Experimental Neurology 320 (2019) 112969

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Research Paper

Depression-like behavior corresponds with cardiac changes in a rodent model of spinal cord injury

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ARTICLE INFO

Keywords:
Depression
Spinal cord injury
Heart rate
Heart rate variability
Rodent model

ABSTRACT

In previous studies we have shown that approximately 1/3 of male Sprague Dawley rats develop symptoms of depression following a spinal cord injury (SCI). Using established behavioral tests to measure depression in rodents, we found that after SCI, subjects characterized as depressed had decreased sucrose preference, open field activity, social exploration, and burrowing behavior. As some of these tests of depression could be affected by the compromised motor function inherent to the SCI condition, the current study examined whether non-subjective, physiological differences in heart rate and heart rate variability were also associated with depression, as seen in humans. Male Sprague Dawley rats were implanted with radiotelemetry devices and either received a moderate contusion injury or remained intact. The implanted telemetry devices recorded home cage activity, body temperature, heart rate, and heart rate variability for 5 min/h throughout a 30-day post-injury assessment period. Depression behavior was evaluated using a battery of tests conducted on days 9–10 and 19–20 post-injury. Locomotor recovery and pain reactivity were also examined. Hierarchical clustering, based on the behavioral scores collected on the tests of depression, revealed that 28% of the SCI subjects displayed symptoms of depression, relative to the remaining 72% of SCI subjects. The subjects characterized as depressed had significantly lower social interaction and burrowing activity than the group that was not depressed. Interestingly, the subjects behaviorally characterized as depressed also had significantly lower heart rate variability than the not-depressed intact group. There was no difference between not-depressed SCI and intact rats on this measure. Therefore, in addition to behavior, depressed and not-depressed rats differ on measures of physiological function that are associated with depression in humans. These physiological differences further validate the rodent model of depression after SCI.

1. Introduction

In addition to effects on physical and physiological function, spinal cord injury (SCI) significantly impacts psychological wellbeing. Compared to the rate of 8.6% in the general population (Kessler et al., 2005; Kessler et al., 2012), epidemiological studies report that the incidence of major depressive disorder (MDD) following SCI ranges from 11 to 24% (Krause et al., 2000), with an additional 16–34% of SCI patients reporting clinical symptoms of depression without meeting the criteria for MDD (Bombardier et al., 2012; Krause et al., 2000; Migliorini et al., 2009). Commensurate with the incidence of depression, suicide risk and ideation following SCI is estimated to be 3 or more times greater than in the general population (Cao et al., 2014; DeVivo et al., 1991; Soden et al., 2000), with depression constituting a significant risk factor (McCullumsmith et al., 2015).

Depression after SCI not only affects psychological wellbeing, it also affects physical function. The fatigue and lack of motivation associated with depression significantly reduce adherence to rehabilitation protocols, and, as such, depression is associated with negative long-term complications in recovery (Elliott and Frank, 1996; Herrick et al., 1994; Malec and Neimeyer, 1983) and lower functional independence (Abdul-Sattar, 2014; Kennedy et al., 2011). Urinary tract infections, pressure ulcers, and autonomic dysreflexia are also more common in depressed SCI patients (Krueger et al., 2013), and depression can result in

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https://doi.org/10.1016/j.expneurol.2019.112969
Received 25 January 2019; Received in revised form 8 May 2019; Accepted 30 May 2019
Available online 31 May 2019
0014-4886/ Published by Elsevier Inc.

unhealthy weight deviations, anxiety, insomnia, and substance abuse. Additionally, in the clinical population, patients with depression often have higher heart rates and lower heart rate variability (Brown et al., 2018; Chang et al., 2017; Paniccia et al., 2017; Schiweck et al., 2018) that, when chronic, can result in cardiovascular problems such as heart attacks, stroke, and coronary artery disease (Agelink et al., 2002; Carney et al., 2005; Rugulies, 2002). Spinally injured patients are already at a higher risk for cardiovascular disease than the general population (Myers et al., 2007). Depression may compound the risk for cardiovascular problems.

Unfortunately, there is no single, effective treatment for depression. Patients diagnosed with MDD are faced with a trial-and-error approach in treatment, where 2–3 treatments are often trialed before achieving remission from depression symptoms (Rush et al., 2006). Further, 10–30% of treated depressed patients will not achieve remission with any of the available antidepressants (Holtzheimer III and Mayberg, 2010; Rush et al., 2006). Despite this unmet need, there have been very few studies of depression in animal models of SCI. The studies that have been conducted (Luedtke et al., 2014; Maldonado-Bouchard et al., 2016; Wu et al., 2016; Wu et al., 2014; Zhao et al., 2014) indicate that depression develops in rodents after SCI despite the absence of psychosocial factors, such as loss of independence, which also contribute to the development of depression in humans. However, it is also recognized that, as in humans, motor function deficits and fatigue associated with SCI may confound characterization of depression in animal models (Wu et al., 2016; Wu et al., 2014). Decreased heart rate variability has been associated with stress and depression in animal models (Grippo et al., 2012; Grippo et al., 2003; Hildreth et al., 2008). To further validate the characterization of depression in SCI rodents, the current study tested whether subjects behaviorally characterized as depressed also displayed changes in heart rate and heart rate variability.

Radiotelemetric devices, that have already proved to be effective tools for monitoring autonomic dysfunction in multiple animal models of SCI (Inskip et al., 2009), were used to monitor heart rate, heart rate variability, home cage activity, and body temperature in the rodent model of spinal cord injury. As described for humans, rats that were behaviorally characterized as depressed after SCI had decreased heart rate variability (HRV) compared to not-depressed SCI and intact controls.

2. Methods

2.1. Subjects

Thirty-three ($n = 25$ contused and $n = 8$ intact) male Sprague Dawley rats (Harlan, Houston, TX) between 90 and 110 days old (300-350 g) were single-housed in plexiglass cages (45.7 (length) x 23.5 (width) x 20.3 (height) cm) with food and water available ad libitum. Food consumption and body weights were recorded daily. Subjects that received the contusion surgery lost bladder function and were manually expressed daily in the morning (7:00–9:30) and evening (16:30–18:00) until an empty bladder was observed for 3 consecutive days. A 12-h light/dark cycle was maintained, with behavioral testing conducted during the light cycle. All of the experiments were reviewed and approved by the Institutional Animal Care and Use Committee at Texas A &M University and all NIH guidelines for the care and use of animal subjects were followed.

2.2. Surgery

2.2.1. Telemetry implantation

Radiotelemetric transmitters (model TA11CTA-F40; Data Sciences International, St Paul, MN) were used to track changes in home cage activity, abdominal temperature, heart rate, and heart rate variability prior to and after the spinal contusion injury. For implantation of the devices, subjects were anesthetized with 5% isoflurane gas. When subjects reached a stable level of anesthesia, the isoflurane gas concentration was lowered to 2–3%, and the surgical fields (right lateral flank, right pectoral, and left lower rib) were shaved and cleaned with iodine and isopropyl alcohol. A 4 cm incision was made through the skin, the abdominal muscle, and the peritoneum to implant the transmitter. Inside the peritoneal space, the transmitter was stitched to the abdominal muscles. The 2 electrodes used to record the electrocardiogram (ECG) were placed under the skin and firmly anchored over the right pectoral muscle and the left caudal rib region. The two cutaneous incisions allowing placement of the ECG electrodes were closed with surgical adhesive and the larger incision with Michel clips. Following surgery, subjects were administered 100,000 units/kg Pfizerpen (penicillin G potassium), Meloxicam (2 mg/kg) for pain, and filtered saline (3 ml, 0.9%) to replenish fluids loss. Subjects were returned to their home cages and the transmitters were activated to begin sampling. Telemetry sampling occurred daily for the first five minutes of every hour throughout the postoperative recovery period. Michel clips were removed 14 days after surgery.

2.2.2. Spinal contusion

Seven days after implantation of the telemetry devices, subjects received a moderate contusion injury at the T12 vertebral level using the Infinite Horizon (IH) spinal cord impactor (PSI, Fairfax Station, VA). Subjects were anesthetized with 5% isoflurane 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 isopropyl alcohol, 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. To compensate for fluid loss, subjects were also given 3 ml of saline after surgery. For the first 24 h after surgery, rats were placed in a recovery room maintained at 26.6 °C. Michel clips were removed 14 days after surgery.

2.3. Behavioral assessment of depression

2.3.1. Behavioral timeline

Depression was assessed with a variety of established tests thought to evaluate symptoms of depression in rats that are analogous to those observed in human patients (Table 1). As shown in Fig. 1, baseline scores were obtained prior to the spinal contusion injury (both before and after the telemetry devices were implanted). Following injury, depression behaviors were re-assessed on days 9–11 and 19–22 (Fig. 1). All testing took place during the light cycle. At the end of the

Table 1
Clinical symptoms associated with rodent depressive behaviors.

Behavioral test	Clinical symptom
Sucrose Preference Test	Loss of interest or pleasure (anhedonia)
Open Field Activity	Psychomotor retardation
Burrowing	Fatigue or loss of energy
Social Activity	Loss of interest or pleasure (anhedonia)

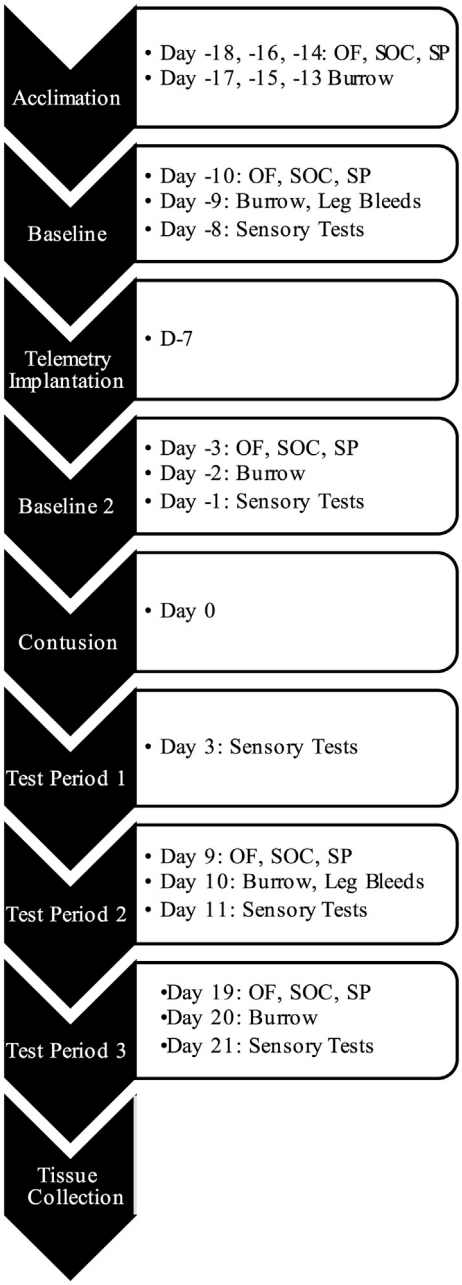

Fig. 1. Experimental timeline. All tests are listed in the order performed on their respective days. OF: Open field activity, SOC: Social activity, SP: Sucrose preference test.

assessment period, subjects received a lethal injection of pentobarbitol (100 mg/kg, i.p.) and the injured spinal cord (1 cm section centered on the lesion) was collected.

2.3.2. Sucrose preference test

Sucrose preference tests were conducted in the subject's home cage between 14:00 and 16:00 h. For testing, one pre-weighed water bottle filled with approximately 250 ml of a 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 h. 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-h period, to prevent any positional biases from confounding results. At the end of the 2-h test period, the change in the weight of each bottle was determined.

Sucrose preference was calculated using the following formula: SP = sucrose solution intake (g) / [sucrose solution intake (g) + water intake (g)], with lower sucrose preference values being indicative of depression. Baseline sucrose preference levels were assessed three days prior to implantation and injury. Following injury, sucrose preference was assessed on days 9 and 19.

2.3.3. Open field activity

The open field test was conducted between 09:00 and 11:00 h in a black plywood box [90 (length) x 60 (width) x 20 (height) cm]. The box was partitioned into 54 squares [10 (length) x 10 (width) cm] delineated with a silver marker. A layer of clear plexiglass was used to cover the top of the box. Subjects were acclimated to the dark testing room for 10 min prior to testing. The subject was placed in the center of the box at the start of testing, and the 5-min test session was video recorded from above.

The number of squares that the subject moved into, operationalized as having at least the front two paws and the head in the square, was scored from post hoc video analysis. Total number of squares crossed was divided by 300 s to obtain a squares-per-second value, with lower values being indicative of depression. Baseline activity was measured 3 days prior to both the implantation of the telemetry device and the injury. Open field activity was assessed 9 and 19 days post-injury.

2.3.4. Social exploration

Social exploration was assessed immediately after open field activity, between 09:00 and 11:00 h. A subject was placed in the center of the open field described above and allowed to explore for 5 min. A single-housed, age and weight-matched, conspecific, that had not been exposed to any experimental treatment, was then placed in the open field as far from the test subject as possible. The subject and the novel rat were video recorded from above for 5 min. From post hoc video analysis, the time that the experimental subject spent performing social behavior (physical contact, angiogenital sniffing, and close pursuit of the novel rat) was recorded and divided by 300 s to derive % social exploration, with lower percentages being indicative of depression. Baseline activity was measured 3 days prior to implantation and injury. Social exploration was re-assessed on days 9 and 19 post-injury, using the same conspecific.

2.3.5. Burrowing

Burrowing is a natural activity that rodents will initiate even when unnecessary, indicating that they find it rewarding (Deacon, 2006). It is decreased when the hippocampus or frontal cortex is lesioned and under a variety of stressful conditions and sicknesses, and has been validated as a measure of anxiety and sickness behavior (Deacon, 2006). Our burrowing apparatus was a polyvinyl chloride (PVC) tube [45 (length) x 15 (diameter) cm] closed on one end and filled with aspen wood chips. For testing, pre-weighed burrowing tubes were filled with 500 g of wood chips and placed in the subject's cage between 12:00 and 14:00 h. After 2 h, the tubes and the wood chips remaining in the tube were weighed.

The change in weight of the tube, along with its initial empty weight, was used to determine the percentage of woodchips burrowed out, with lower scores being indicative of depression. Baseline burrowing scores were collected 2 days prior to implantation of the telemetry devices and injury. After injury, burrowing was assessed on days 10 and 20 post-injury.

2.4. Behavioral assessment of recovery

2.4.1. Locomotor recovery

The recovery of hindlimb function was scored using the Basso, Beattie, and Bresnahan (BBB) scale (Basso et al., 1995). Subjects were acclimated to a circular open field enclosure (99 cm diameter, 23 cm deep) and allowed to move freely in it for 5 min on 3 consecutive days

prior to surgery. All subjects stepped normally (BBB = 21) prior to SCI. After injury, the locomotor capacity (BBB) of subjects was observed for 5 min and scored by a trained observer on days 1–7, 9, 11, 13, 15, 18, and 21 post-SCI. Care was taken to ensure that all investigators' scoring behavior had high intra- and inter- observer reliability (all r's > 0.89). 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, and scores 14–21, which are very seldom used under the present injury parameters. By pooling these scores, an ordered scale was created that is relatively continuous, with units of approximately equivalent interval spacing. Meeting these criteria allows for application of metric operations (computation of mean performance across legs), improves the justification for parametric statistical analyses, and increases statistical power.

2.4.2. Thermal nociceptive reactivity

Thermal reactivity was assessed with the tail flick test. Subjects were restrained in clear plexiglass tubes (7.00 cm [internal diameter] × 20.00 cm [length]) and placed on the tail-flick apparatus (IITC Inc., Life Science, CA). Their tails were positioned in the 0.5 cm deep groove on the apparatus so that the area 1.5 in. from the tip of the tail was directly under a thermal light source. Subjects were allowed to acclimate to the apparatus for 15 min. Prior to testing, the temperature of the focused light was set to elicit a baseline tail-flick response in 3–4 s (average) in intact subjects. This pre-set temperature was maintained across all subjects. In testing, the latency to flick the tail away from the radiant heat source 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 test. No false alarms were recorded.

2.4.3. Mechanical nociceptive reactivity

Subjects were placed back into the restraining tubes and, after a 15 min acclimation, mechanical reactivity was assessed using von Frey stimulation. In this test, nylon monofilaments (Semmes–Weinstein Anesthesiometer, Stoelting Co., Chicago, IL) of increasing strength were applied sequentially at approximately 2 s intervals to the L5 dermatome on the plantar surface of the hind paws. Each subject was tested twice on each foot in a counterbalanced ABBA order. The stimulus was presented until subjects exhibited a motor withdrawal (spinal) and vocal (supraspinal) 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 no response was observed with 300 g of force, the experiment was terminated and this maximum force used was reported.

2.4.4. Girdle test

At-level allodynia was also assessed using the girdle test. To ensure that the rats remained calm for testing, they were handled for 5 min immediately before beginning the girdle test. In testing, a von Frey hair with a bending force of 204.14 mN (26 g force) was applied to a 4 × 11 grid across the girdle region of each subject. Because animals do not normally vocalize with this stimulus, a vocalization response indicates that a noxious stimulus was experienced. Vocalization responses were recorded and mapped onto a grid map for each animal. In mapping the area of response, the number of vocalizations are recorded (Nv) and normalized by the following formula: (Nv · 100)/total number of applications (44), indicating the percent vocalizations out of the total number of applications.

2.5. Radiotelemetric acquisition

Measures of home cage activity (arbitrary units, AU), body temperature (° C), heart rate (HRT, beats per minute, bpm), and heart rate variability (HRV, SD/IBI) were recorded from telemetry devices using DSI Data Exchange Matrices (Data Sciences International). Data were transmitted using radio frequency waves at 455 kHz and stored on a computer using DataQuest ART 4.3 (Data Sciences International) software. Waveform sampling was scheduled to occur automatically every hour for 5 min at an acquisition rate of 1 kHz for each animal. Heart rate variability (HRV) was obtained from a time domain analysis. A coefficient of variability was calculated by the software and expressed as a ratio of SD/IBI, where SD is the standard deviation of the inter-beat intervals (IBI), and IBI is the average time distance in milliseconds between consecutive heartbeats observed over the 5 min sampling period. After acquisition, the hourly data collected were averaged to obtain daily values and these were exported to Excel files for further statistical analyses.

2.6. Lesion analysis

At the end of the assessment period, subjects were anesthetized with pentobarbital (100 mg/kg, i.p.) and perfused (intracardially) with phosphate-buffered saline (1 × PBS) followed by 4% paraformaldehyde (PFA). A 1 cm section of the spinal cord, centered over the injury site, was collected. The spinal cord was post-fixed in 4% PFA at 4 °C for 48 h after collection, then transferred to a 30% sucrose solution, and stored at 4 °C until sectioning and tissue analysis.

Histological analyses were conducted to examine spinal lesion size and extent. Spinal cord segments were sectioned from the rostral to the caudal end in 20 μm thick sections, and every 10th slice was preserved for staining. All sections were stained with cresyl violet for Nissl substance and luxol fast blue for myelin. The total cross-sectional area of the cord and spared tissue was assessed at the lesion center using MicroBrightField software (MBF Bioscience, Williston, VT). Assessments were made by an experimenter who was blind to the subject's treatment condition. Four indices of lesion magnitude were derived: damage (lesion); residual gray matter (GM); residual white matter (WM); and width. To determine the area of damage, an experimenter blind to the experimental treatments traced around the boundaries of cystic formations and areas of dense gliosis. Nissl-stained areas that contained neurons and glia of approximately normal densities denoted residual GM. WM was judged spared in myelin-stained areas lacking dense gliosis or swollen fibers. The total area of each cross-section was derived by summing the areas of damage, GM, and WM. Width was determined from the most lateral points along the transverse plane. To control for variability in section area across subjects, we applied a correction factor derived from standard undamaged cord sections, taken from age-matched controls (Grau et al., 2014). This correction factor is based on section widths and is multiplied by all area measurements to standardize area across analyses. By standardizing area across sections, we were able to estimate the degree to which tissue was missing (i.e. tissue loss from atrophy, necrosis, or apoptosis). An accurate assessment of the degree to which a cord has been affected includes both the remaining damaged tissue as well as resolved lesioned areas. When we sum the amount of missing tissue and the measured damaged area, we derive an index of the relative lesion (percent relative lesion) in each section that is comparable across sections. We also compute the relative percent of GM and WM remaining in each section, relative to intact controls.

2.7. Statistical analysis

To identify changes in depressive behavior caused by SCI, we first calculated change from baseline scores. For each of the behavioral tests, scores collected on Days 9 and 19 were subtracted from the baseline

scores collected after telemetry device implantation, and immediately prior to the spinal contusion injury (Baseline 2). A hierarchical cluster analysis (HCA) was then performed to group subjects into cohorts based on their change from baseline scores (average of days 9 and 19) across tasks (sucrose preference, burrowing, open field activity, and social activity). As described in Luedtke et al. (2014), the HCA was performed using Ward's method and applying squared Euclidean distance as the distance measure. The number of appropriate clusters 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. After identifying the number of clusters depicted in the dendrogram, the HCA was repeated using the same parameters but requesting a single solution of two clusters. A new variable, cluster membership, was generated for all subjects.

Analysis of variance (ANOVA) tests were then conducted comparing the change from baseline scores for each of the clusters identified, across behavioral tests. Based on the pattern of behaviors exhibited by each cluster, they were labeled as “depressed” and “not-depressed.” Repeated measures ANOVAs, with day as the repeated factor and group as the between subjects variable, were used to compare the converted BBB locomotor scores, and telemetric data on activity, temperature, heart rate, and heart rate variability across depressed and not-depressed groups.

3. Results

3.1. Assessment of behavioral differences

Average change from baseline scores (derived from days 9–10 and 19–20) for each of the behavioral tests were used in the hierarchical cluster analysis. The dendrogram produced by this analysis showed that the subjects separated into two clusters (Fig. 2), with 26 subjects (18 contused, 8 intact controls) in cluster 1, and 7 subjects (all contused) in cluster 2. To determine the behavioral pattern characteristic of the clusters, the two groups were compared on each of the depression measures. Based on significantly lower social exploration ($F(1,31) = 6.61$, $p = 0.015$) and burrowing ($F(1,31) = 4.89$, $p = 0.035$), subjects in cluster 2 were labeled as “depressed” and subjects in cluster 1 were labeled as “not-depressed.” Therefore, 7 of 25 contused subjects (28%) were found to exhibit depression-like symptoms. Because none of the uninjured controls clustered in the “depressed” group, all subsequent analyses compared “depressed,” “not-depressed SCI,” and “not-depressed intact” groups.

3.2. Behavioral tests

Behavioral assays of depression were assessed for all subjects and compared at testing time points pre- and post-injury. Baseline 1 measurements, collected before implantation of the telemetry recording device, did not differ among the groups on any of the behavioral tests (Fig. 3). After telemetry implantation but before SCI (Baseline 2), the depressed group decreased their sucrose preference but were similar to the other groups across all other behavioral tests (Fig. 3). The depressed group's decreased sucrose preference at Baseline 2 resulted in an artificially inflated change from baseline score in subsequent comparisons.

Indeed, there was a main effect of group on sucrose preference on both Days 9 and 19 ($F(2,30) = 7.20, 22.13$, respectively, $p < 0.005$). However, contrary to an anhedonia phenotype, the depressed group had significantly higher change from baseline scores, relative to the not-depressed SCI subjects on Day 9, and to both SCI and intact not-depressed groups at Day 19 post-injury ($p < 0.005$, Fig. 4A). Despite similar Baseline 1 sucrose preference scores prior to implantation of the telemetry devices, the depressed subjects had lower Baseline 2 sucrose preferences after implantation of the telemetry devices ($F(2,30) = 9.7$, $p = 0.001$, Fig. 3A), which inflated their subsequent change-from-baseline scores. Commensurate with a depression phenotype, there was also a main effect of group on burrowing on Day 9 post-injury ($F(2,30) = 4.72$, $p = 0.017$). The depressed group burrowed more than the not-depressed intact group at this time point (Fig. 4B). There was a significant effect of group on social exploration on Day 9 post injury ($F(2,30) = 4.87$, $p = 0.015$), but not on Day 19 ($F(2,30) = 1.89$, $p > 0.05$). Post hoc Tukey tests revealed that the depressed group spent less time engaging in social activity than the not-depressed SCI group on Day 9 post-injury ($p < 0.005$, Fig. 4D).

3.3. Assessment of locomotor recovery, pain, and general health

An ANOVA revealed a main effect of group on recovery of locomotor function ($F(2,30) = 34.02$, $p < 0.001$). Both the not-depressed and depressed SCI subjects had lower BBB scores than the intact subjects throughout the post-injury assessment period ($F = 59.29, 136.09$, respectively, $p < 0.001$). There were no differences in locomotor recovery, however, across the depressed and not-depressed SCI groups. Day 1 BBB scores did not differ, and a repeated measures ANOVA revealed no differences between groups in locomotor function across the 21-day post injury assessment period ($F(1, 23) = 1.395$, $p > 0.05$, Fig. 5).

The contusion injury also acutely increased reactivity thresholds on the girdle test of allodynia. There were no differences in girdle reactivity prior to SCI, but there was a significant difference between groups on Day 3 post injury ($F(2,30) = 4.24$, $p < 0.05$). As can be seen in Fig. 6, the not-depressed SCI subjects made significantly more vocalizations to girdle stimulation than the not-depressed intact group ($p < 0.05$). The depressed SCI group also vocalized more than the intact subjects with stimulation, but this difference was not significant. There were no significant differences between the groups on the later tests of girdle reactivity, although girdle responses remained higher in the SCI groups than intact controls (Fig. 6A).

While the contusion injury per se did not appear to influence sensory reactivity thresholds with mechanical stimulation below the level of injury, there was an effect of depression on nociceptive responses. There were no group differences for mechanical or vocal reactivity to von Frey stimulation prior to injury. On both Days 11 and 21 post injury, however, reactivity to von Frey stimulation of the hind paws differed across groups ($F(2,30) = 3.94, 3.87$, respectively, $p = 0.03$ on both days, Fig. 6B). Eleven days after injury, the depressed group had lower motor reactivity thresholds than either of the not-depressed groups ($p < 0.05$), and they reacted at lower pressures than the intact controls on day 21 post-injury ($p < 0.01$, Fig. 6B). There were no differences between the not-depressed SCI and not-depressed intact groups. There were also no differences across groups in the vocal reactivity thresholds with von Frey stimulation, or on the tail-flick test of thermal reactivity.

As in many different injury models, our spinally injured groups lost weight immediately post-injury and then gained significantly less weight than the intact controls during the recovery period ($F(2,22 = 10.13$, $p = 0.001$, Fig. 7A). While there were no overall differences in food consumption between the groups, there is a visible dip in food consumption for the first 3 days after SCI, which corresponds to the weight decrease in the SCI groups (Fig. 7B). After the first three days of recovery, the SCI groups did return to a normal rate of weight gain, but their overall weights remained lower than the intacts due to their initial weight loss. Recovery of bladder function did not differ between the depressed and not-depressed SCI rats. Twenty-eight percent (2/7) of the depressed subjects recovered bladder function within 30 days of injury, and 33.3% (6/18) of the not-depressed SCI subjects recovered bladder function.

3.4. Assessment of physiological data

Repeated measures ANOVAs revealed differences in home cage activity and heart rate across the groups ($F(2, 29) = 17.66$, $p < 0.001$; F

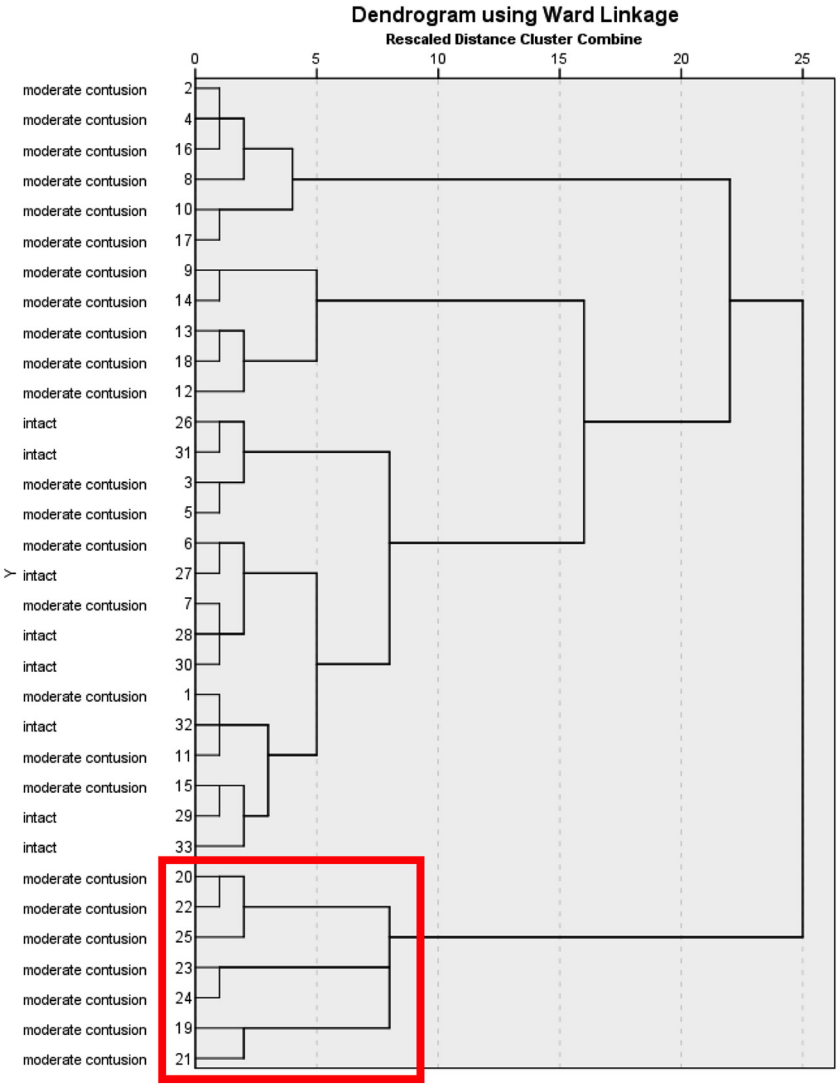

Fig. 2. 28% of SCI subjects display depression-like behavior.
Hierarchical clustering results in a dendrogram displaying linkages. Each line on the left represents one subject; each subject is coded with its injury condition. The red box contains the cluster that displayed depression-like behaviors. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(2,29) = 4.56, $p = 0.019$, respectively, Fig. 8A, B). Post hoc analyses revealed that both SCI groups had significantly lower home cage activity than the intact group ($p < 0.001$, Fig. 8B). Both the depressed and not-depressed SCI groups also had higher heart rates than the not-depressed intact group (F (1, 13) = 6.21, $p < 0.05$, F (1, 23) = 8.70, $p < 0.01$ respectively, Fig. 8A). There was no main effect of group on heart rate variability (F (2, 27) < 1.0, $p > 0.05$). However, as can be seen in Fig. 8C, the depressed SCI group had lower heart rate variability than the other two groups. Planned pairwise comparisons confirmed that heart rate variability was significantly reduced in depressed SCI subjects relative to not-depressed intact controls (F (1,13) = 7.07, $p < 0.05$). Heart rate variability did not differ significantly between not-depressed SCI and intact controls, nor between depressed and not-depressed SCI groups, though that is likely due, in part, to the dip in HRV in the not-depressed SCI group immediately after SCI. There was no effect of depression or SCI on abdominal body temperature across the recovery period (Fig. 8D).

3.5. Assessment of spinal cord injury

Percent spared gray and white matter, percent damage, and percent relative lesion (missing + damaged tissue) across a 1 cm section of spinal cord containing the lesion was assessed at the end of the study. There were no significant differences in any of the measures between the depressed and not-depressed groups.

Correlations were also performed on the histological outcomes at the center of the lesion, and measures of pain, depression behavior, and locomotor recovery. Commensurate with our previous studies and the original analysis of Basso et al. (1995), percent damage was negatively correlated with BBB scores at the end of the recovery period ($r = -0.41$, $p < 0.05$). None of the other histological outcomes correlated with pain or depression behaviors at the end of the experiment.

4. Discussion

Using a comprehensive battery of behavioral tests, we have identified depression-like symptoms in 28% of our spinally injured rats. This is consistent with the incidence of major depressive disorder in human patients after SCI, and it replicates previous studies from our laboratory (Luedtke et al., 2014; Maldonado-Bouchard et al., 2016). Importantly, also replicating our previous studies, there was no difference in locomotor recovery in depressed and not-depressed rats, and there were no

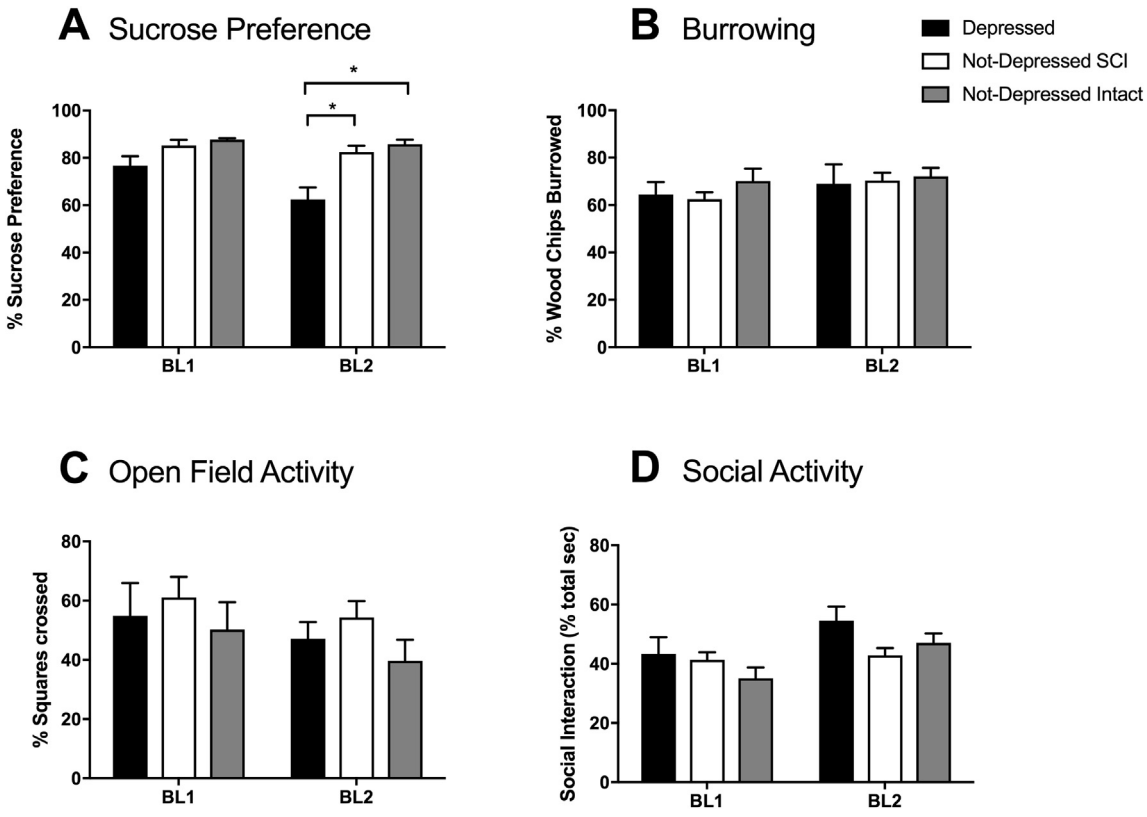

Fig. 3. Baseline behaviors did not vary among groups.
Baseline behaviors before telemetry device implantation (BL1) and after device implantation but before SCI (BL2) were measured. No differences were found between the groups that would later cluster as depressed SCI, not-depressed SCI, or not-depressed intact, except for sucrose preference at BL2. The rats that would later be the depressed SCI group had lower sucrose preference at that time point than either of the other groups. $^{*}p < 0.05$.

correlations between lesion size and depression behaviors (Luedtke et al., 2014). Instead, as found for humans, the current study showed that rats behaviorally characterized as depressed display decreased heart rate variability relative to their not-depressed conspecifics. These data not only validate our behavioral model of depression, they also suggest that heart rate variability may be a valid biomarker for vulnerability to depression and anxiety in the rodent spinal contusion model. Diagnosing depression is more difficult in both people and animals with SCI than in an able-bodied population, because many of depression's somatic symptoms (psychomotor retardation, weight changes, low energy, sleep disturbances) can also be attributed to SCI itself (Bombardier et al., 2004). Heart rate variability may be a useful addition to the behavioral assessment of depression in the clinical population.

In this experiment, we found that while our depressed cluster of SCI rats exhibited lower social activity and burrowing behavior than the not-depressed clusters, they did not differ in open field activity and, in fact, increased their sucrose preference. The depressed group's large increase in the change from baseline sucrose preference scores was likely due to their decreased sucrose preference at baseline (after telemetry device implantation and before SCI). This decreased sucrose preference after telemetry device implantation may reflect a susceptibility to depression that can be triggered by physical or psychological stressors, such as surgery. With no effects of telemetry device implantation on baseline behavior, the lowered burrowing and social activity post injury are indicative of a depression phenotype in the SCI model.

Like other researchers, we also saw that pain after injury was related to depressive outcome in both humans and animals (Cairns et al., 1996; Maldonado-Bouchard et al., 2016; Rintala et al., 1998). People with chronic pain are at a higher risk for depression than those with low pain, but recently there has been a concerted effort to find the mechanistic relationship between pain and depression (Walker et al., 2014). With increasing evidence for a role for inflammation in the development of depression, it has become apparent that inflammatory cytokines, such as IFN-α, TNF-α, and IL-1β are involved in both pathways, as well a number of neuroimmune mediators, such as indolamine dioxygenase, glutamate, and GABA (Walker et al., 2014). It is likely that pain and depression are often comorbid because they have biologically similar mechanisms.

For the physiological measures, all subjects, irrespective of depression or injury phenotype, showed an elevation in heart rate, a spike in body temperature, decreased home cage activity, and decreased heart rate variability immediately after surgery, indicating that surgery is a physiological stressor. For the intact controls, however, all physiological measures quickly returned to baseline. In the not-depressed SCI group, the decreased home cage activity and increased heart rate persisted across the recovery period, likely reflecting decreased locomotor ability and elevated inflammation relative to intact controls. Intriguingly, the decreased heart rate variability persisted in the depressed SCI group only. The subjects characterized as depressed had decreased heart rate variability, relative to the not-depressed groups, from the time of the telemetry device implantation to the end of the post-SCI assessment period. Heart rate variability represents cardiac ability to adapt to various internal and external stimuli, and thus measures the health and homeostatic adaptability of the autonomic nervous system (Acharya et al., 2006). It has been used to measure and predict autonomic dysfunction in a variety of clinical situations, most notably in response to both physical and psychological stressors (Acharya et al., 2006; Sztajzel, 2004). The current study suggests that cardiac responses to a stressor may also predict susceptibility to development of depression.

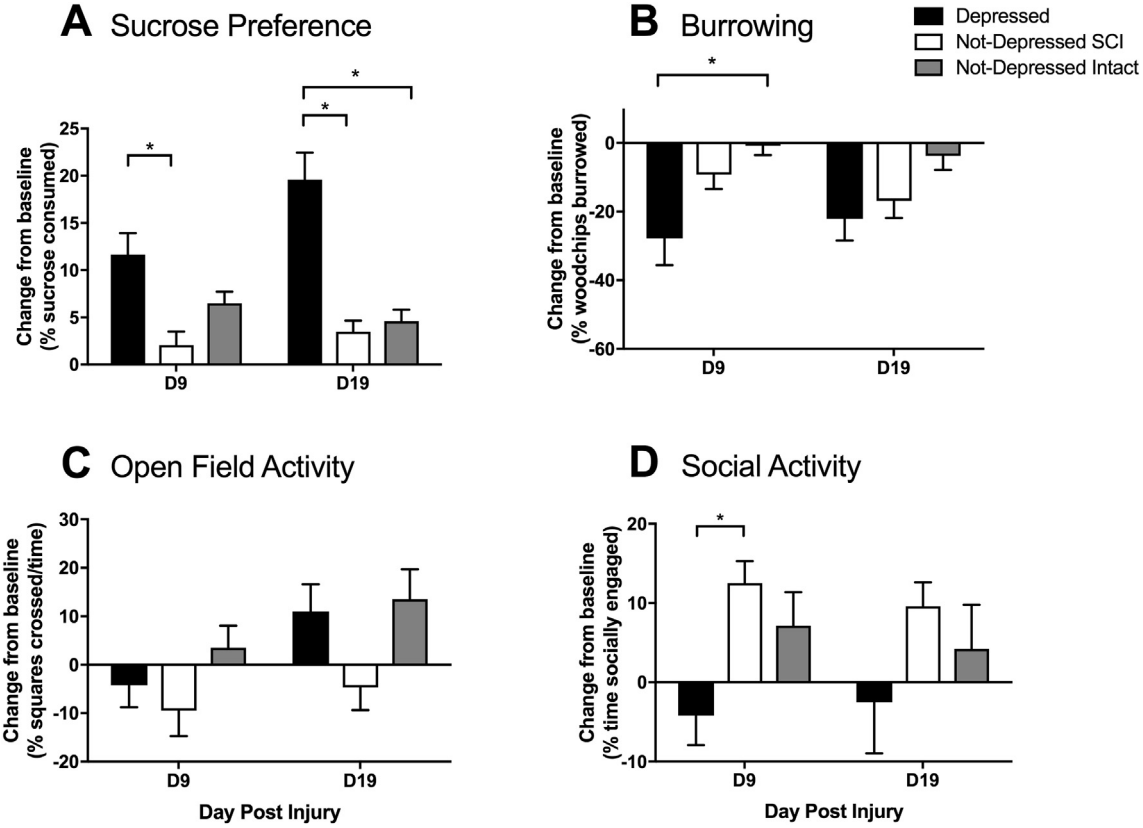

Fig. 4. A subset of SCI rats develops depression-like behaviors. Change from baseline scores were compared across the depressed and not-depressed SCI groups, as well as the not-depressed intact group for days 9 and 19 post-SCI. The depressed SCI group had higher sucrose preference on both days (A), driven by lower sucrose preference during the Baseline 2 assessment, (see Fig. 3). The depressed SCI group burrowed less than the intact controls on Day 9 post-injury (B), and they engaged in less social activity than the not-depressed SCI group (D). They did not differ in the distance travelled in 5 min in the total arena (C). * $p < 0.05$.

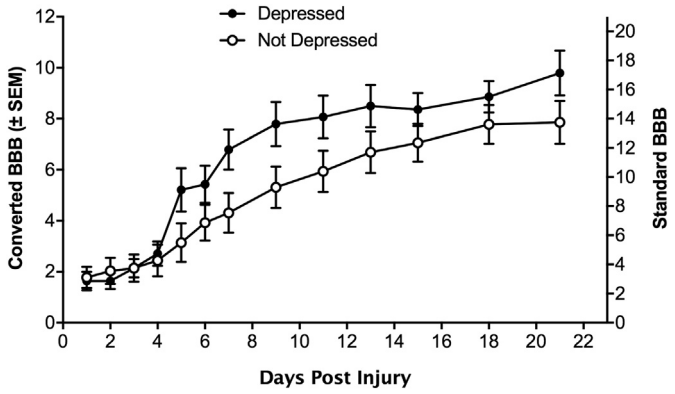

Fig. 5. Locomotor recovery does not determine depressive behavior. Average (± SEM) BBB scores are shown across the 30 days post injury assessment period. There were no significant differences in locomotor recovery across the depressed and not-depressed SCI groups.

In fact, in both humans and animals, low heart rate variability during or after stressors is associated with other predictors of susceptibility to depression, such as increased inflammation, cortisol and glucocorticoid dysregulation, and low circulating norepinephrine (Thayer and Sternberg, 2006; Weber et al., 2008). A growing number of human studies have also linked heart rate variability to self-regulatory capacity (Geisler et al., 2010; Reynard et al., 2011; Segerstrom and Nes, 2007), emotional regulation (Appelhans and Luecken, 2006; Geisler et al., 2010), and coping styles (Ramaekers et al., 1998). Individuals with lower levels of resting heart rate variability have been found to have poorer emotion regulation abilities and deficits in attentional control and working memory (Appelhans and Luecken, 2006; Thayer and Brosschot, 2005). By contrast, individuals with higher levels of resting heart rate variability have greater emotion regulation and responding, as well as executive functioning (Appelhans and Luecken, 2006; Thayer and Brosschot, 2005). Higher resting heart rate variability is also associated with reduced indices of distress in children watching an upsetting film (Fabes et al., 1993), and with greater self-reported emotion regulation and constructive coping strategies in university students (Fabes and Eisenberg, 1997). These data suggest that lowered heart rate variability may predict susceptibility to depression and anxiety, rather than being a consequence of these affective disorders.

Lowered heart rate variability is also widely recognized as a prognostic risk factor for coronary artery disease, as well as mortality following myocardial infarctions (Carney et al., 2005; Dekker et al., 2000; Tsuji et al., 1996; Udupa et al., 2007; van der Kooy et al., 2006). People with depression and reduced heart rate variability are at higher risk for myocardial infarction (Nicholson et al., 2006; O'Neil et al., 2016), even after controlling for confounding variables such as increased body mass index, physical activity, hypertension, and hypercholesterolemia (Anda et al., 1993; Barefoot et al., 1996; Penninx et al., 2001). Alarmingly, the development of depression prior to or after myocardial infarction is also associated with a significant increase in mortality (Bush et al., 2001; Egeberg et al., 2016; Meijer et al., 2013). It has been posited that reduced heart rate variability may be indicative of decreased vagus nerve activity and the loss of cholinergic inhibition of pro-inflammatory cytokine production (Huston and Tracey, 2011). With activation of an immune response, as would occur with SCI, this may lead to significantly enhanced cytokine production and cardiac tissue damage from inflammation (Huston and Tracey, 2011). Elevated pro-inflammatory cytokine expression is also associated with depression in

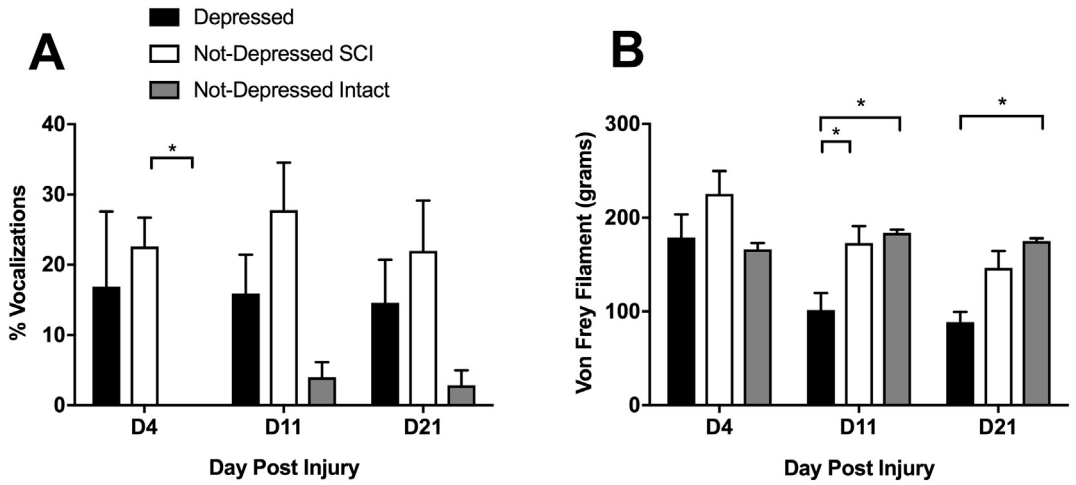

Fig. 6. Pain is associated with SCI and depression. On day 4 post-SCI, the not-depressed SCI group vocalized more in response ot light touch in the girdle test than did intact controls (A). The depressed SCI group showed decreased mechanical reactivity thresholds, relative to the not-depressed groups, on day 11 post-SCI, and compared with the intact group, on day 21 post-SCI (B). * $p < 0.05$.

humans (Liu et al., 2012; Myint et al., 2005), and we have previously shown that SCI rats characterized as depressed have higher serum cytokine levels than those characterized as not-depressed (Maldonado-Bouchard et al., 2016). Alternatively, depression may impact cardiac function through activation of the hypothalamic-pituitary-adrenal (HPA) axis, which will increase heart rate and promote hypertension and atherosclerosis (Joynt et al., 2003). Further research on the role of inflammation and other mechanisms mediating the associations between heart rate variability, depression, and cardiovascular disease is warranted. This is particularly relevant to SCI, in which cardiovascular complications are among the leading causes of mortality and morbidity (Cragg et al., 2013; Garshick et al., 2005; Myers et al., 2007; Sabre et al., 2013).

Whereas heart rate variability appears to be associated with a depression phenotype in the rodent model, heart rate appeared to be elevated by the lower thoracic SCI per se. This is commensurate with a previous study of female Sprague Dawley rats that found transient increases in resting heart rate following a T10 SCI (Harman et al., 2018). In rodent models, lower thoracic injury appears to increase resting heart rates for the first week post injury, with the heart rate returning toward pre-injury levels by weeks 2–3 post injury. While bradycardia is most often associated with SCI in humans, this typically occurs in patients with injuries above T6. In these injuries, the intact parasympathetic control of the heart, which is responsible for lowering the heart rate, is largely unopposed by the sympathetic innervation of the heart (Biering-Sørensen et al., 2018; Weaver et al., 2012). With a T12 SCI, however, the sympathetic innervation of the heart remains intact and bradycardia is less common. Whereas decreased heart rate variability may be a biomarker for susceptibility to depression post SCI in the rodent model, changes in heart rate may be due to trauma per se.

Clearly, cardiac health is a concern for both spinally injured and depressed patients, and the association of these physiological correlates of depression with more nuanced behavioral changes in our SCI rats highlights the importance of a physiologically relevant model of depression after SCI. It shows that depression behaviors after SCI are linked to specific biological changes associated with the injury, rather than only psychosocial factors. Very few researchers have investigated depression after SCI in animal models, and currently, there are few published papers identifying biological predictors of depression after SCI. Further research is needed, but alterations in cardiovascular function after injury may be able to predict susceptibility to depression-like behaviors.

In sum, we have developed an effective method for characterizing depression after SCI in an animal model. This will be useful in further understanding the molecular mechanisms underlying the development of depression, as well as in investigating the individual differences

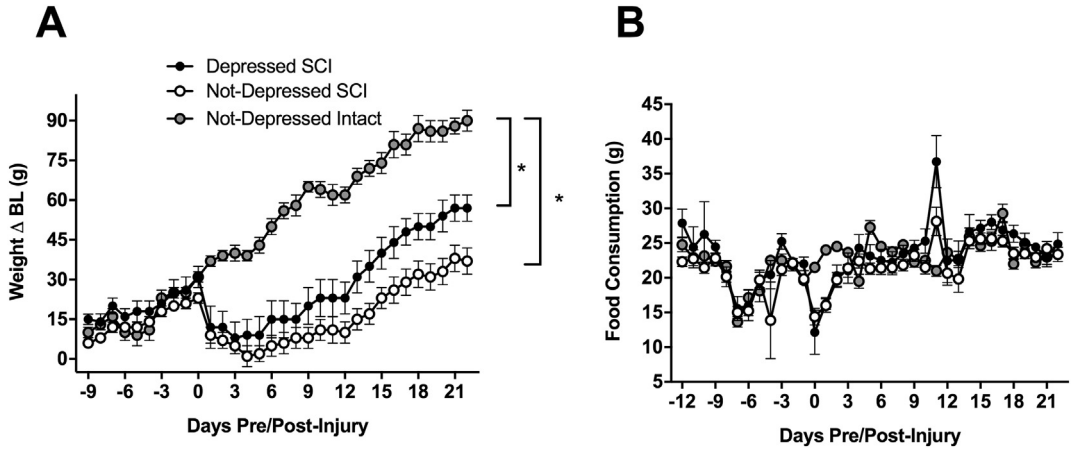

Fig. 7. Weight changes and feeding behavior.
Change from baseline (12 days prior to SCI) weights were tracked. Both SCI groups decreased in weight immediately after SCI and did not recover to a weight commensurate with the intact subjects (A). Weight changes were not associated with daily food consumption, which did not differ among the groups (B). * $p < 0.05$.

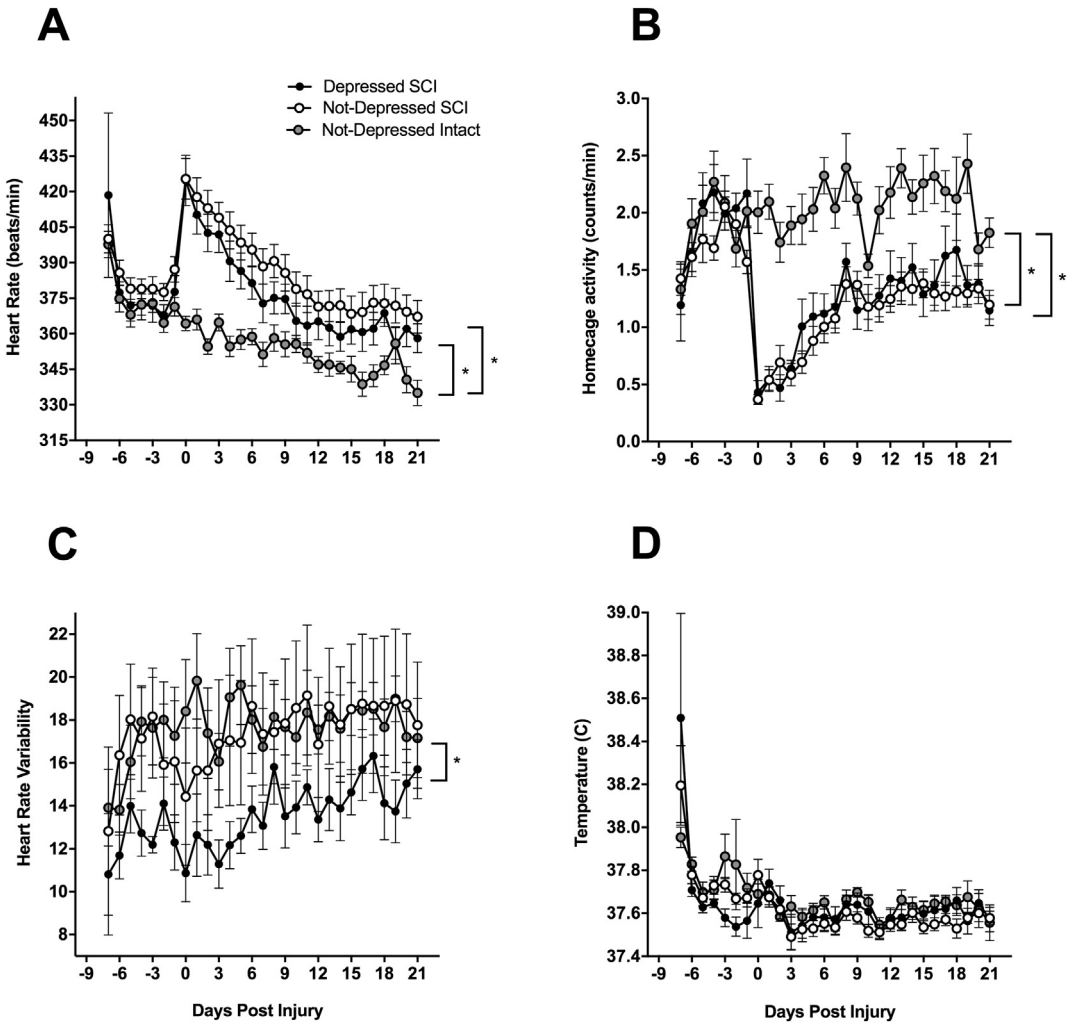

Fig. 8. SCI increases heart rate, but depression is associated with changes in heart rate variability.
Changes in physiological measures are depicted. Intact controls differed significantly from both the depressed and not-depressed SCI groups in both heart rate and home cage activity after SCI (A-B). The depressed SCI subjects also had lower heart rate variability than the intact controls (C). Internal body temperatures did not differ among the groups (D). * $p < 0.05$.

among subjects that may increase vulnerability to this disorder.

Funding

This work was supported by Mission Connect and the Gillson Longenbaugh Foundation.

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