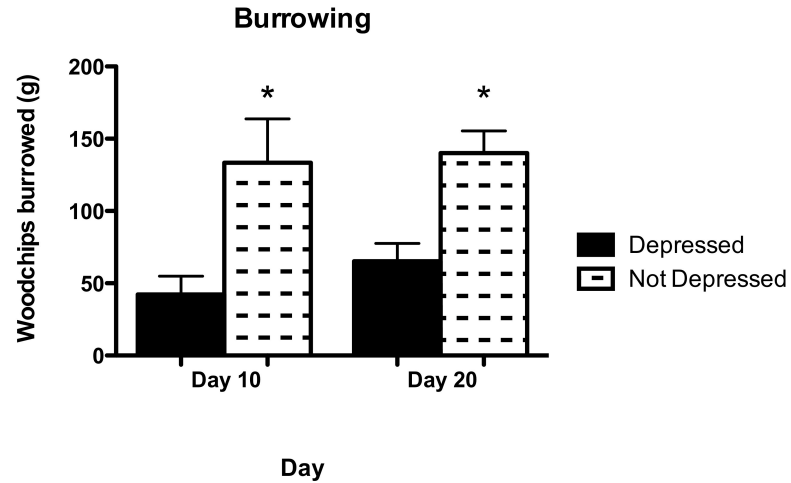


Fig. 6. Comparison of the average amount of woodchips burrowed (Mean  $\pm$  SEM) by the depressed and non-depressed clusters. Depressed subjects burrowed significantly less woodchips than non-depressed subjects on day 10 and day 20.  $*p < .05$

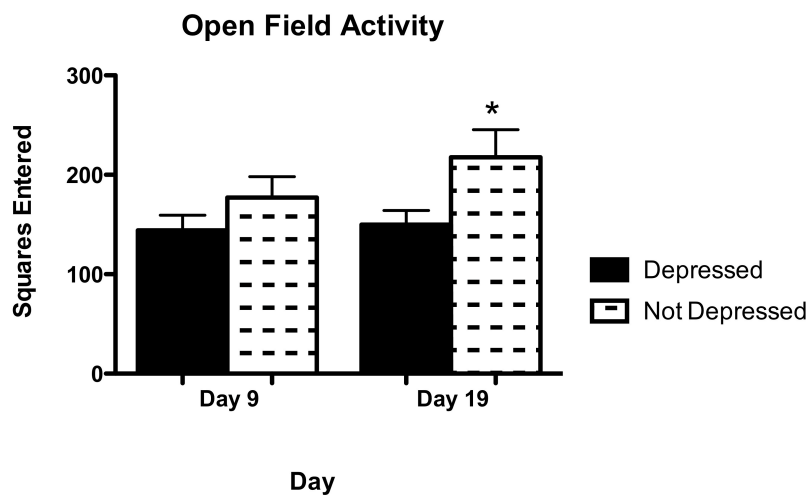


Fig. 7. Comparison of the average number of squares entered (Mean  $\pm$  SEM) by the depressed and non-depressed clusters in the open field activity test. Depressed subjects entered significantly fewer squares than non-depressed subjects on day 19.  $*p < .05$

Table 3. Summary of results from independent-samples *t* test comparing subject means in cluster 1 and cluster 2.

|  | Behavioral test         | Cluster 1 "Not Depressed"<br>Mean | Cluster 2 "Depressed"<br>Mean | Significant |
|--|-------------------------|-----------------------------------|-------------------------------|-------------|
| Loss of interest/pleasure<br>or motivation | Sucrose Preference Test | Day 9: 77.33%                     | Day 9: 59.72%                 | Yes         |
|  |                         | Day 19: 76.87%                    | Day 19: 67.24%                | No          |
|  | Social Exploration      | Day 10: 190.57s                   | Day 10: 140.44s               | Yes         |
|  |                         | Day 20: 215.71s                   | Day 20: 186.89s               | No          |
| Forced Swim Test                           | 293.86s                 | 233.56s                           | Yes                           |             |
| Psychomotor<br>Agitation/Retardation       | Open Field Test         | Day 9: 177.14 squares             | Day 9: 144.00 squares         | No          |
|  |                         | Day 19: 217.71 squares            | Day 19: 149.89 squares        | Yes         |
|  | Burrowing               | Day 10: 133.43g                   | Day 10: 42.22g                | Yes         |
|  |                         | Day 20: 140.00g                   | Day 20: 65.00g                | Yes         |

### Effects of depression on functional recovery after SCI

Results from a repeated measures ANOVA indicated that depressed subjects did not have significantly different recovery of locomotor function than non-depressed subjects ( $F(1, 14) < 0.1$ ,  $p > .05$ ). Depressed subjects gained less weight but this weight gain was not significantly different from non-depressed subjects weight gain ( $F(1, 14) = 0.52$ ,  $p > .05$ ).



## CHAPTER IV

### CONCLUSIONS AND SUMMARY

Of the seven symptoms of depression that are assessable in rodents, this study examined five: loss of interest of pleasure, psychomotor retardation, fatigue or loss of energy, changes in weight gain, and behavioral despair/hopelessness. Assessment of 3 of these 5 symptoms, with the tasks used in the present study, was confirmed in an initial cluster analysis: loss of interest of pleasure, psychomotor agitation or retardation, and changes in weight gain. Based on these behaviors, a second cluster analysis categorized 9 of the 16 subjects as depressed. Subjects that clustered in the “depressed” group displayed a loss of interest or pleasure; they had significantly lower preferences for sucrose, decreased social exploration and increased immobility in the forced swim test compared to “non-depressed” rats. Depressed rats also displayed psychomotor retardation, demonstrating significantly reduced open field and burrowing activity when compared to their non-depressed counterparts.

The rate of depressive-symptoms, which was found to be 56%, is closer to the rate of presentation of clinical symptoms of depression in SCI patients (37%; Migliorini et al., 2009) who do not meet the criteria for Major Depressive Disorder than to the rate of MDD diagnosis in SCI patients (11-24%, Kishi et al., 1994, Judd et al., 1989, Frank et al., 1992, Krause et al., 2000). This could be due to the limitation of diagnostic criteria applicable to animal models and, in the present study, the categorization of depression

based only two of the critical symptoms. The high rate of "depressed" subjects can be explained by the requirement that the subjects present only on 2 out of 7, rather than 5 out of 9 symptoms. According to the American Psychiatric Association (*DSM-IV-TR*), five of the nine, or more than half of, the symptoms of depression must be met in order for a person to receive a diagnosis of Major Depressive Disorder (2000). While the lowered criteria for categorizing depression may be seen as a limitation of the current ethogram, this less stringent criterion may also allow researchers to assess the severity of depression following SCI, and identify treatments for patients who may not meet the criteria for MDD but still present a number of clinical symptoms.

While the incidence of depression in the SCI rats does not concur with that of the clinical population, there does appear to be agreement between the time of expression of symptoms of depression. The significant loss of pleasure or interest present on days 9 and 10, assayed with the sucrose preference and social exploration tests respectively, is consistent with research that has shown that depression, as measured by the Beck Depression Inventory Scale is most severe in SCI patients in the acute phase (Kennedy, 2000). Days 9 and 10 post-injury represents the acute phase of injury in rodents. Significantly lower burrowing performance by depressed subjects on day 10 is also supported by this research. Depressed subjects burrowed more on day 20 than on day 10, though consistently less than non-depressed subjects, perhaps indicating that depression was more severe in the acute phase. Significant differences between the depressed and non-depressed subjects in the open field test on day 19 is likely due to the elimination of

interferences by locomotor impairment present in the acute phase, when subjects had not regained as much hindlimb stepping ability and coordination. To summarize, depressive-like symptoms should be measured using the sucrose preference test, the social exploration test, and the burrowing test in the acute phase on days 9 and 10, and using the open field test, burrowing test, and forced swim test at the beginning of the chronic phase on days 19-21

Despite the effects of depression in the acute phase of injury when spontaneous recovery of function is most robust, depression did not appear to have a significant effect on recovery of locomotor function or on weight gain following injury. Interestingly, clinical research has also shown that severity of injury or the magnitude of functional impairment does not predict the likelihood that a patient will develop depression following spinal cord injury (Bombardier, et al., 2004). This could explain why subjects with lower scores on the BBB scale of locomotor recovery did not display more depressive-like symptoms than subjects with higher BBB scores. Depressed subjects did gain less weight back after injury compared to non-depressed subjects, however the results were not significant. Research has shown that somatic symptoms such as poor appetite are positively correlated with overall depression severity in clinical populations with MDD (Denninger et al., 2006). The results from the present study show a similar positive direction of decreased weight and the presentation of depressive-like symptoms.

With respect to validation of the behavioral ethogram, the present study met three of the five criteria for an animal model proposed by McKinny and Bunny (1969). As previously discussed, this behavioral model of depression in rodents following spinal cord injury studied five of the seven symptoms of depression that are capable of being measure in rodents. Based on previous studies and the cluster analysis in the present study, the behavioral tests employed measure behaviors that are reasonably analogous to those seen in human depression. Based on the significant differences between the behavior of depressed and non-depressed subjects in the sucrose preference, social exploration, open field, burrowing, and forced swim tests, there were observable behavioral changes following spinal cord injury. These changes could be objectively evaluated using the five tests. Each of these tests, excluding burrowing, have been previously used to measure depression in rodents, thus meeting the third criteria that independent observers should agree on objective criteria for drawing conclusions about the subjective depressive state.

The fourth and fifth criteria proposed by McKinny and Bunny (1969) is that (4) the treatment modalities effective in reversing depression in humans should reverse the changes seen in animals and (5) the system should be reproducible by other investigators. Future studies are planned to examine the effects of antidepressant drug administration on the behaviors exhibited in the current ethogram for depression. These studies will address the fourth criteria. To address the fifth criteria, the study needs to be replicated by other investigators in different laboratories. Other limitations of the present

study include a small sample size ( $n = 16$ ). We have also planned to increase the sample size assessed in the current study, further validating this behavioral ethogram. Another limitation is that the present study did not assess the remaining two symptoms of depression capable of being measured in rodents: a decreased ability to think or concentrate and sleep disturbances. Future studies should examine these symptoms. As previously discussed, an EEG or EMG could be used to measure sleep disturbances and a novel object recognition task could be used to assess subjects' cognitive abilities.

Despite these limitations, the present study holds important implications for future research on depression following spinal cord injury. This is the first attempt at the development of a rodent model of depression after SCI. The tests and day of testing that have been identified as appropriate for measuring depression in a rodent model of spinal cord injury will be valuable in future studies. Further, the results of the present study condense the number of tests into clusters that can differentiate between depressed and non-depressed SCI rats. Future studies could select one or two tests from each cluster, loss of interest/motivation and psychomotor retardation, and test subjects on days that have been shown to be significant. This significantly reduces the time needed to conduct behavioral testing for depression and more subjects can be run in a shorter time. This is clinically relevant because thorough research on the effectiveness of different antidepressants on particular symptoms of depression following spinal cord injury are necessary. The animal model developed in the present study will enable future studies to perform these investigations.

Future studies should also consider the effects of anti-depressant drug administration on recovery of locomotor, sensory, and bladder function. The potential for anti-depressant drugs to undermine recovery of function following spinal cord would be an important consideration for SCI patients who present symptoms of depression and for clinicians who treat these patients. Future studies that examine the molecular changes in the spinal cord after injury that may predict the development of depression in patients are also necessary. These studies may lead to early detection and possible new drug treatments for depression following spinal cord injury.

### **Summary**

The present study developed an ethogram for measuring depression in a rodent model of spinal cord injury. Behaviors measured in this ethogram for depression are analogous to the symptom criteria for Major Depressive Disorder. Depression should be measured in the acute phase of injury using the sucrose preference test, burrowing task, and social exploration test. Depression should also be measured at the start of the chronic phase using the open field test, burrowing task, and the forced swim test. These tests are able to distinguish between depressed and non-depressed subjects. Consistent with previous research, depressed subjects demonstrated stronger indications of depression in several of these behavioral tests during the acute phase compared to the chronic phase. While severity of depression varies with the phase of injury, depressive-state does not have an effect on locomotor recovery or on weight gain following spinal cord injury. Additional work is needed to examine the ability of anti-depressant medications to reverse the

behavioral effects observed in the present study. Future work should also consider the effects of anti-depressant drug administration on recovery of function. Future studies should also investigate sleep disturbances and cognitive impairments as additional measures of depression in a rodent model of spinal cord injury.

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