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Spinal cord injury disrupts circadian rhythms in rats

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Abstract

Traumatic spinal cord injury affects 282,000 people in the United States alone. Individuals often suffer from partial paralysis and chronic pain along with physiological impairments, such as abnormal thermoregulation, decreased motor function, and poor sleep quality. Current clinical therapies focus on symptomatic treatments. Despite extensive research, all of the physiologic effects and the molecular mechanism underlying spinal cord injury, and how they affect each other, remains elusive. Here, we tested whether spinal cord injury disrupted circadian rhythms, which could prolong the recovery process. We used implanted transmitters in male and female rats to measure a marker, body temperature, and an entrainer, activity, of circadian rhythms. In a separate experiment, we use blood samples taken from rats at certain timepoints throughout spinal cord injury recovery to analyze concentrations of glucocorticoids. We found that both male and female injured rats were slightly hypothermic immediately after surgery and hyperthermic in the following days, but body temperature recovered at later time points. Activity was disrupted shortly after injury and similarly recovered in the following days post-injury and glucocorticoids were acutely increased in the days following injury. These results suggest the disruption of circadian rhythms from spinal cord injury at acute timepoints with a recovery in function seen at later timepoints. Measures to keep the circadian rhythm synchronized, such as regulating body temperature, encouraging activity, and limiting light pollution at certain times of day may facilitate recovery from spinal cord injury.
Introduction

Background

There are 17,000 new cases of spinal cord injuries (SCIs) within the U.S. each year, of which 80% are men. These SCIs often occur from falls and vehicular accidents. The initial injury is caused by the damage of central nervous system (CNS) tissue from compression and is followed by inflammation, causing secondary injury. Depending on the level of injury, motor weakness or paralysis and loss of sensation often occur. SCI can have a myriad of motor and sensory difficulties that often result in paralysis in extremities and chronic pain. As such, individuals suffering from SCI often report a decreased quality of life. Many individuals report impairments like pressure sores, hypotension, urinary tract infections, lessened ability to perform daily tasks, and difficulties sleeping. An overall disruption of circadian rhythm and homeostasis may contribute to these impairments.

Spinal Cord Injury Recovery

Under normal circumstances, the spinal cord allows bi-directional signaling to occur between the brain and the rest of the body. When the spinal cord is damaged, this communication is impaired, which can contribute to the pain and secondary impairments mentioned above. The effects seen after spinal cord injury are largely dependent on the severity of injury, and where the injury occurs.

In thoracic injuries, like the SCI studied in this paper, control of the sympathetic nervous system is impaired, particularly below the level of the lesion. Loss of sympathetic control often results
in abnormal temperature regulation, especially when sensory input to thermoregulation centers is also reduced\textsuperscript{7,8}. These injuries also cause a large decrease in muscle mass below the level of the injury. This can lead to decreased metabolic rate, which may also be adjusted by sympathetic nervous system activity\textsuperscript{9}.

Many individuals suffering from SCI also complain about decreased sleep quality. Many of these patients experience insomnia, irritability, or problems falling asleep and waking up\textsuperscript{10,11}. Part of the sleep disturbances experienced may be explained by pain and discomfort preventing sleep or by Restless Leg Syndrome, in which individuals feel the need to move their legs in order to dissipate the pain, which disturbs consistent sleep\textsuperscript{12}. Good sleep quality is involved with preventing obesity and heart diseases, promotes cell repair, and promotes functioning of the immune system\textsuperscript{13}.

**Circadian Rhythms**

Sleep is an important aspect of circadian rhythms, which are part of the body’s biological clock, in charge of restricting activity to certain hours of the day. The biological clock measures time on a scale of 24 hours and is one of the most well-studied molecular mechanisms.

The center of the circadian clock, or the “master oscillator,” is the suprachiasmatic nucleus (SCN), a small brain region within the anterior hypothalamus. The SCN receives light information directly from light-sensitive ganglion cells in the retina via the retinohypothalamic tract\textsuperscript{14,15}. The SCN then coordinates the timing of multiple circadian oscillators in other CNS areas and peripheral organs, which then regulate internal timing in local areas\textsuperscript{16}. However, the
SCN is not the only source of timing in the multiple circadian oscillators. These oscillators can be additionally entrained by rhythmic behaviors, like feeding, social influences, or exercise\(^{17,18}\).

External cues, or zeitgebers, like feeding, exercise, and especially light influence circadian rhythms. These rhythms can then feedback and further influence timing of feeding and exercise\(^{19,20}\). Another major function of circadian rhythms is to regulate the sleep-wake cycle through body temperature and hormonal cues\(^{21,22}\). Disruptions to the communication for these biological clocks can alter sleep patterns and food intake, resulting in stress responses and metabolic changes\(^{23,24}\).

**Core Body Temperature Regulation**

Body temperature regulation is important for the maintenance of homeostasis and the function of the sleep-wake cycle\(^ {25}\). Neurons in the preoptic area and anterior hypothalamus maintain body temperature and receive information from the SCN for rhythmic activity\(^ {26}\). These neurons also receive information from skin receptors about the temperature of the external environment. Body temperature is also regulated by the sympathetic nervous system through vasoregulation and sweat gland activity\(^ {27}\).

Under normal conditions, body temperature is usually highest during the day, when individuals are active and eating in stimulating environments. Conversely, core temperature is lowest at night when individuals are inactive or sleeping, are fasting, and are in a quieter environment\(^ {28}\).
Body temperature peaks in the early evening before dropping at nighttime to initiate sleep and then achieves its minimum before the individual wakes\textsuperscript{29,30}.

When body temperature is not synchronized with circadian rhythms, for example an individual goes to sleep when core body temperature is high, the individual often experiences difficulties falling asleep and constant fatigue\textsuperscript{31,32}. Jetlag is a common form of this misalignment, which results from the internal clock not being aligned with the local timezone (i.e., sleep/wake times). On a larger time scale, this desynchrony can have many physiologic effects, like metabolic and cardiac dysfunction as well as immune deficiency\textsuperscript{33,34}.

**Corticosterone**

Glucocorticoids are steroid hormones that are released by the adrenal gland cortex. Release is rhythmically timed, following cues from the SCN. Glucocorticoids further contribute to synchronization of cell-intrinsic clocks to coordinate physiological and metabolic dynamics\textsuperscript{35,36}. For example, glucocorticoids have been shown to inhibit phase-shifts from food-intake in circadian oscillators\textsuperscript{37}. Glucocorticoids are a major player in the hypothalamic-pituitary-adrenal (HPA) axis, which is responsible for the stress response\textsuperscript{38}.

Cortisol is the main glucocorticoid in humans regulated by circadian rhythms. In rats, corticosterone is the main circadian glucocorticoid (both cortisol and corticosterone are referred to as CORT in this manuscript). CORT is involved in glucose metabolism, to increase concentrations of glucose in the blood, immune function suppression, and activation of the stress response\textsuperscript{39}. As mentioned before, food can entrain peripheral circadian rhythms. It can also
entrain the HPA axis and individuals will have an increase in plasma CORT before a meal is expected\textsuperscript{40}. CORT is released following pulses of ACTH secretion\textsuperscript{41}. Over the 24-hour period, CORT levels decrease before resting and increase to prepare for activity\textsuperscript{42,43}.

**Effect of SCI on Peripheral Oscillators**

Previous literature has studied the relationship between SCI and individual peripheral oscillators. Much of the information regarding SCI and thermoregulation focuses on athletes with SCI and more specifically, the impaired ability of sweating and vasodilation in thermoregulation following exercise\textsuperscript{44,45}. Thermoregulation has also been studied in individuals suffering from complete SCI\textsuperscript{46}. Other studies performed have shown that activity-based therapy promotes recovery and overall health following incomplete SCI\textsuperscript{47,48}. Additionally, there have been studies evaluating circadian fluctuations of cortisol and melatonin and variations following SCI\textsuperscript{49}. These studies show that peripheral oscillators are affected by SCI; however, whether their daily rhythms are disrupted and whether this impairs feedback on circadian rhythms still remains unclear, which could have broader health and recovery effects.

**Hypothesis**

There is some evidence suggesting that SCI disrupts whole-body homeostasis; however, research directly relating SCIs and circadian rhythms is still understudied. Here, we evaluate the effects of thoracic SCI on body temperature and glucocorticoid rhythms.

Male and female rats were subjected to experimental contusion SCI after implantation of a transmitter that recorded body temperature and daily locomotor activity. A separate cohort was
given SCI to measure glucocorticoid concentration in plasma throughout acute recovery. We hypothesize that, due to the impaired communication between body systems, SCI will cause an acute circadian rhythm disruption that may be partially brought on by a dysregulation in glucocorticoid release.

**Materials and Methods**

**Telemetry Experiment**

*Subjects*

12 adult male and female Sprague Dawley rats were used for the telemetry experiment. Animals were housed individually with standard rat chow and water *ad libitum*. Environment was temperature controlled with a 12-hour light/dark cycle (lights on at 7:00 AM/lights off at 7:00 PM). Subjects were allowed at least one week to acclimatize prior to experimental procedures. All procedures were conducted in accordance to the University of Colorado Boulder *Institutional Animal Care and Use Committee* (IACUC) regulations.

*Mini-Mitter Implantation*

Rats were anesthetized with isoflurane. The abdomen was shaved and sterilized. A midline incision was made and the TA-E mitter (STARR Life Sciences, PA) was positioned in the abdominal cavity according to E-Mitter Implantation Procedure protocol. Suture material was passed through the tubing attached to the surface of the transmitter capsule and sutured to the body wall. The organs were allowed to settle before closing the incision via sutures. The animals
were monitored before being placed in their home cages on top of the receiving boards. These boards are part of the VitalView system allowing data collection of body temperature and activity in 30-minute increments. The implanted transmitters measured body temperature and activity counts. Activity counts were determined by changes in signal strength when the rat moved on the receiver board. These counts did not differentiate between changes in posture or physical location\textsuperscript{50}. Animals were allowed two weeks to recover prior to further experimental procedures and to collect baseline body temperature and activity measures.

**Spinal Cord Injury**

There were two surgery groups: injured (6 male, 6 female) and sham (6 male, 6 female). Two weeks following the mini-mitter implantation, rats were once again anesthetized with isoflurane. Animals were placed in clean environment and a partial T8 laminectomy was performed prior to injury. Animals were then stabilized, using the Impactor fixation plate, to clamp T7 and T9 with Addson micro-forceps. Injured group animals were subjected to a moderate contusion injury (150 kDyn, 1 s dwell) using the Infinite Horizon Impactor (Precision Systems and Instrumentation). Sham groups were only subjected to the laminectomy and did not receive any spinal cord contusion. The dorsal incision on all animals was then closed with sutures and staples.

**Post-Surgery Animal Care**

Post-operative animal care included daily administration of saline solution (5, 5, 4, 3, 2 mL on the first five days post-surgery for both sham and injured animals to prevent dehydration. Injured animals required manual compression of the bladder, to facilitate emptying, twice per day until
bladder function recovered, which was around 14 dpi. Recovery of bladder function was determined by decreasing amounts of urine voided during manual compressions. Animals were monitored daily for signs of suboptimal recovery or infections, especially those of the bladder.

Statistics
At 56 days post-surgery, rats were perfused with phosphate-buffered saline and tissue samples from sham and SCI rats were collected for possible future analysis. Data was collected from the VitalView system, which included measurements of body temperature and activity in 30-minute intervals. Timepoints when handling occurred were omitted for clarity, since rats had stress-related hyperactivity and hyperthermia. 30-minute interval datapoints were consolidated into 3-hour averages using Microsoft Excel and omitting the spikes in body temperature and movement that followed handling and post-operative care. This data was plotted and statistics analyzed using SigmaPlot (Systat Software). Data at defined timepoints from baseline to day 56 for sham and injured groups was analyzed using a two-way analysis of variants (ANOVA) followed by Bonferroni post-hoc test and was considered significant if \( p < 0.05 \). All data is reported as mean ± standard error.

Glucocorticoid Experiment

Subjects
16 adult male and female Sprague Dawley rats were used for the corticosterone experiment. Animals were housed in pairs with standard rat chow and water ad libitum. Environment was temperature controlled with a 12-hour light/dark cycle (lights on at 7:00 AM/lights off at 7:00 PM). Subjects were allowed at least one week to acclimatize prior to experimental procedures.
All procedures were conducted in accordance to the University of Colorado Boulder *Institutional Animal Care and Use Committee* (IACUC) regulations. SCIs and post-operational animal care was performed as mentioned above (both males and females sham n=6, SCI n=10).

*Tail Nicks*

Rats were handled prior to initiation of the experiment. Rat serum was collected one week prior to spinal cord surgery every six hours for 24 hours. After surgery, blood was collected every six hours over a 24-hour period at 2, 4, and 7 days post-injury (dpi). To collect blood, the rat was restrained and a scalpel blade was used to cut off 1 mm of the distal tail. The tail was lightly massaged to encourage blood flow. The tail nick process was under 3 minutes to prevent any increase in corticosterone due to stress. After the initial cut, following blood samples were obtained by gently removing the scab to limit additional cuts. When blood samples were taken in the dark cycle, a red-light headlight was the only light used.

*Analysis of Blood Samples via ELISA*

Immediately after blood samples were collected, they were centrifuged at 10,000 g for 10 minutes. From the separated layers, the top layer of serum, was collected and stored at -80ºC. Some samples from various rats at different timepoints did not have enough serum after being centrifuged and were thus omitted from analysis. The remaining samples were used to analyze levels of corticosterone through a CORT ELISA kit (Cat. No. ADI-901-097; Enzo Life Sciences, Farmingdale, NY, USA).

*Statistics*
Sham and injured animals were sacrificed at 7 days post-surgery and tissue samples were collected for possible future analysis. Corticosterone plasma levels were recorded in Microsoft Excel. These data were plotted and analyzed using SigmaPlot (Systat Software). Data from baseline to day 7 for sham and injured groups was analyzed using a two-way ANOVA followed by Bonferonni post-hoc tests and were considered significant if $p < 0.05$. All data is reported as mean ± standard error.

Results

**Body Temperature Rhythms are Disrupted at Acute Post-SCI Times**

Figure 1: Effect of spinal cord injury on body temperature in both male and female rats. Timepoints are represented on Zeitgeber time (ZT0 = 7 AM/start of light phase) and are 3 hour averages for 30-minute measurements. Experiment groups include female SCI (n=6), female sham (n=6), male SCI (n=6), and male sham (n=6). (* $p < 0.05$)
First, we assessed how SCI affected core temperature rhythms. Rats with small transmitters were subjected to sham surgery or SCI, and body temperature was studied prior to and after surgery (Figure 1). Pre-surgery measurements revealed the expected pattern in daily body temperature, with higher body temperature during the active (dark) phase and lower body temperature during the inactive (light) phase. Immediately post-surgery, both female and male SCI animals experienced significant hypothermia (compared to sham rats). (female, sham v. SCI hypothermia: immediately post-surgery: ZT12, ZT15, ZT18, ZT21, ZT24; 1 dpi: ZT3, ZT6, ZT12, ZT15 – p < 0.05) (male, sham v. SCI hypothermia: immediately post-surgery: ZT12, ZT15, ZT18, ZT21, ZT24; 1 dpi: ZT3, ZT6, ZT12, ZT15, ZT18 – p < 0.05). From 2-7 dpi, SCI rat body temperature was de-synchronized from those of the sham rats (main effect of treatment: female: $F_{1,203} = 22.156, p < 0.001$; male: $F_{1,186} = 20.535, p = 0.001$) (female, sham v. SCI: 2 dpi: ZT3, ZT6, ZT9; 3 dpi: ZT3, ZT6, ZT9; 4 dpi: ZT3, ZT6, ZT9; 5 dpi: ZT3, ZT6, ZT9; 6 dpi: ZT18 – p < 0.05) (male, sham v. SCI: 2 dpi: ZT0, ZT12, ZT 15; 4 dpi: ZT3, ZT6, ZT9; 5 dpi: ZT6, ZT9, ZT18, ZT24; 6 dpi: ZT9, ZT12; 7 dpi: ZT3, ZT6, ZT9, ZT12 – p < 0.05). Body temperature regulation largely recovered at 13-14 dpi (no main effect of treatment: female: $F_{1,203} = 1.147, p > 0.05$; male: $F_{1,186} = 2.794, p > 0.05$) (female, sham v. SCI: 14 dpi: ZT3, ZT6, ZT12, ZT15 – p < 0.05) (male, sham v. SCI: 14 dpi: ZT21 – p < 0.05).
Daily average body temperatures were also analyzed as average body temperature during the inactive (light) phase and active (dark) phase. Whereas sham rats had relatively typical body temperature immediately after surgery, female and male SCI rats displayed significant hypothermia in the inactive phase immediately after surgery. In addition, female and male rats also had slightly increased body temperature in the inactive phase shortly following SCI and normalized around 7 dpi (Figure 2). (female, sham v. SCI: 2 dpi (sham: 37.60°C ± 0.08°C, SCI: 37.86°C ± 0.08°C; p < 0.05), 3 dpi (sham: 37.54°C ± 0.08°C, SCI: 37.88°C ± 0.08°C; p < 0.05), 4 dpi (sham: 37.36°C ± 0.08°C, SCI: 37.80°C ± 0.08°C; p < 0.05), and 5 dpi (sham: 37.36°C ± 0.08°C, SCI: 37.75°C ± 0.07°C; p < 0.05) (male, sham v. SCI: 4 dpi (sham: 37.27°C ± 0.09°C, SCI: 37.6°C ± 0.1°C; p < 0.05) and 5 dpi (sham: 37.29°C ± 0.09°C, SCI: 37.7°C ± 0.1°C; p < 0.05).

In the active phase, SCI rats displayed this hypothermia immediately following surgery also. Female and male SCI active phase temperature was similar to sham animals in the days following.

![Figure 2: Effect of spinal cord injury on inactive and active phase body temperature in both male and female rats. Timepoints are daily body temperature averages from baseline to 42 dpi. Experiment groups include female SCI (n=6), female sham (n=6), male SCI (n=6), and male sham (n=6). (* p < 0.05)
Activity Rhythms are Disrupted at Acute Post-SCI Times

Immediately after surgery and at 2 dpi, SCI rats exhibited comparable activity to the sham animal activity during their inactive phase (total counts at 2 dpi – female-sham: 13000 ± 2000, female-SCI: 7000 ± 2000, p < 0.05; male-sham: 13200 ± 800, male-SCI: 3200 ± 800, p < 0.001).

As expected after SCI, both male and female injured rats had lower activity counts than their sham counterparts (Figure 3). The significant decrease in activity occurred until 13-14 dpi where activity counts were comparable (total counts in active phase at 14 dpi – female-sham: 9000 ± 900, female-SCI: 7000 ± 1000, p > 0.05; male-sham: 8500 ± 500, male-SCI: 6600 ± 500, p < 0.05).

Figure 3: Effect of spinal cord injury on cumulative activity counts over 3-hour periods in both male and female rats. Timepoints are represented on Zeitgeber time (ZT0 = 7 AM/start of light phase) and are 3 hour averages for 30-minute measurements. Experiment groups include female SCI (n=6), female sham (n=6), male SCI (n=6), and male sham (n=6). (* p < 0.05)
Activity counts were further analyzed as average activity during the inactive (light) phase and active (dark) phase (Figure 4). In the inactive phase, both male and female SCI animals had largely decreased activity immediately following surgery (average counts in inactive phase immediately post-surgery – female-sham: 290 ± 50, female-SCI: 130 ± 90, male-sham: 200 ± 10, male-SCI: 70 ± 30). This discrepancy was potentiated in male SCI animals until 3 dpi (male, sham v. SCI: 2 dpi (sham: 173 ± 38, SCI: 70 ± 21, p < 0.05) and 3 dpi (sham: 184 ± 36, SCI: 78 ± 24, p < 0.05)).

In the active phase, significant differences were also revealed between the two treatment groups in both male and female rats. Activity counts were the lowest following surgery in all groups. As expected, SCI rats displayed less activity overall. This discrepancy was evident until 6-7 dpi when both male and female rats increased activity to near sham levels. This regaining of locomotor activity follows typical recovery after SCI in rats.

**Figure 4:** Effect of spinal cord injury on inactive and active phase activity in both male and female rats. Timepoints are daily activity averages from baseline to 42 dpi. Experiment groups include female SCI (n=6), female sham (n=6), male SCI (n=6), and male sham (n=6). (* p < 0.05)
Plasma Corticosterone Dysregulation at Acute Post-SCI Times

Tail nicks were performed on sham/SCI female and male rats every 6 hours to collect serum for corticosterone analysis. Pre-surgery serum CORT levels showed the expected rhythmic expression patterns; with increased CORT at the start of the active (dark; ZT12) phase (females: 1.4 ng/mL ± 0.3 ng/mL; males: 0.6 ng/mL ± 0.1 ng/mL) and decreased CORT at the start of the inactive (light; ZT0) phase (females: 0.6 ng/mL ± 0.2 ng/mL; males: 0.4 ng/mL ± 0.1 ng/mL). CORT concentrations significantly increased in both male SCI rats, at 2 and 7 dpi, and female SCI rats, at 7 dpi, compared to pre-surgery CORT levels (Figure 5a) (female, pre-surgery v. SCI: 7 dpi: ZT0, ZT24 – p < 0.05) (male, pre-surgery v. SCI: 2 dpi: ZT0, ZT6, ZT24; 7 dpi: ZT0, ZT24 – p < 0.05). Additionally, the time at which CORT should be lowest (ZT0), CORT is strongly dysregulated at 7 and 14 dpi. Most timepoints for pre-surgery and 7 dpi, over the 24-hour period, are comparable in the same cohort of animals. Female SCI animals displayed increased CORT concentrations in plasma at the start of the light cycle/end of the dark cycle at 7

Figure 5: a) Effect of spinal cord injury on glucocorticoid rhythms in both male and female rats. Timepoints are on Zeitbeger time (ZT0 = 7 AM, start of light/inactive phase) and track concentration of corticosterone over a 24 hour time period from baseline to 14 dpi. (* p < 0.05 when compared to baseline measurements) b) Effect of spinal cord injury on daily average corticosterone concentration from baseline to 14 dpi. Experiment groups include female SCI (n=10), female sham (n=6), male SCI (n=10), and male sham (n=6). (* indicates SCI vs. pre-surgery, p < 0.05, † indicates SCI vs. sham, p < 0.05)
dpi (vs. pre-surgery; \( p < 0.05 \)). In male SCI animals, CORT concentrations at 2 (vs. pre-surgery; at ZT0 and ZT6; \( p < 0.001 \)) and 7 dpi (at ZT0; \( p < 0.05 \)) were significantly increased in comparison to baseline measurements.

Average daily corticosterone levels were calculated (Figure 5b). Serum CORT concentration significantly increased in SCI animals at 2 dpi in both male and female rats (female, sham v. SCI: 2 dpi (sham: 1.9 ng/mL ± 0.5 ng/mL, SCI: 3.6 ng/mL ± 1.3 ng/mL, \( p < 0.05 \)) (male, sham v. SCI: 2 dpi (sham: 0.9 ng/mL ± 0.1 ng/mL, SCI: 2.7 ng/mL ± 0.5 ng/mL, \( p < 0.05 \)) and pre-surgery v. SCI: 2 dpi (pre-surgery: 0.4 ng/mL ± 0.1 ng/mL, SCI: 2.7 ng/mL ± 0.5 ng/mL, \( p < 0.05 \)). The SCI and sham corticosterone concentrations were similar in the days post-surgery.

CORT levels were further analyzed as average CORT concentration during the inactive and active phase (Figure 6). CORT significantly increased at 2 dpi in male SCI rats during the inactive phase (vs. pre-surgery \( p < 0.05 \); vs. sham \( p < 0.05 \)). Female SCI rats had no such increase during the inactive phase. 14 dpi samples were comparable to pre-surgery samples in both SCI and sham animals.

Figure 6: Effect of spinal cord injury on inactive and active corticosterone concentrations in male and female rat plasma. Timepoints are daily CORT averages from baseline to 14 dpi. Experiment groups include female SCI (n=10), female sham (n=6), male SCI (n=10), and male sham (n=6). (* indicates SCI vs. pre-surgery, \( p < 0.05 \), † indicates SCI vs. sham, \( p < 0.05 \))
In the active phase, both male and female SCI animals had increased CORT plasma concentrations when compared to their sham counterparts at 2 dpi (vs. pre-surgery $p < 0.05$). Samples taken from pre-surgery, 7 dpi, and 14 dpi timepoints were comparable between the two treatment groups in both male and female cohorts.

**Discussion**

The present research sought to determine whether SCI disrupts circadian rhythms in male and female rats. Our previous experiments found that SCI rats had more fecal activity during the inactive phase at acute timepoints. This dysregulation normalized at 42 dpi$^{51}$. Thus, we explored whether the rhythms of markers for circadian rhythm were similarly disrupted. Specifically, we asked, does SCI (i) change core body temperature throughout recovery (ii) disrupt rhythm of daily activity and (iii) desynchronize peaks in plasma CORT. The inquiries were based on previous findings in male and female SCI rats.

To test for circadian rhythm disruption after SCI, male and female rats were subject to a moderate T8 spinal cord contusion. The first experiment included males and females that were implanted with transmitters to measure body temperature and activity every 30 minutes for 56 dpi. The second experiment consisted of male and female rats that were subject to tail nicks every 6 hours on specific post-surgery days until 14 dpi to measure CORT concentration in the plasma. The experiments revealed disruption of body temperature and activity rhythms, particularly at acute timepoints. Acute plasma levels of CORT were also dysregulated. These physiological functions recovered over time.
SCI disrupts body temperature and activity rhythms at acute timepoints

The Mini-Mitter transmitter used in this experiment recorded body temperature and activity. The consolidated data revealed a pattern of desynchronized body temperature where both male and female rats with acute SCI exhibited warmer core temperatures in the inactive phase, when compared to their sham counterparts. Most often, SCI in humans is followed by chronic hypothermia, or cooler body temperatures, after initial hyperthermia, similar to what was seen in the rats in this study\textsuperscript{46}. However, few individuals experienced potentially fatal fevers in acute timepoints\textsuperscript{52}. Similarly, the warmer body temperatures, seen in this experiment during the first few days following injury, occurred during the inactive phase. The active phase core body temperatures at acute post-surgery times (e.g., 2-7 dpi) and beyond were similar between SCI and sham male and female rats. It is also possible that body temperature during surgery could have additional effects on body temperature during recovery, like the effects seen in this experiment\textsuperscript{53}.

While it is not surprising that SCI rats show reduced activity, given acute paralysis with these injuries, the gradual recovery of movement may be important for circadian rhythms. It is also worth mentioning that when locomotor ability recovered, activity rhythms were strong again, similar to pre-surgery activity. As mentioned previously, circadian rhythms influence exercise, but exercise also provides feedback to further entrain these rhythms. In one study, rats were exposed to forced running, which resulted in circadian phase-shifts\textsuperscript{54}. The recovery of activity rhythms, through specific timing of exercise, could strengthen synchronization of circadian rhythms.
SCI disrupts normal glucocorticoid functioning at acute timepoints

Plasma CORT was significantly increased at 2 dpi in male and female SCI rats, when compared to their baseline pre-surgery CORT levels. Both male and female rats also displayed increased plasma CORT at the beginning of the light cycle at 2 and 7 dpi. CORT levels at 14 dpi were comparable between SCI and pre-surgery measurements in addition to SCI and sham measurements. A previous study found that CORT was increased after injury with evidence of diurnal CORT synthesis\(^{55}\). Here, recovery similarly suggests acute disruption of normal glucocorticoid functioning.

At all dpi, male SCI rats exhibited increased CORT at ZT0, or the start of the light cycle. Females exhibited the same, except for at 7 dpi where CORT levels were generally increased. These results are not what usually occurs under normal conditions: plasma CORT concentrations are generally highest before wake, to prepare the body for wake. Conversely, CORT concentrations are generally lowest before sleep, to aid in sleep-initiation. This suggests that the SCI disrupted CORT concentration regulation. However, it is possible that the method used in this study to collect and measure CORT may not be detailed enough to visualize the CORT rhythms.

When plasma CORT was analyzed as concentrations during the light and dark phase, males exhibited raised concentration at 2 dpi compared to their pre-injury baseline. Females only displayed this increase during their active phase at 2 dpi. This also suggests brief acute disruption of normal CORT rhythms. However, it is worth noting that CORT is also entrained by ultradian
cycles. Under normal functioning, glucocorticoids follow ultradian rhythms in addition to circadian rhythms. Ultradian rhythms are cycles that occur multiple times within a 24-hour period. Because of the existence of smaller rhythms within the 24-hour cycle, our results may also show these ultradian fluctuations of CORT concentrations in the body, exaggerating the perceived extent of CORT disruption. Other experiments may reveal more about the extent of the disruption. For example, previous studies have shown that increased CORT has resulted from SCI, where the SCI-induced excess was more pronounced in higher-thoracic (T1) SCIs compared to lower-thoracic (T9) SCIs.

**Conclusions and Future Direction**

Both male and female rats with SCI displayed acute homeostatic disruption, including dysregulated body temperature, activity, and glucocorticoids. As mentioned previously, some of the outputs of circadian rhythms occurs via the sympathetic nervous system, which plays a large role in thermoregulation. Body temperature may be desynchronized due to impaired communication between the SCN and sympathetic nervous system.

Male and female rats with SCI had expectedly reduced activity in the days following injury. Exercise further entrains spinal cord injury. Since the SCI animals were unable to exercise or move around with ease, due to partial paralysis of the hind limbs, it is possible that circadian rhythms were desynchronized. Few studies in humans have showed the timing of exercise as a pathway for functional recovery. This modulation of the circadian rhythms, using exercise as a timed external cue, could potentially be useful in resynchronizing circadian rhythms to facilitate better sleep and long-term recovery.
Male and female rats with SCI displayed highest CORT at the start of the light (inactive) cycle. Generally, corticosterone is increased before wake, to prepare the body for activity. A potential limitation to the glucocorticoid experiment is the timeframe. CORT, in addition to being released by circadian timers, is also released on ultradian (within-day) rhythmic cycles. Since CORT was only taken in 6 hour intervals, it may not be the most accurate depiction of the CORT rhythms after SCI. Because of this, it is possible that a blood sample was taken when the CORT ultradian rhythm was decreasing. This would provide researchers with the misconception that CORT has been steadily decreasing for those 6 hours. These rats also exhibited significantly higher CORT levels at 2 dpi. CORT is one of the main hormones of the HCA axis, which is largely responsible for initiating the stress response. The acute increase in CORT levels may be part of a stress response to SCI rather than a larger scale circadian disruption.

**Figure 7:** Under normal conditions, circadian rhythms and peripheral oscillators, such as body temperature, activity, and glucocorticoids, act on each other to synchronize the body. However, SCI disrupts the regulatory factors of these peripheral oscillators and further disrupts the ability for these oscillators and circadian rhythms to be synchronized.
Taken together, the above evidence highlights the widespread effects of circadian rhythm dysfunction (Figure 7). However, there are still questions that need to be answered to determine practices that would aid in recovery after SCI. Would external regulation of temperature aid in sleep and recovery? If so, is there a way to mimic the body’s temperature fluctuation to facilitate sleep initiation and wake-up? Given that individuals suffering from SCI have limited movement, is there a way to introduce timed exercise to adjust circadian rhythms? Is glucocorticoid release desynchronized and if it is, is there a pharmacological option to mimic biological rhythms to realign the internal clock with the external environment? Answers to these could help determine if “resetting” the circadian rhythm after SCI would facilitate recovery.

In order to address these questions, future discovery science studies could observe if body temperature during surgery and anesthesia wake-up is correlated to recovery and if forced wheel-running re-aligns body temperature and activity rhythms with the light/dark cycle. Additionally, glucocorticoids could be measured in one hour increments to better visualize the ultradian rhythms at play, along with the overarching circadian rhythm. When these practices have been optimized and the effect of exercise established, we can determine if extrinsically adapting the circadian rhythm would promote recovery. Overall, our data suggest that SCI may broadly disrupt the circadian system, which could slow recovery. Therefore, future studies could assess whether strengthening circadian rhythms, using strategies discussed above, could help expedite recovery.
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