S-KETAMINE AS AN ANTI DEPRESSANT IN A RODENT SPINAL CORD CONTUSION MODEL

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

Testing the Effect of Ketamine on Recovery and as an Antidepressant in an Spinal Cord Contusion Model

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In addition to the physical effects, spinal cord injury (SCI) also impacts quality of life and psychological wellbeing. Compared to the rate of 8.6% in the general population, the incidence of major depressive disorder (MDD) ranges from 11 to 24% in SCI patients (Krause et al., 2000). Currently, depression after SCI is treated with selective serotonin reuptake inhibitors (SSRIs), but these drugs have side effects that include attenuation of functional recovery after SCI (Coyle et al., 2015).

S-Ketamine is a novel drug that has shown potential as an antidepressant, but has not been assessed following SCI. To address this, we used a battery of established behavioral tests (sucrose preference, forced swim, open-field activity, social exploration, and burrowing tasks) to assess depression in a rodent SCI model. Subjects were acclimated to the behavioral tasks and baseline scores were collected two days before a moderate contusion injury. Twenty-four hours

later, subjects were given an injection of 0, 5, 10, or 20 mg/kg S-ketamine (i.p.). Depression was re-assessed on days 2, 9-10, and 19-21 post-injury.

To characterize subjects as depressed or not-depressed, initial analyses focused on data collected on days 9-11 and 19-21 post-injury, after any potential antidepressant effects of S-ketamine should have dissipated. Using cluster analyses, we found that 13 out of 32 subjects (41%) displayed depression-like behaviors (decreased sucrose preference and open-field activity). Next, we examined the effects of S-ketamine. Analyzing data collected on day 2 post-injury, and within the window for S-ketamine's efficacy, we found that 20 mg/kg ketamine increased social behavior, relative to all other dose groups. Importantly, depressed subjects treated with 20 mg/kg S-ketamine showed the highest level of social interaction 24 hours after administration. These data suggest that S-ketamine may be an effective antidepressant after SCI. Further studies are warranted to elucidate the molecular mechanisms mediating these effects and to optimize administration schedules.

DEDICATION

We would like to dedicate this thesis to our parents for their love and support.

ACKNOWLEDGMENTS

We would like to thank our faculty advisor Dr. Michelle Hook. Without her encouragement and guidance our project would not have been possible. She has enriched our experience in undergraduate research with her mentorship and insight. We would also like to extend our warmest thanks to Alejandro Aceves for his selfless support in helping us complete our project. Finally, we would like to thank Miriam Aceves for performing the contusion surgeries necessary for this study.

NOMENCLATURE

BBB	Basso, Beattie, and Bresnahan
DSM – IV–TR	The American Psychiatric Association Diagnostic and Statistical Manual Fourth Edition, Revised
MDD	Major Depressive Disorder
SCI	Spinal Cord Injury
SSRI	Selective Serotonin Reuptake Inhibitor

CHAPTER I

INTRODUCTION

Spinal cord injury

In the United States alone, approximately 276 000 individuals live with a spinal cord injury (SCI), with 12 500 new cases occurring every year (NSCISC, 2015). The majority (82%) of SCI patients are males, with injury occurring most often between the ages of 16-30 years. Depending on the severity of the injury, and the vertebral level at which it occurs, SCI will result in paralysis, loss of sensory function, loss of bowel or bladder control, dysreflexia or spasms, loss of sexual function/sensitivity and fertility, chronic pain, and difficulty breathing, coughing, or clearing secretions from the lungs.

In addition to physical effects, SCI decreases quality of life and impacts psychological wellbeing. Compared to the rate of 8.6% in the general population, the incidence of major depressive disorder (MDD) ranges from 11 to 24% in patients after SCI (Krause et al., 2000). Decreased psychological wellbeing is one of the most significant, and often overlooked, consequences of injury. The significance of depression on quality of life is underscored by data showing that suicidal ideation and attempts are three times greater in the SCI population, relative to the non-injured population (Teasdale et al., 2001). Depression also has significant implications for physical recovery post SCI. For example, the incidence of urinary tract infections, pressure ulcers, autonomic dysreflexia, and acute, uncontrolled hypertension are significantly increased in

SCI patients with depression, relative to non-depressed patients (Al Taweel et al., 2015). Depression significantly affects the wellbeing and recovery of SCI patients.

Diagnosis of depression

According to the American Psychiatric Association (*DSM-IV-TR,* 2000), MDD is diagnosed when at least five of the following symptoms are observed for at a two-week period minimum (*DSM-IV-TR*, 2000). In all cases, individuals either experience a depressed mood or a loss of interest or pleasure.

Symptoms of major depressive disorder include (*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

Although not all patients meet the criteria for MDD, 37% display significant symptoms of depression after SCI (Migliorini et al., 2009). Antidepressant drug therapy is commonly prescribed for these individuals (Siddal et al., 2015). Unfortunately, however, frequently prescribed selective serotonin reuptake inhibitors (SSRI) do not reverse all signs of depression

and have side effects that can include attenuation of functional recovery, as well as a paradoxical *potentiation* of symptoms of depression (Coyle et al., 2015). Moreover, current antidepressants act on the monoamine systems of the brain and are slow to elicit antidepressant effects (Anisman et al., 2009). There is also evidence that these treatments may attenuate recovery of function for patients. For example, Fullerton (1981) reported that 3 SCI patients were diagnosed with major depression and treated with amitriptyline. Two of the patients developed adverse side effects (including autonomic dysreflexia), and the remaining individual improved by discharge from the inpatient rehabilitation program rather than the anti-depressant treatment itself (Fullerton et al., 1981).

Notably, studies also indicate that 23% of patients suffer intractable depression (Zorumski et al., 2015), which means that they do not experience relief from depression irrespective of the treatments prescribed. In fact, they often feel discouraged after seeing no significant results and this can worsen their mental condition. Therefore, further research on the etiology of depression and its interaction with SCI, as well as the molecular effects of conventional pharmacotherapeutics, is needed. The development of animal models is essential for elucidating the molecular causes of depression following SCI. To address this, the current study tested the potential of S-ketamine, as an antidepressant, in an animal model of SCI.

A rodent model of depression after spinal cord injury

Tests for behaviorally assessing depression have been developed for rodent models of various 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 stroke (Wang et al., 2009). The behaviors measured in these paradigms are analogous to some, though not all, of the core symptoms of major depressive disorder (outlined in Table 1), including 'helplessness', loss of interest in pleasurable activities, and decreases in activity or changes in appetite and sleep patterns. Indeed, McKinney and Bunney (1969) indicated that valid animal models of depression should:

1. Have induced symptoms of depression that are reasonably analogous to those seen in human depression.
2. Have observable behavioral changes, which can be objectively evaluated.
3. Independent observers should agree on objective criteria for drawing conclusions on 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

Addressing these criteria, Luedtke et al. (2011) recently validated a behavioral ethogram for assessing depression in an animal model of SCI. Using established behavioral tests for depression, Luedtke et al. (2011) found that 35% of rats with SCI exhibited symptoms of depression, an incidence commensurate with SCI patients reporting clinical symptoms of depression without a diagnosis of MDD (Migliorini et al., 2009). 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, which signifies helplessness, compared to "non-depressed" rats. Depressed rats also displayed psychomotor retardation, demonstrating significantly reduced exploratory and pleasurable activities when compared to their non-depressed counterparts (Luedtke et al., 2011).

Further validating this model of depression, Luedtke et al. (2011) demonstrated that a clinically-relevant antidepressant, fluoxetine, reversed depressive symptoms in the forced swim test. Thus, the pharmacological therapies effective at reversing depression in humans were also efficacious in the depressed SCI rats. These data are important as they demonstrate that even in the absence of the confounding psychosocial variables (i.e. loss of independence, economical stressors) the molecular changes inherent to SCI exacerbate depression in rodents and may impact psychological wellbeing in humans.

Meeting the 5th criterion for an animal model of depression, evidence of depression after SCI has been reported by independent laboratories. Using the procedures outlined by Luedtke et al. (2011), Maldonado et al. (2016) examined depression in a rodent SCI model to test the relationship between inflammation and depression/anxiety post injury. The data from this study supported the hypothesis that changes in molecular mediators of inflammation are associated with decreased psychological wellbeing following SCI. In an independent laboratory, Wu et al. (2014) used a similar animal model to find that SCI caused spatial and retention memory impairment and depressive-like behavior. Therefore, these independent laboratories were able to see evidence of depression using similar models. Thus, the criteria for accessing depression in our rodent injury model has been met and will be used to test for S-ketamine as a potential antidepressant following SCI.

S-Ketamine as an anti-depressant following spinal cord injury

Currently, depression after SCI is treated with SSRI's. These medications, however, do not reverse all signs of depression and have potential side effects that include attenuation of functional recovery as well as the paradoxical potentiation of symptoms of depression (Coyle, et al., 2015). Moreover, the efficacy of current pharmacological treatments differs not only across patients, but also across injury and disease conditions (i.e. SCI and post-traumatic stress disorder). It is likely that there are diverse molecular changes that underlie the multi-faceted symptoms of depression. New and effective treatments must also be developed. Indeed, the incidence of intractable depression in the human population underscores the need for the development of alternative anti-depressant therapies.

In recent years, ketamine has shown potential as an anti-depressant for MDD (Coyle and Laws 2015). In contrast to commonly prescribed anti-depressants, ketamine is an N-methyl-D-aspartate antagonist, thereby affecting the action of glutamate a major excitatory neurotransmitter in the brain (aan het Rot et al., 2012), with purported rapid antidepressant properties that are sustained beyond ketamine's 3-hour half-life (Young, 2013; Salvadore and Singh, 2013). In a meta-analysis, Coyle and Laws (2015) reviewed the results of 21 different experiments using ketamine as an anti-depressant for MDD and bipolar disorder (BO). This meta-analysis showed that ketamine reduced depressive symptoms, at variable time points in patients with MDD and BO (Coyle and Laws, 2015).

In research with SCI patients, ketamine has also shown effectiveness in managing neuropathic pain and allodynia (an experience of pain from a non-painful stimulation of the skin) (Tam et al., 2012). Since pain is often comorbid with depression (Nicholson et al., 2009), indicating that it may lead to the development of depression or vice versa, ketamine may also be useful in treating depression after SCI. From a molecular perspective, one of the key changes associated with the pain and depression is an increase in pro-inflammatory cytokines post injury (Maldonado et al., 2016). Research indicates that ketamine has neuroprotective and anti-inflammatory effects post-SCI, reducing the over-expression of inflammatory cytokines such as IL-6 (Niesters et al., 2014). We hypothesize that ketamine, by reducing levels of pro-inflammatory cytokines such as IL-1β and IL-6 mRNA, may be an effective treatment for depression after SCI, as well as recovery post injury (Bette et al., 2004). To date, however, no research has directly assessed the effects of ketamine on depression following SCI.

Importantly, despite the purported benefits of ketamine treatment it is also possible that it will have adverse effects in SCI patients. Ketamine is a dissociative psychedelic drug (Muetzelfeldt et al., 2008). Consequently, ketamine also has the potential to cause hallucinations and to possibly exacerbate anxiety symptoms that are linked to depression in many patients (Irwin et al., 2013). The effects of ketamine must be empirically assessed in an SCI model. Thus, the proposed experiment will test the effects of S-ketamine in the rodent SCI model, comparing the manifestation of depression-like symptoms in subjects treated with S-ketamine and vehicle-treated controls. We will examine the effects of S-ketamine, rather than ketamine, in the rodent

SCI model because it is the stronger NMDA antagonist between the R and S isomers, with twice the analgesic potency compared to the clinically used racemic mixture (Muller et. al., 2016).

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

Depression Symptom in the DSM-IV-TR	Measure of Symptom-Like Behavior in Rats	References
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 Hilakivi et al., 1990
Feelings of worthlessness or excessive or inappropriate guilt	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 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 Lamkin et al., 2010
Psychomotor agitation or retardation	Open Field Test Stressor Recognition 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

CHAPTER II

METHODS

Subjects

Subjects were male Sprague-Dawley rats (Harlan, Houston, TX) between 90-110 days old. They were individually housed in Plexiglas bins (45.7 (length) x 23.5 (width) x 20.3 (height) cm) with *ad libitum* food and water. Food consumption and subject weights 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 (5:30-7:00 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 the Infinite Horizon spinal cord impactor (PSI, Fairfax Station, VA). 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%. The subject's back was shaved and disinfected with iodine and a 5.0 cm incision was made over the spinal cord. Two incisions were made on the vertebral column on each side of the dorsal spinous processes, extending about 2 cm rostral and caudal to the T12 segment. Muscle and connective tissue were then dissected to expose the underlying vertebral segments.

Musculature around the transverse processes was cleared to allow for clamping of the vertebral spinal column. Next, the dorsal spinous process at T12 was removed (laminectomy), and the spinal tissue exposed. The dura remained intact. The vertebral column was fixed within the IH device using two pairs of Adson forceps. A moderate injury was produced using an impact force of 150 kilodynes and a 1 s dwell time. After injury, the wound was closed using Michel clips. To help prevent infection, subjects were treated with 100 000 units/kg Pfizerpen (penicillin G potassium) immediately after surgery and again 2 days later. For the first 24 h after surgery, rats were placed in a recovery room maintained at 26.6 °C. To compensate for fluid loss, subjects were given 3 ml of saline after surgery. Michel clips were removed 14 d after surgery.

S-ketamine administration

Drug administration occurred 24 h following SCI. First, baseline locomotor function was determined using the Basso, Beattie, and Bresnahan (BBB) scale (Basso et al. 1995). Balancing BBB scores across groups, subjects were then assigned to one of four treatment conditions: 0, 5, 10, and 20 mg/kg of S-ketamine obtained from Cristalia Produtos Quimicos Farmaceuticos Ltda., (San Paulo, Brazil). Drug was administered via an intraperitoneal (i.p.) injection. An equivalent volume of filtered 0.9% saline was administered to vehicle controls. To evaluate the analgesic efficacy of S-ketamine, sensory reactivity was assessed before drug treatment and 30, 60, 120 and 180 min post-treatment using the von Frey and tail-flick tests (described below).

Assessment of depression

The following time schedule was used in all experiments (Figure 1). Tests of depression evaluated symptoms analogous to those observed in human patients (Table 1). 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

The sucrose preference test can be used to test for anhedonia- a loss of interest in pleasurable activities. Anhedonia is a core symptom of MDD. Rats exhibiting anhedonia display a decreased preference for a sucrose solution over plain water, when compared to baseline measures taken prior to injury or disease. Sucrose preference tests were conducted in the subject's home cage. For testing, one pre-weighed water bottle filled with approximately 200 mL of 2% sucrose solution and a pre-weighed bottle filled with an equal amount of filtered water were placed on either side of the subject's cage, and left for 2 hours. 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 the 2-hour period, to prevent any positional biases from confounding results. At the end of the 2-hour test 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 = sucrose solution intake (mL) / [sucrose solution intake (g) + 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 2, 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.

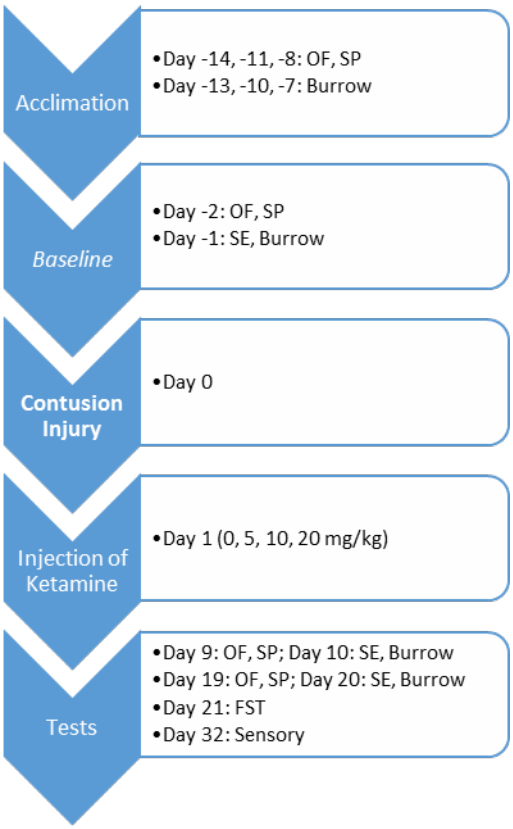


Figure 1. Experiment Schedule

Open field activity

Since rats are naturally social and curious animals, the open field test is a simple measure for the depressive-like symptom of decreased interest or pleasure-seeking. When placed in a large open field environment, rats with symptoms of depression will display decreased locomotor activity and reduced exploratory behavior.

The open-field environment was a black plywood box [100 (length) x 50 (height) x 100 (width) cm]. The floor of the box was partitioned into 100 squares [10 (length) x 10 (width) cm] delineated with a 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 scored *post hoc* using video tracking software (EthoVision, Noldus). 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 2, 9, and 19 days post injury. Decreased activity in the open field, compared to baseline, was interpreted as an indication of depression-like behavior.

Burrowing

The burrowing task is a behavioral test developed by Deacon et al. (2006) that was initially used to screen for prion disease in mice and IL-1β-expressing replication-deficient adenovirus in rats. Normal rats demonstrate vigorous burrowing activity, which suggests that it is a rewarding activity (Deacon et al., 2006). Therefore, burrowing may be a useful measure for depressive-like symptoms of diminished pleasure or loss of energy in subjects (Deacon et al., 2006).

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. 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

Rats are highly sociable creatures (McKibben et al., 2014), and a decrease in social interaction is indicative of decreased interest or pleasure. Social exploration was assessed in the open-field environment described above. 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 then 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 (moving toward, anogenital sniffing, close following) and nonsocial behavior (resting, moving away, self-grooming, and exploration of the open field) was scored by two independent observers as described previously by Swain and Le (1998).

Forced swim test

The forced swim test (FST) is commonly used for assessing depression in rodent models (Posolt, 1977). For the test, subjects are placed into a plastic container filled with water from which they cannot escape. If an animal is not depressed, it will be mobile throughout the test. However, if a subject has depressive symptoms it will remain immobile throughout some portion of the test. The duration of this immobility is interpreted as a measure of behavioral despair or "hopelessness." Hopelessness has been positively correlated with suicide ideation, one of the major criteria of depression (Minkoff et al., 1973).

For this 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 clear rectangular container [57 (Length) x 40 (Width) x 33 (Height) cm] filled with water, maintained at 23 ± 1˚C. The forced swim test is traditionally conducted using a 10-minute acclimation period and a 5-minute test period 24 hours later. However, Abel and 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, and the testing period consisted of the first 10-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*)*.

Locomotor recovery

The recovery of hind limb stepping ability was scored using the Basso, Beattie and Bresnahan

(BBB) scale (Basso et al., 1995). Briefly, subjects 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 prior to surgery for 5 minutes on 3 consecutive days. 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, 21, 24, and 27 post-SCI. Care was taken to ensure that all investigators' scoring behavior had high intra- and inter- observer reliability (all r's > 0.89) and that they were blind to the subject's experimental treatment.

Sensory reactivity

Thermal reactivity was assessed using radiant heat in the tail-flick test. Subjects were placed in the restraining tubes with their tail positioned in a 0.5 cm deep groove, cut into an aluminum block, and allowed to acclimate to the apparatus (IITC Inc., Life Science, CA) and testing room (maintained at 26.5 °C) for 15 min. Thermal thresholds were then assessed. Thermal reactivity was tested using a halogen light that was focused onto the rat's tail. Prior to testing the temperature of the light, focused on the tail, was set to elicit a baseline tail-flick response in 3-4 s (average). This pre-set temperature was maintained across all subjects. In testing, the latency to flick the tail away from the radiant heat source (light) was recorded. If a subject failed to respond, the test trial was automatically terminated after 8 s of heat exposure. Two tests occurred at 2-min intervals, and the second test tail-flick latencies were recorded. To confirm that subjects did not respond in the absence of the heat stimulus, blank trials were also performed. A 'false alarm' was recorded if subjects made a motor movement or vocalization response during the

blank tests. The blank trials were performed 1 min before or after each sensory test (in a counterbalanced fashion). No false alarms were recorded. After assessment of thermal reactivity, subjects were returned to their home cages for a minimum of 2 h.

After 2 h, subjects were placed back into restraint tubes and presented with von Frey stimulation (von Frey stimuli formed from nylon monofilaments; Semmes–Weinstein Anesthesiometer, Stoelting Co., Chicago, IL) to test tactile reactivity. Filaments of increasing strength were applied every 2 s to the plantar surface of the paw. The stimuli was presented until subjects exhibited a paw withdrawal/motor and vocalization response. The intensity of the stimuli that produced the responses was reported using the formula provided by Semmes–Weinstein: Intensity = log10 (10,000 * g force). If one or both responses were not observed, testing was terminated at a force of 300*g*. Each subject was tested twice on each foot in a counterbalanced ABBA order.

Statistical analysis

For the identification of depression, first a Principal components analysis (PCA) was used to determine which behavioral measures were correlated with each other. PCA is a variable reduction technique that transforms a group of observations from a related set of variables into a (typically) smaller set of linear uncorrelated variables. These uncorrelated variables are referred to as principal components. Change from baseline (Test day score- Baseline score) scores for each of the subjects on each of the behavioral measures (sucrose preference, forced swim, open field activity, social exploration, burrowing, food deviation change) were averaged across days 9/10 and 19/20 and then subjected to a principal components analysis using orthogonal Varimax

rotation. Scores were averaged so that the equation derived in subsequent analyses would characterize symptoms that persisted into the chronic phase as well as having strong predictive value for the early phase of SCI. Factors with loadings of ≥ 0.32 on a component were retained and used in subsequent cluster and discriminant function analyses. Variables with a complex structure, which loaded onto more than one component, were removed from the PCA and the analysis was repeated (Tabachnick and Linda, 2007).

A hierarchical cluster analysis was then performed using the measures with moderate-strong loadings on the components retained in the PCA. Hierarchical cluster analysis is a statistical procedure used to separate a sample into clusters that the experimenter can operationally define. Average change from baseline scores (derived from both Days 9-10 and 19-21), on each of the retained behavioral tests, were used in this test. The hierarchical cluster analysis was performed using Ward's method and applying squared Euclidean distance as the distance measure. The number of appropriate clusters, based on the behavioral measures, was obtained by looking for a break in the agglomeration coefficient change and by observing the dendrogram, which visually depicts the distance between linked clusters. It must be noted that the cluster sizes need not be even. After identifying the number of clusters depicted in the dendogram, the cluster analysis was repeated using the same parameters but requesting a single solution of two clusters. A new variable, cluster membership, was generated for all subjects.

The two clusters' performance on each of the behavioral measures collected (depression and recovery) were compared across the recovery period (days 9-10 and 19-20), after any potential antidepressant effects of S-ketamine should have been dissipated. Repeated measures analysis of variance (ANOVA) tests were conducted using change from baseline scores (Test day score-Baseline score) for each of the behavioral tests. Based on the pattern of behaviors exhibited by each cluster, they were labeled as "depressed" and "non-depressed."

Repeated-measures ANOVAs were also used to compare the locomotor and sensory recovery across S-ketamine dose groups and across depressed and non-depressed subjects. First, BBB scores were transformed to help assure that the data were amendable to parametric analyses (Ferguson, et al. 2004). This transformation pools BBB scores 2–4, removing a discontinuity in the scale. The transformation also pools scores from a region of the scale (scores 14–21) that is very seldom used under the present injury parameters. By pooling these scores, we obtain an ordered scale that is relatively continuous with units that have approximately equivalent interval durations. Meeting these criteria allows us to apply metric operations (computation of mean performance across legs), improves the justification for parametric statistical analyses, and increases statistical power. Additional statistical power was achieved by obtaining a measure of locomotor performance 24 h after injury, prior to shock treatment. This provides a behavioral index of the injury extent that is correlated with long-term recovery ($r>0.41$, $p > 0.05$; Hook et al., 2004). By using this factor as a covariate in an analysis of covariance (ANCOVA), we substantially reduce unexplained variance. Group differences on dichotomous variables (e.g.,

mortality) were evaluated using the Fisher exact probability test. This test allows for comparisons of simple (2 x 2) frequency tables with relatively small samples.

We also examined the effects of S-ketamine using ANOVAs, with S-ketamine dose as the dependent variable. Remaining within the window of S-ketamine's efficacy, we analyzed data collected on day 2 post-SCI. Next, using the depressed and non-depressed clusters that we obtained from day 9-10 and 19-20, we used multivariate ANOVA's to find any significant interactions between dose and depression on day 2 post-SCI.

CHAPTER III

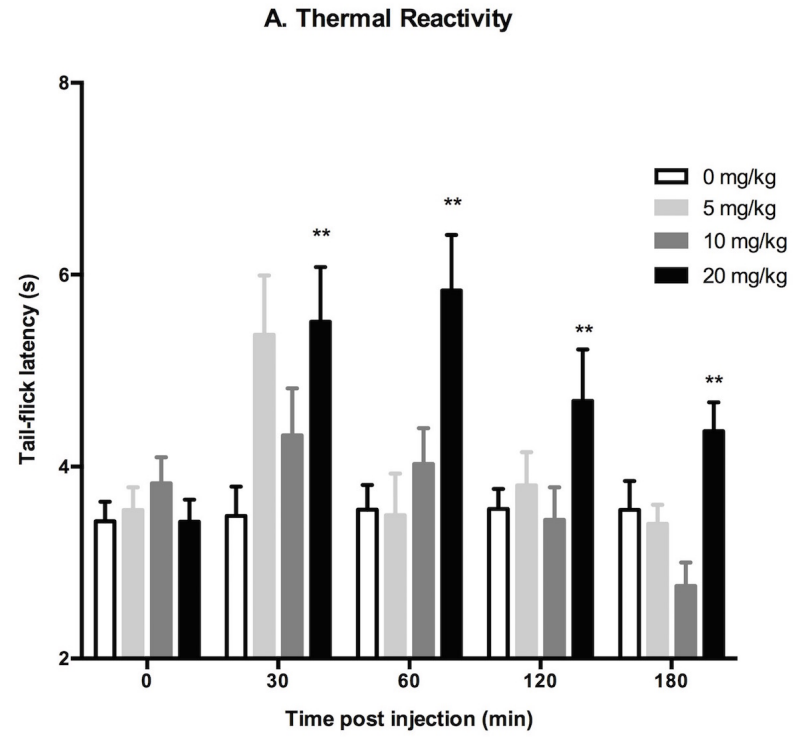
RESULTS

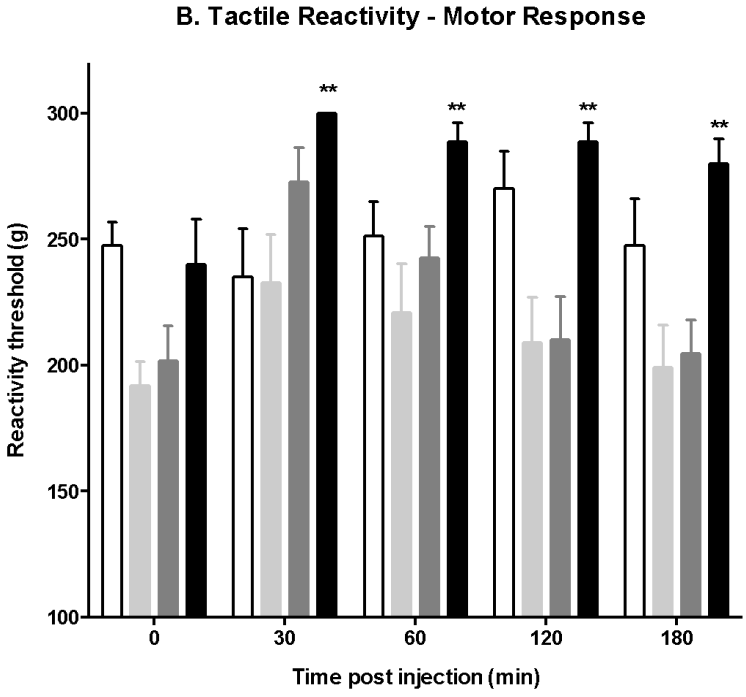
Effects of S-ketamine on sensory and motor recovery

Recognizing that changes in motor and sensory function may confound performance on the tests of depression, our initial analyses focused on the effects of S-ketamine on sensory reactivity and recovery of locomotor function.

Analgesic efficacy of S-ketamine

As shown in Figure 2, a single dose of S-ketamine (i.p.) produced robust analgesia on both the thermal and tactile reactivity tasks. Using baseline reactivity (prior to treatment, time 0 min) as a covariate, there was a significant main effect of dose on the tail-flick test (F (3, 33)= 8.27, p < .001) as well as the motor response to tactile stimulation (F (3, 33)= 3.48, p = .03). Planned comparisons indicated that the 20 mg/kg of S-ketamine produced significant analgesia, relative to vehicle controls on all tasks (Tail flick, F (1, 13) =16.73, p < .001; Tactile motor, F (1, 13) =7.63, p < .02; Tactile vocal, F (1, 13) = 7.25, p < .02).


A. Thermal Reactivity
Tail-flick latency (s)
8
6
4
2
0 mg/kg
5 mg/kg
10 mg/kg
20 mg/kg
**
**
**
**
0
30
60
120
180
Time post injection (min)


B. Tactile Reactivity - Motor Response
Reactivity threshold (g)
300
250
200
150
100
**
**
**
**
0
30
60
120
180
Time post injection (min)

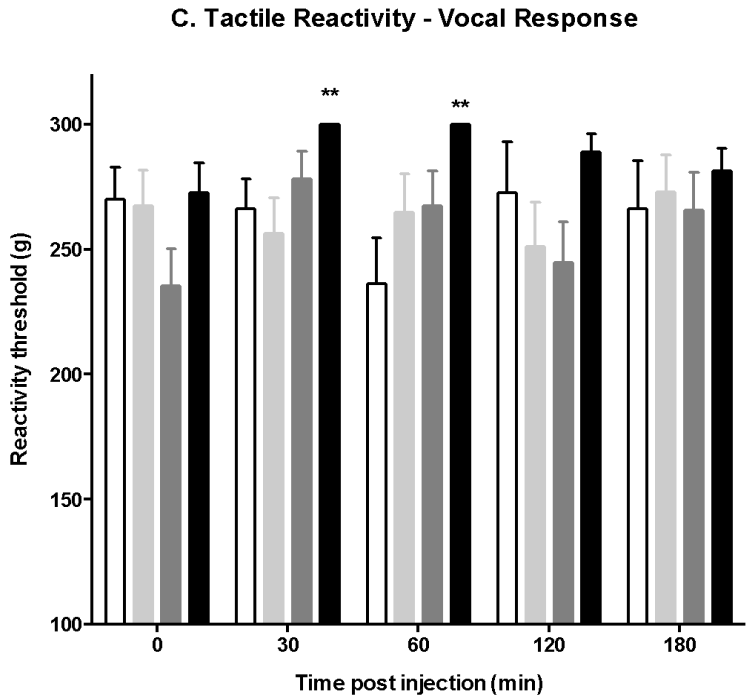


Figure 2. Subjects receiving 20 mg/kg S-ketamine displayed significant analgesia for 60-180 mins post administration on all sensory reactivity tasks, relative to vehicle controls. Prior to S-ketamine administration, there were no differences in motor or vocal reactivity across dose groups. Following administration of 20 mg/kg S-ketamine, however, subjects showed decreased motor reactivity to a thermal (A) and mechanical (B) stimulus for 180 mins post administration. Decreased vocal reactivity to mechanical stimulation was also observed for the 20 mg/kg group for 60 mins post S-ketamine administration (C). **$p < 0.05$.

Recovery of locomotor function

There was no effect of S-ketamine on the recovery of locomotor function (F (3, 28) < 1.0, p > 0.05). Baseline BBB scores were balanced across dose groups and final BBB scores ranged from 8 ± 1.02 for the 0 mg/kg dose group to 9.125 ± .63 for the 5 mg/kg and 20 mg/kg group (Figure 3).

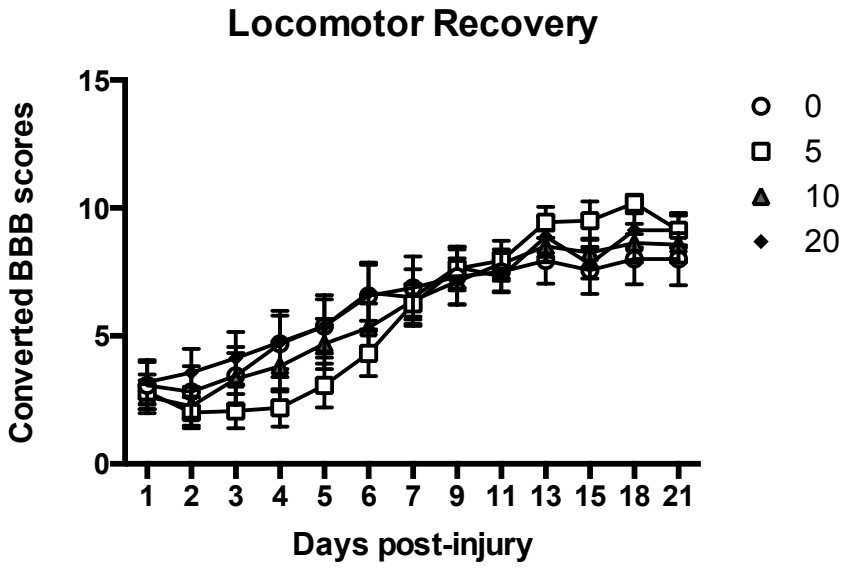


Figure 3. S-ketamine had no effect on locomotor recovery. BBB scores were balanced prior to administration and, irrespective of dose, all groups recovered similar levels of locomotor function.

Recovery of sensory function

ANOVAs were used to examine the effects of S-ketamine dose on long-term recovery of sensory function. Acute administration of S-ketamine significantly affected tail-flick latency on day 21

post-injury (F (3, 31) = 3.11, p < .05). Further comparisons revealed that the 5 mg/kg group displayed significantly longer tail-flick latencies than all other groups at this timepoint (Fig. 4A). The dose of S-ketamine did not affect tail-flick latencies on Days 3 or 11 post injury. There were also no effects of S-ketamine dose on motor reactivity thresholds with mechanical stimulation across the 21-day recovery period. However, vocal response thresholds differed across groups on Day 21 (F (3, 31) = 3.99, p < .05). Both the 0 and 5 mg /kg displayed higher vocal reactivity thresholds than the 10 mg/kg group at this time point (Fig. 4B).

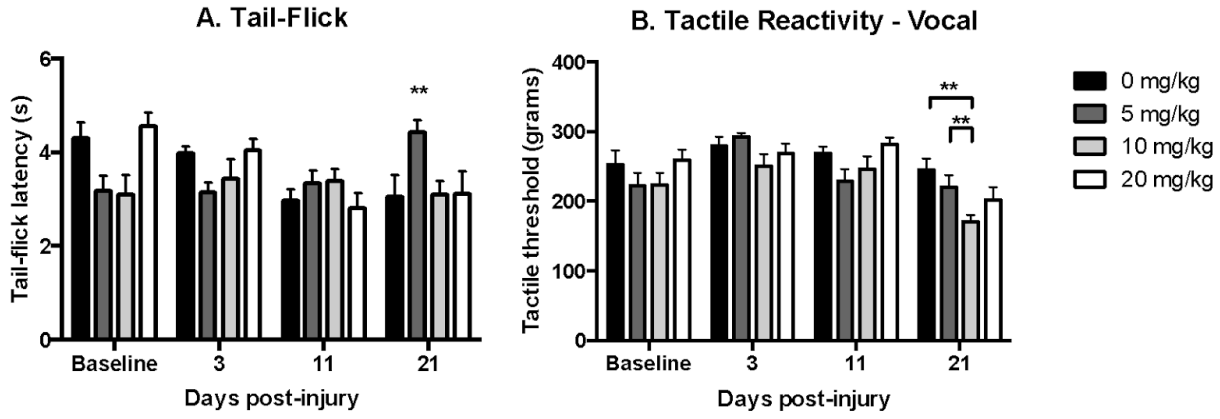


Figure 4. The 5 mg/kg dose of S-ketamine decreased reactivity to both thermal (A) and mechanical (B) stimuli 21 days after administration. ** p< .05.

Depression following spinal cord injury

To characterize subjects as depressed or not-depressed, initial analyses focused on symptoms of depression measured on Days 9-11 and 19-21 post-injury, after any anti-depressant effects of the S-ketamine should have dissipated.

First, a principal components analysis (PCA) was used to assess the validity of the individual tests for identifying symptoms of depression. Using the PCA, two principal components were identified. The first component contained burrowing activity and food deviation, while forced swim and open-field activity loaded on the second component. Sucrose preference and social activity loaded on both components, and were removed from further cluster analyses.

Table 2. Rotated using orthogonal Varimax rotation with Kaiser normalization. Only component loadings ≥ 0.32 are shown.

Principal Component Loadings

Behavioral Test	***Component 1***	***Component 2***
Burrow Activity	-.783	
Food Deviation	.769	
Forced Swim Test		.734
Open-Field Activity		-.594

Average change from baselines scores obtained for days 9-10 and 19-20, for each of the behavioral tasks retained in the PCA, were used in a hierarchical cluster analysis (HCA). The HCA was used to classify the subjects into depressed and non-depressed groups. The dendogram

produced by the analysis identified two subject cohorts, with 19 subjects in the first cluster and 13 in the second.

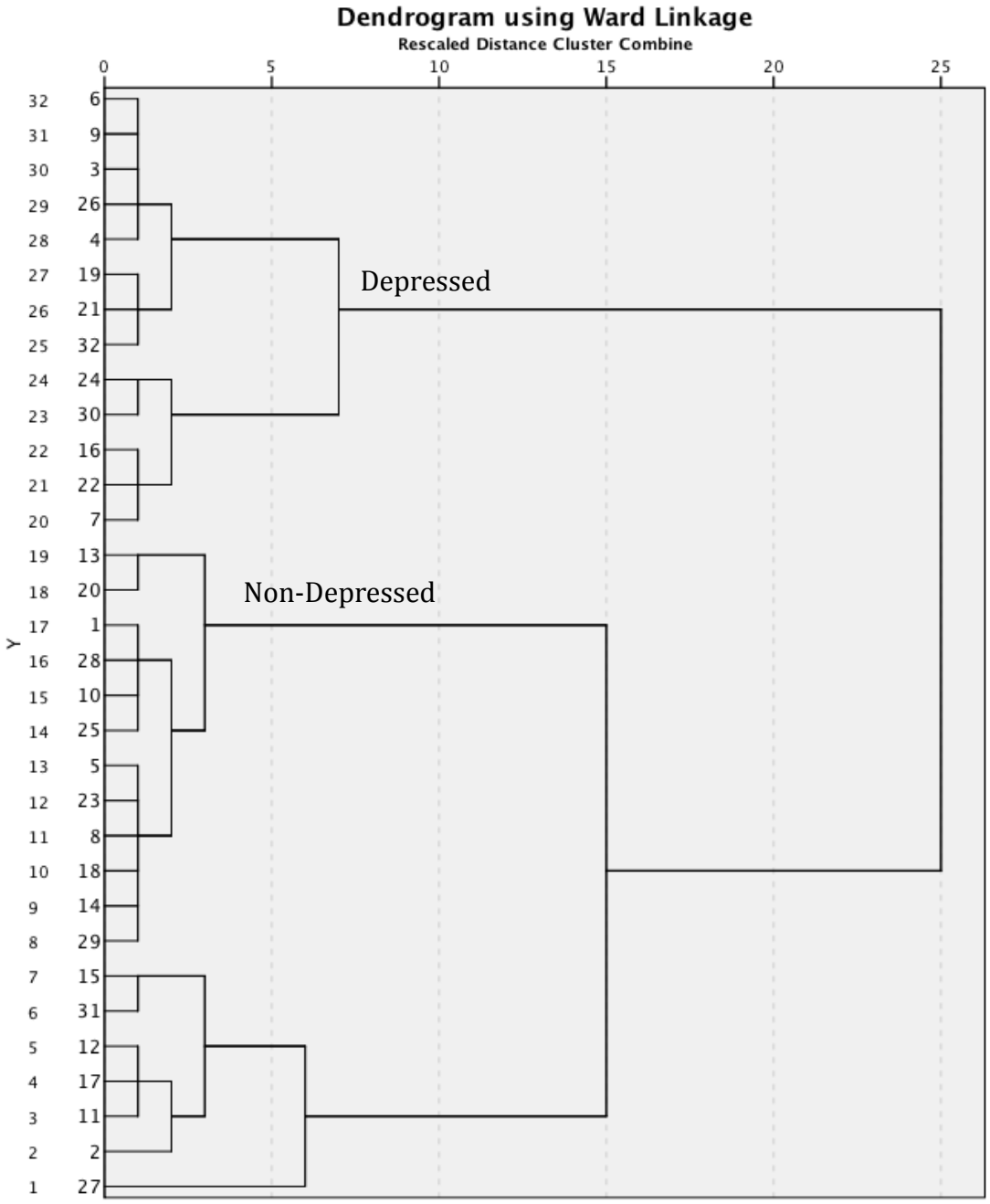


Figure 6. The dendogram shows the result of the hierarchical cluster analysis. Two clusters were formed based on depression-like behaviors that persisted across the acute and chronic phases of injury.

Individual ANOVAS were used to examine differences between the two clusters on Days 9 and 19. All results are portrayed as a change from baseline (Figs 7A-F). As shown in Fig. 7A, subjects in Cluster 2 displayed a larger decrease in sucrose preference on Day 9 post-injury relative to Cluster 1. The differences between the cohorts, however, only approached significance (F (1, 31) = 3.16, p= .06). Sucrose preference did not differ across clusters on Day 19 post-injury. As shown in Fig. 7B, open field activity was also significantly different between the two clusters on days 9-10 and 19-20 (F (1, 31) = 22.9 and 33.8 respectively, $p < .05$). Cluster 2 showed significantly decreased open field activity on both days when compared to Cluster 1 subjects. Burrowing scores were not significantly different between the two clusters at either timepoint (F (1, 31) = 0.02, 1.04 respectively, $p > .05$) although, as can be seen in Fig. 7C, Cluster 2 showed decreased burrowing activity on days 19-20 when compared to Cluster 1. Similarly, social activity did not differ significantly between the clusters (F (1, 31) < 1.0 for both timepoints, $p > 0.05$). Subjects in Cluster 2 subjects showed higher social activity on days 9-10, while Cluster 1 subjects showed increased social behavior on days 19-20 (Fig. 7D). Food deviation between the two clusters did not differ significantly (F (1, 31) < 1.0 for both timepoints, $p > .05$; Fig. 7E). Cluster 2 subjects displayed comparatively more mobility in the forced swim test than subjects in Cluster 1, however these results were not significant (F (1, 31) =2.13, $p > .05$). Based on lower average burrowing activity, decreased sucrose preference and

decreased open-field activity, subjects in Cluster 2 were designated as "depressed," while Cluster 1 was "non-depressed." Therefore 13 of 32 subjects (41%) were found to show signs of depression or depression-like behavior.

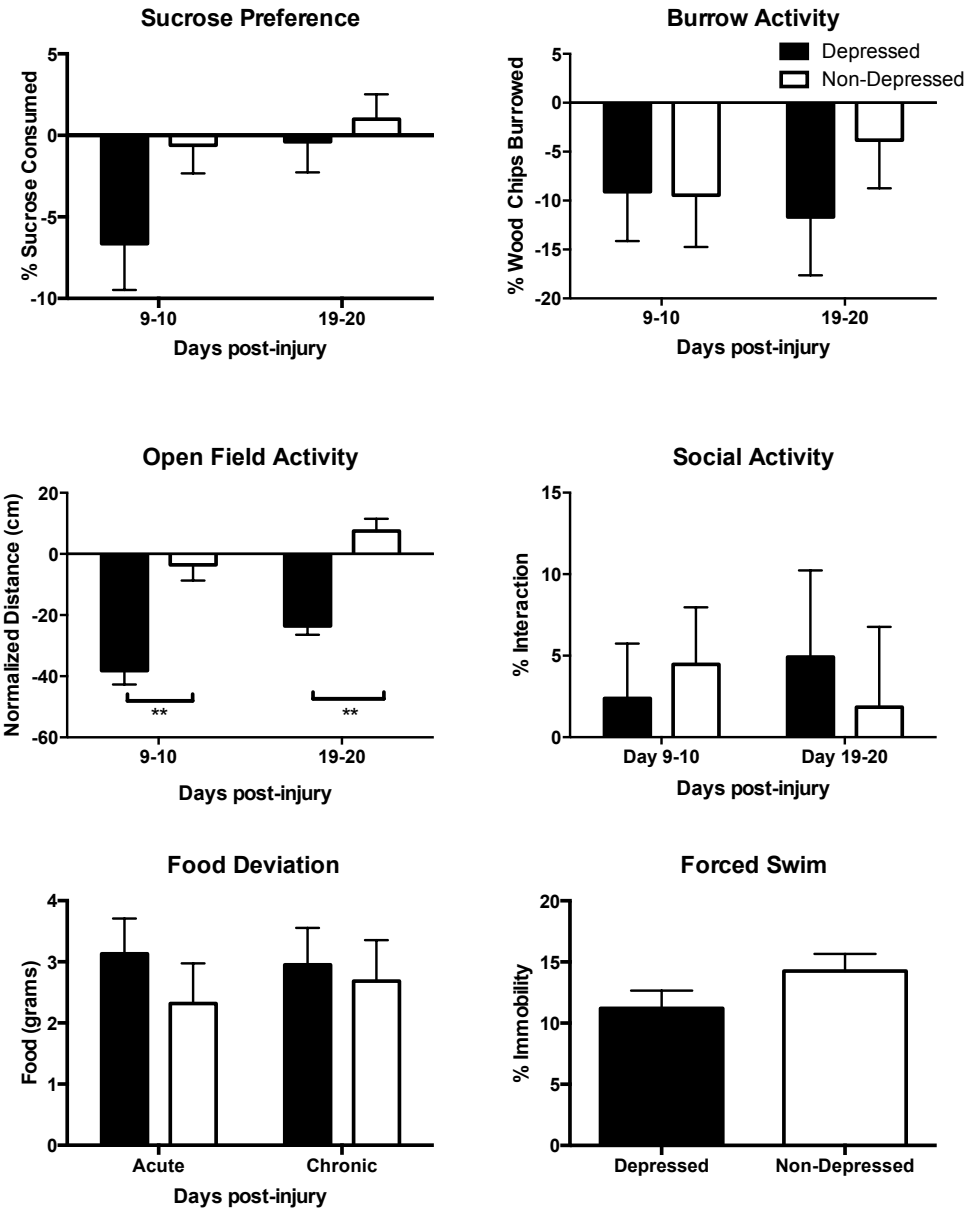


Figure 7. Subjects categorized as depressed displayed behavioral changes indicative of anhedonia and fatigue. Relative to non-depressed subjects, depressed subjects had decreased sucrose preference (A), burrowing behavior (B) and open field activity (C) on Days 9-10 and 19-20 post injury. Social activity (D), food deviation (E) and immobility in the forced swim test (F) did not differ across the groups. All data is shown as change from baseline. **p< .05.

Effects of depression on locomotor and sensory recovery

Recovery of locomotor function

There were no significant differences between the depressed and non-depressed groups in locomotor recovery (F (3, 28) = . 123, p > .05). (Fig. 8). Final BBB scores were 9.18 ± .49 for the non-depressed group, and 8 ± .54 for the depressed group.

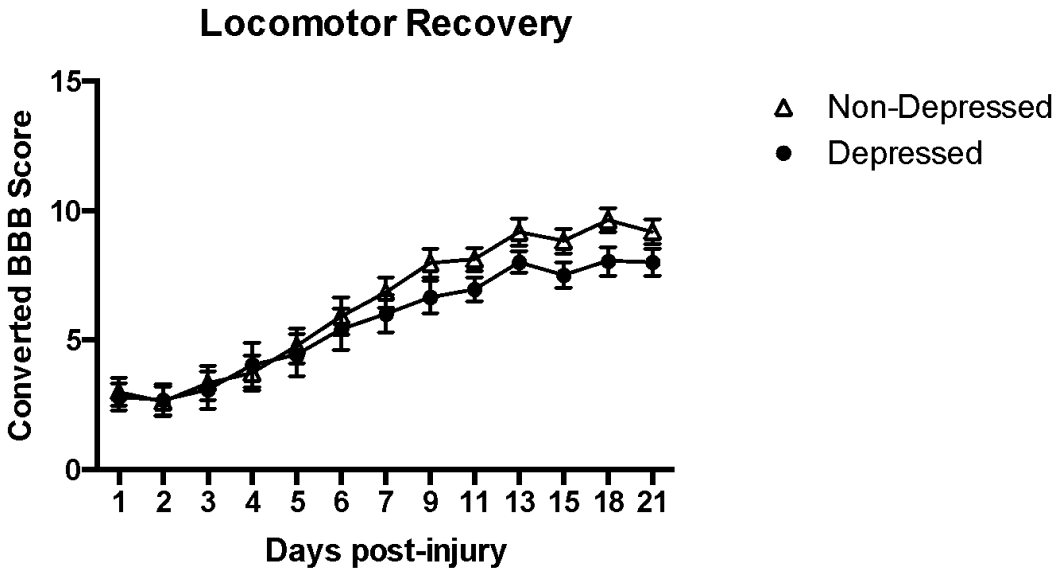
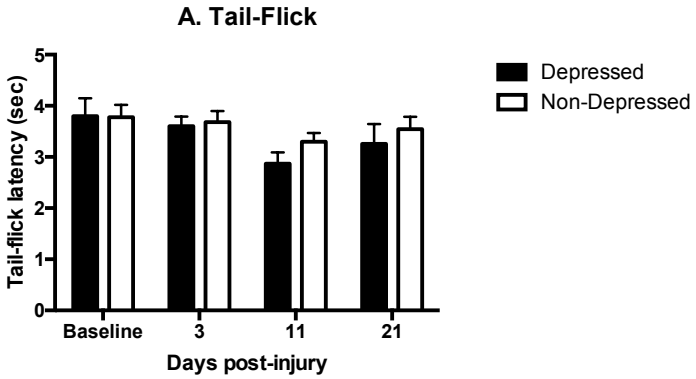


Figure 8. Locomotor recovery did not differ significantly between depressed and non-depressed subjects.

Recovery of sensory function

Tactile and tail flick tests were used to assess long-term recovery of sensory function in the depressed and non-depressed groups. Prior to treatment, there were no significant differences across the groups on any of the sensory reactivity tasks. Similarly, the depressed and non-depressed groups did not differ on the tail-flick test at 21 days post-injury (F (1, 31) = .456, p > .05; Fig. 9A). Using repeated measures ANOVAS, however, we found significant differences between the depressed and non-depressed groups on Day 11 for both tactile motor and vocal reactivity. At Day 11 post injury depressed subjects, overall, displayed higher motor and vocal reactivity thresholds (F (1, 30) = 9.71 and 9.60, respectively, p < .05) with mechanical stimulation than non-depressed subjects (Fig. 9B and C, respectively).



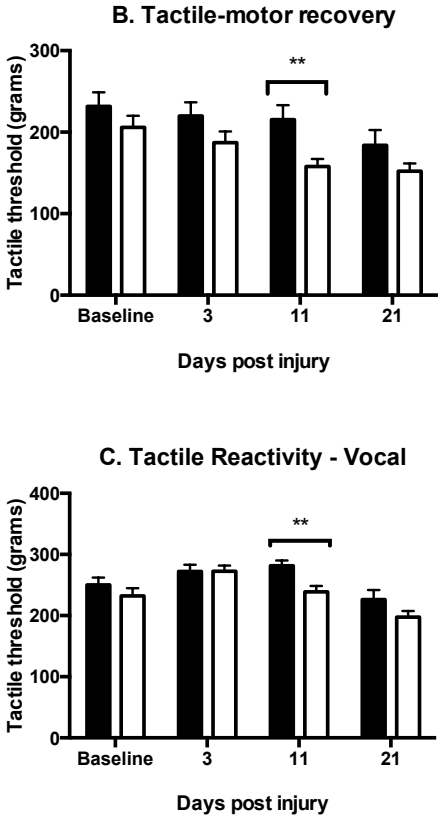


Figure 9 (A-C). Depressed subjects show higher motor and vocal thresholds on Day 11 post injury compared to non-depressed subjects. ** $p < 0.05$.

Effects of S-ketamine on depression

After identifying the depressed and non-depressed cohorts, we examined the effects of S-ketamine on depression-like behaviors. The incidence of depression, assessed on Days 9-10 and 19-20, was equal across the four S-ketamine dose groups (Fig. 10). At this point, however, any potential antidepressant effects of S-ketamine would have dissipated (Murrough et al., 2013). Analyzing the behavioral data collected on Day 2 post-injury, and within the clinical window for

S-ketamine's efficacy, we first compared the expression of depression-like behaviors across dose groups, and then using multivariate ANOVAS looked at potential interactions between S-ketamine and depression.

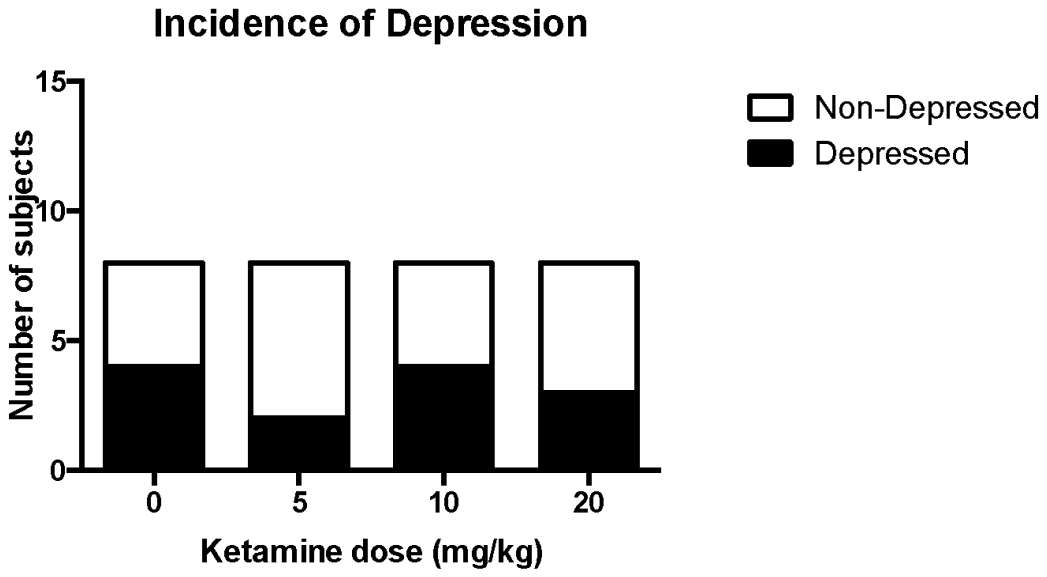


Figure 10. The graph shows the number of depressed and non-depressed subjects within each treatment group.

Analysis of Day 2 data for sucrose preference showed no significant differences between the drug treatments (F (3, 31) = .691, $p > .05$). Depression, however, did significantly impact sucrose preference scores on Day 2 (F (1, 31) = 4.56, $p < .05$). While the subjects later categorized as depressed displayed a large decrease in sucrose preference on Day 2, relative to baseline, the non-depressed subjects did not (Fig. 11A).

There were no significant differences in burrowing activity on Day 2 between the dose treatment groups (F (3, 31) = .002, p > .05). However, there was a significant interaction between depression and drug treatment for burrowing behavior (F (3, 31) = 4.93, p < .05). The 5 and 10 mg/kg doses of S-ketamine appeared to decrease burrowing behavior on Day 2 post-injury, relative to baseline, in the depressed subjects whereas these doses increased burrowing behavior in the non-depressed subjects.

There were no effects of dose on open field activity on Day 2 post-injury (F (3, 31) = 1.52, p > .05). However, there were significant differences between depressed and non-depressed subjects at this timepoint (F (1, 31) = 5.76, p = .023). Depressed subjects displayed lower open field activity across all dose treatments relative to non-depressed subjects (Fig. 11C).

Neither drug dose nor depression significantly affected social behavior on Day 2 post injury (drug dose, F (3, 31) = 2.58, p = .077; depression, F (3, 31) = 0.518, p > .05). However, as shown in Fig. 11D, subjects that received 20 mg/kg of S-ketamine displayed comparatively more social behavior than the other dose groups. Planned comparisons confirmed that social behavior in the 20 mg/kg groups was significantly higher than that displayed by the 0 and 10 mg/kg dose groups (p< .05). Although not biologically active at 24 hrs post administration, the 20 mg/kg dose of S-ketamine appears to have protracted effects on depression; 20 mg/kg S-ketamine increased social interactions in the depressed cohort relative to all other groups.

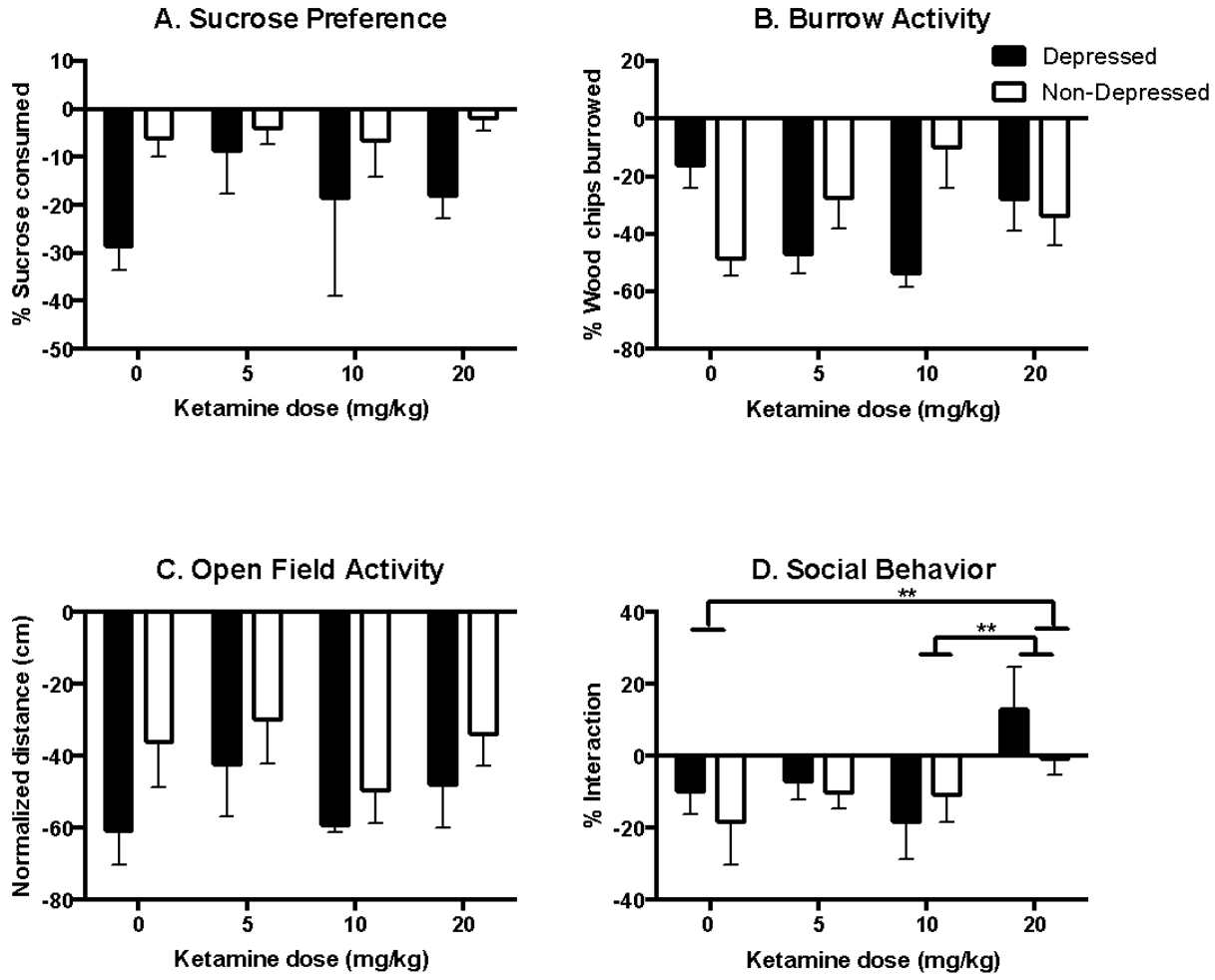


Figure 11. S-ketamine (20 mg/kg) increased social behavior on Day 2 post injury. Twenty-four hours after administration, ketamine appears to decrease the effects of depression on sucrose preference (A) and the 20 mg/kg dose reversed effects on the social interaction task. None of the doses of S-ketamine tested affected burrowing (B) or open field activity (C). ** $p < .05$

CHAPTER IV

CONCLUSION

.Based on the battery of tests performed and subsequent statistical analysis, we found that 41% of our subjects were depressed. Depression-like behavior was associated with lower average burrowing activity, decreased sucrose preference and decreased open-field activity on days 9-10 and 19-20 post-SCI. The administration of S-ketamine initially reversed symptoms of depression. Analyzing data collected on day 2 post-injury, and within the window for S-ketamine's efficacy, we found that 20 mg/kg S-ketamine increased social behavior, relative to all other dose groups. Importantly, depressed subjects treated with 20 mg/kg S-ketamine showed the highest level of social interaction 24 hours after administration. These data suggest that S-ketamine may be an effective antidepressant after SCI.

The incidence of depression found in our study is commensurate with that reported for the clinical SCI population. Although only 11-24% of patients are diagnosed with MDD after injury (Krause et al., 2000), 37 % of patients display significant symptoms of depression after SCI without meeting the criteria for MDD (Migliorini et al., 2009). In our study, 13 of the 32 subjects displayed characteristics of depression on some, but not all, tasks. Characterization of depression in this study therefore is more akin to the patients that have depression symptoms but are not diagnosed with MDD. Further, other SCI studies conducted in rodent models have shown a 35% depression rate, similar to ours (Luedtke et al., 2014). Unfortunately, S-ketamine did not affect the development of depression-like behaviors. As shown in Fig.9 the incidence of depression was

similar across the four S-ketamine dose groups on Days 9-21. Even though the literature suggests that the effects of a single dose of ketamine post SCI are long-lasting (Young, 2013; Salvadore and Singh, 2013) the effectiveness of S-ketamine would not have persisted over 21 days. Indeed, the established terminal elimination half-life of ketamine is 100-200 minutes (Niciu et al., 2014), therefore it would not have been biologically active at these later stages of injury. Our data, therefore, suggest that S-ketamine affects the expression rather than the development of depression.

Indeed, in our study S-ketamine did reverse symptoms of depression 24 hours after drug administration, which was evident in the social behavior task. This finding is significant, as social behavior is the most salient symptom for the human population. Studies conducted in the human population have shown that at 24 hours post administration, a single dose of ketamine produces an antidepressant response rate of 64%, compared to 28% in controls treated with the anesthetic midazolam (Murrough et al., 2013). These antidepressant effects appear to subside by Day 7 (Murrough et al., 2013). Notably, however, Murrough (2013) also reported some adverse side effects with ketamine administration, including dizziness, blurred vision, headache, attention deficits, and poor coordination. Furthermore, 17% of the patients experienced significant dissociative symptoms immediately after infusion (Murrough, 2013). Another study tested the effects of repeated infusions of ketamine, and showed that the response duration was much greater than that of a single infusion (aan het Rot et al., 2010). This study only involved patients that had positively responded to single infusions of ketamine in the past, however, and was severely limited by a small sample size (n=9). In addition, the duration of effective remission of

symptoms varied across patients; ranging from a relapse for less than 1 week post-treatment, to remaining symptom-free for more than 3 months (aan het Rot et al., 2010). Further experiments need to be conducted to determine the long-term efficacy of S-ketamine with repeated infusions, in addition to establishing the dose of S-ketamine that is safe and has limited side effects. Importantly, S-ketamine had no effects on locomotor recovery, sensory function or long-term neuropathic pain in our study. In fact, studies have shown that S-ketamine administered in the acute phase of SCI exerts neuroprotective effects in rats, by reducing levels of tumor necrosis factor α (TNF- α), interleukin-6 (IL-6), spinal cord malondialdehyde (MDA), and TUNEL-positive cells (Kose et al., 2013). This is in contrast to, currently prescribed selective serotonin reuptake inhibitors (SSRI) that have side effects that can include attenuation of functional recovery, as well as a paradoxical potentiation of symptoms of depression (Coyle et al. 2015). It is important to develop treatment strategies that reduce symptoms of depression without compromising functional recovery for SCI. S-ketamine may be a viable treatment option.

S-ketamine also appeared to be analgesic while biologically active. A clinical study looking at neuropathic pain below the level of spinal cord injury reported that 50% of patients responded positively to S-ketamine treatment, compared to 0% in a placebo group and 10% in a lidocaine-treated group (Kvarnström et al., 2004). In addition, S-ketamine administration did not change temperature thresholds or affect sensory function (Kvarnström et al., 2004). In our study, depression also reduced pain in the early phase of injury. Specifically, the 20 mg/kg dose reduced evoked pain on the thermal and tactile tasks for up to 180 minutes. For chronic pain, whereas non-depressed subjects appeared to develop mechanical sensitivity across the days post injury (with a decrease in mechanical thresholds), depressed subjects showed no change in motor or

vocal thresholds. These effects run counter to most empirical studies. Pain is usually comorbid with depression (Korff and Simson, 1996). Further data is needed to conclusively determine the relationship between pain, depression and S-ketamine in our rodent model.

Our data suggest that S-ketamine may be an effective antidepressant with limited side-effects after SCI. Further studies are warranted, investigating the effects of multiple infusions and alternate routes of administration (i.e. intravenous to emulate the clinical setting more closely). The results are promising. This alternative antidepressant may be effective for treating depression not only after SCI, but also for the10-30% of depressed patients that do not achieve remission with available antidepressants (Rush et al. 2006; Mathew & Charney, 2009; Holtzheimer & Mayberg, 2010).

REFERENCES

aan het Rot, M., Collins, K. A., Murrough, J. W., Perez, A. M., Reich, D. L., Charney, D. S., & Mathew, S. J. (2010). Safety and efficacy of repeated-dose intravenous ketamine for treatment-resistant depression. *Biological psychiatry*, *67*(2), 139-145.

aan het Rot, M., Hogenelst, K., & Schoevers, R. A. (2012). Mood disorders in everyday life: A systematic review of experience sampling and ecological momentary assessment studies. *Clinical psychology review*, *32*(6), 510-523.

Al Taweel, W., & Seyam, R. (2015). Neurogenic bladder in spinal cord injury patients. *Research and reports in urology*, *7*, 85.

Anisman, H. (2009). Cascading effects of stressors and inflammatory immune system activation: implications for major depressive disorder.*Journal of psychiatry & neuroscience: JPN*, *34*(1),

APA. (1994). Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV). American Psychiatric Association: Arlington, VA

Cao, Y., Massaro, J. F., Krause, J. S., Chen, Y., & Devivo, M. J. (2014). Suicide mortality after spinal cord injury in the United States: injury cohorts analysis. *Archives of physical medicine and rehabilitation*, *95*(2), 230-235.

Coyle, C. M., & Laws, K. R. (2015). The use of ketamine as an antidepressant: a systematic review and meta-analysis. *Human Psychopharmacology: Clinical and Experimental*, *30*(3), 152-163.

Deacon, R. M. (2006). Burrowing in rodents: a sensitive method for detecting behavioral dysfunction. *NATURE PROTOCOLS-ELECTRONIC EDITION-*, *1*(1), 118.

Fromm, L., Heath, D. L., Vink, R., & Nimmo, A. J. (2004). Magnesium attenuates post-traumatic depression/anxiety following diffuse traumatic brain injury in rats. *Journal of the American College of Nutrition*, *23*(5), 529S-533S.

Fullerton D.T., Harvey R.F., Klein M.H., and Howell T. (1981). Psychiatric disorders in patients with spinal cord injuries. Arch. Gen. Psychiatry 38, 1369–1371

Hilakivi-Clarke, L.A., Wozniak, K.M., Durcan, M.J, and Linnoila, M. (1990). Behavior of streptozotocin-diabetic mice in tests of exploration, locomotion, anxiety, depression and aggression. Physiol. Behav. 48, 429-433.

Holtzheimer, P.E. and Mayberg, H.S. (2010). Deep brain stimulation for treatment-resistant depression. *Am J Psychiatry*. 167(12): 1437-44.

Hook, M. A., Moreno, G., Woller, S., Puga, D., Hoy Jr, K., Balden, R., & Grau, J. W. (2009).

Intrathecal morphine attenuates recovery of function after a spinal cord injury. *Journal of neurotrauma*, *26*(5), 741-752.

Itier, J. M., Ibáñez, P., Mena, M. A., Abbas, N., Cohen-Salmon, C., Bohme, G. A., ... & Ret, G. (2003). Parkin gene inactivation alters behaviour and dopamine neurotransmission in the mouse. *Human molecular genetics*,*12*(18), 2277-2291.

Jones, N. C., Salzberg, M. R., Kumar, G., Couper, A., Morris, M. J., & O'Brien, T. J. (2008). Elevated anxiety and depressive-like behavior in a rat model of genetic generalized epilepsy suggesting common causation.*Experimental neurology*, *209*(1), 254-260.

Krause, J. S., Kemp, B., & Coker, J. (2000). Depression after spinal cord injury: relation to gender, ethnicity, aging, and socioeconomic indicators.*Archives of Physical Medicine and Rehabilitation*, *81*(8), 1099-1109.

Kvarnström, A., Karlsten, R., Quiding, H., & Gordh, T. (2004). The analgesic effect of intravenous ketamine and lidocaine on pain after spinal cord injury.*Acta anaesthesiologica scandinavica*, *48*(4), 498-506.

Lamkin, D. M., Lutgendorf, S. K., Lubaroff, D., Sood, A. K., Beltz, T. G., & Johnson, A. K. (2011). Cancer induces inflammation and depressive-like behavior in the mouse: modulation by social housing. *Brain, behavior, and immunity*, *25*(3), 555-564.

Lu, P., Woodruff, G., Wang, Y., Graham, L., Hunt, M., Wu, D., ... & Goldstein, L. S. (2014). Long-distance axonal growth from human induced pluripotent stem cells after spinal cord injury. *Neuron*, *83*(4), 789-796.

Luedtke, K., Bouchard, S., Woller, S., Funk, M., & Hook, M. (2014). Assessment of depression in a rodent model of spinal cord injury. Journal of Neurotrauma, 31(12), 1107-1121.

Maldonado-Bouchard, S., Peters, K., Woller, S. A., Madahian, B., Faghihi, U., Patel, S., ... & Hook, M. A. (2016). Inflammation is increased with anxiety-and depression-like signs in a rat model of spinal cord injury. *Brain, behavior, and immunity*, *51*, 176-195.

Mathew, S.J. and Charney, D.S. (2009). Publication bias and the efficacy of antidepressants. *Am J Psychiatry*. 166(2): 140-45.

McKibben, C. E., Reynolds, G. P., & Jenkins, T. A. (2014). Analysis of sociability and preference for social novelty in the acute and subchronic phencyclidine rat. *Journal of Psychopharmacology*, 0269881114544778.

McKinney W.T., Jr., and Bunney W.E., Jr. (1969). Animal model of depression. I. Review of evidence: implications for research. Arch. Gen. Psychiatry 21, 240–248

Migliorini C.E., New P.W., and Tonge B.J. (2009). Comparison of depression, anxiety and stress in persons with traumatic and non-traumatic post-acute spinal cord injury. Spinal Cord 47, 783–788

Minkoff K., Bergman E., Beck A.T., and Beck R. (1973). Hopelessness, depression, and attempted suicide. Am. J. Psychiatry 130, 455–459

Morgan, C. J., & Curran, H. V. (2012). Ketamine use: a review. *Addiction*,*107*(1), 27-38.

Muller, J., Pentyala, S., Dilger, J., & Pentyala, S. (2016). Ketamine enantiomers in the rapid and sustained antidepressant effects. *Therapeutic Advances in Psychopharmacology*, 2045125316631267.

Murrough, J. W., Iosifescu, D. V., Chang, L. C., Al Jurdi, R. K., Green, C. E., Perez, A. M., ... & Charney, D. S. (2013). Antidepressant efficacy of ketamine in treatment-resistant major depression: a two-site randomized controlled trial. *American Journal of Psychiatry*.

Nicholson, B. (2009). Benefits of Extended-Release Opioid Analgesic Formulations in the Treatment of Chronic Pain. *Pain Practice*, *9*(1), 71-81.

Muetzelfeldt, L., Kamboj, S. K., Rees, H., Taylor, J., Morgan, C. J. A., & Curran, H. V. (2008). Journey through the K-hole: phenomenological aspects of ketamine use. *Drug and alcohol dependence*, *95*(3), 219-229.

Niciu, M. J., Henter, I. D., Luckenbaugh, D. A., Zarate Jr, C. A., & Charney, D. S. (2014). Glutamate receptor antagonists as fast-acting therapeutic alternatives for the treatment of depression: ketamine and other compounds. *Annual review of pharmacology and toxicology*, *54*, 119.

Niesters, M., Martini, C., & Dahan, A. (2014). Ketamine for chronic pain: risks and benefits. *British journal of clinical pharmacology*, *77*(2), 357-367.

Overstreet, D. H. (2012). Modeling depression in animal models. *Psychiatric Disorders: Methods and Protocols*, 125-144.

Paul, R., Schaaff, N., Padberg, F., Möller, H. J., & Frodl, T. (2009). Comparison of racemic ketamine and S-ketamine in treatment-resistant major depression: report of two cases. *The World Journal of Biological Psychiatry*, *10*(3), 241-244.

Pyter, L.M., Pineros, V., Galang, J.A., McClintock, M.K., and Prendergast, B.J. (2009). Peripheral tumors induce depressive-like behaviors and cytokine production and alter hypothalamic-pituitary-adrenal axis regulation. Proc Natl Acad Sci USA 22, 9069-9074.

Rush, A.J., Trivedi, M.H., Wisniewski, S.R., Nierenberg, A.A., Stewart, J.W., Warden, D., Niederehe, G., Thase, M.E., Lavori, P.W., Lebowitz, B.D., McGrath, P.J., Rosenbaum, J.F., Sackeim, H.A., Kupfer, D.J., Luther, J., and Fava, M. (2006). Acute and longer-term outcomes in

depressed outpatients requiring one or several treatment steps: a STAR*D report. Am J Psychiatry. 163(11):1905-17.

Restall, J., Tully, A. M., Ward, P. J., & Kidd, A. G. (1988). Total intravenous anaesthesia for military surgery. A technique using ketamine, midazolam and vecuronium. *Anaesthesia*, *43*(1), 46-49.

Swain, M.G. and Le, T. (1998). Chronic cholestasis in rats induces anhedonia and a loss of social interest. Hepatology 28, 6-10.

Salvadore, G., & Singh, J. B. (2013). Ketamine as a fast acting antidepressant: current knowledge and open questions. *CNS neuroscience & therapeutics*, *19*(6), 428-436.

Siddall, P. J., & Middleton, J. W. (2015). Spinal cord injury-induced pain: mechanisms and treatments. *Pain Management*, *5*(6), 493-507.

Spinal Cord Injury (SCI) Facts and Figures at a Glance. Retrieved from https://www.nscisc.uab.edu/Public/Facts%202015.pdf

Tabachnick, B. G., and Linda, F. S. (2007). *Principal Components and Factor Analysis Using Multivariate Statistics (Fifth ed*., pp. 649). Boston: Pearson Education Inc.

Tam, E., & Furlan, A. D. (2012). Transdermal lidocaine and ketamine for neuropathic pain: a study of effectiveness and tolerability. *The open neurology journal*, *6*(1).

Wang, Y., & Manis, P. B. (2008). Short-term synaptic depression and recovery at the mature mammalian endbulb of Held synapse in mice. *Journal of neurophysiology*, *100*(3), 1255-1264.

Weeks, D. L., Greer, C. L., Bray, B. S., Schwartz, C. R., & White, J. R. (2011). Association of antidepressant medication therapy with inpatient rehabilitation outcomes for stroke, traumatic brain injury, or traumatic spinal cord injury. *Archives of physical medicine and rehabilitation*, *92*(5), 683-695.

Young, S. N. (2013). Single treatments that have lasting effects: some thoughts on the antidepressant effects of ketamine and botulinum toxin and the anxiolytic effect of psilocybin. *J Psychiatry Neurosci*, *38*(2), 78-83.

Zhao, J., Mou, Y., Bernstock, J. D., Klimanis, D., Wang, S., Spatz, M., ... & Li, X. (2015). Synthetic Oligodeoxynucleotides Containing Multiple Telemeric TTAGGG Motifs Suppress Inflammasome Activity in Macrophages Subjected to Oxygen and Glucose Deprivation and Reduce Ischemic Brain Injury in Stroke-Prone Spontaneously Hypertensive Rats. *PloS one*, *10*(10), e0140772.

Zorumski, C. F., Nagele, P., Mennerick, S., & Conway, C. R. (2015). Treatment-resistant major depression: Rationale for NMDA receptors as targets and nitrous oxide as therapy. *Frontiers in psychiatry*, *6*.