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# Inflammation is increased with anxiety- and depression-like signs in a rat model of spinal cord injury

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ABSTRACT

Spinal cord injury (SCI) leads to increased anxiety and depression in as many as 60% of patients. Yet, despite extensive clinical research focused on understanding the variables influencing psychological well-being following SCI, risk factors that decrease it remain unclear. We hypothesized that excitation of the immune system, inherent to SCI, may contribute to the decrease in psychological well-being. To test this hypothesis, we used a battery of established behavioral tests to assess depression and anxiety in spinally contused rats. The behavioral tests, and subsequent statistical analyses, revealed three cohorts of subjects that displayed behavioral characteristics of (1) depression, (2) depression and anxiety, or (3) no signs of decreased psychological well-being. Subsequent molecular analyses demonstrated that the psychological cohorts differed not only in behavioral symptoms, but also in peripheral (serum) and central (hippocampi and spinal cord) levels of pro-inflammatory cytokines. Subjects exhibiting a purely depression-like profile showed higher levels of pro-inflammatory cytokines peripherally, whereas subjects exhibiting a depression- and anxiety-like profile showed higher levels of pro-inflammatory cytokines centrally (hippocampi and spinal cord). These changes in inflammation were not associated with injury severity; suggesting that the association between inflammation and the expression of behaviors characteristic of decreased psychological well-being was not confounded by differential impairments in motor ability. These data support the hypothesis that inflammatory changes are associated with decreased psychological well-being following SCI.



## 1. Introduction

In addition to its effects on physical function, spinal cord injury (SCI) significantly impacts quality of life and psychological well-being. As many as 60% of spinal cord injured patients suffer from depression (Shin et al., 2012), anxiety (Post and van Leeuwen, 2012), and general decreased quality of life (Boakye et al., 2012). Commensurate with these statistics, and the risks associated with depression (McCullumsmith et al., 2015), suicide attempts and suicide ideation are estimated to be 3 or more times greater following SCI, than in the general population (DeVivo et al., 1991; Soden et al., 2000). Depression is also associated with long-term negative outcomes after SCI including an increased incidence of secondary complications (Malec and Neimeyer, 1983; Herrick et al., 1994; Elliott and Frank, 1996) and lower functional independence (Gelis et al., 2011; Abdul-Sattar, 2014). Given the high percentage of spinal cord injured patients experiencing psychological symptoms, and the significant impact of this mood disorder on rehabilitation outcomes and quality of life, it is imperative that we better understand the mechanisms underlying decreased psychological well-being following SCI.

There is compelling evidence to suggest that, in addition to psychosocial stressors, the activation of the immune system may be contributing to the manifestation of depression and anxiety in a subset of the clinical population. Both rodent and human studies support this hypothesis (Smith, 1991; Maes, 1999; van West and Maes, 1999; Capuron et al., 2001, 2003, 2004; Miller et al., 2013;

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Vogelzangs et al., 2013). In the human clinical population, studies have found an association between elevated levels of pro-inflammatory cytokines and depression (Howren et al., 2009; Dowlati et al., 2010; Liu et al., 2012a,b) as well as anxiety (Pace and Heim, 2011; Miller et al., 2013). In animal models, stressed rats were found to display increased spleen and brain (hippocampus, hypothalamus, and cortex) mRNA levels of pro-inflammatory cytokines relative to non-stressed rats (You et al., 2011). Further, in rodent models, central or systemic administration of pro-inflammatory cytokines produces sickness behavior characterized by behavioral and physiological changes resembling depression (Anisman et al., 2005), which can be alleviated with anti-depressants (Merali et al., 2003). More recently, Wu et al. (2014) showed that brain microglia are activated in a mouse model of SCI, and that SCI increases cognitive dysfunction as well as depression-like symptoms. These data suggest that immune system activation may play a pivotal role in the development of depression and anxiety.

A role for the immune system in the development of depression and anxiety may be especially important after SCI. SCI is characterized by inflammation and activation of the immune system. Following injury, microglia, macrophages, and astrocytes are recruited to the site of trauma. Pro-inflammatory cytokine genes and other inflammation-related genes are up-regulated within hours of injury (Dumont et al., 2001; Yip and Malaspina, 2012), and some remain elevated for weeks following the injury (Malaspina et al., 2008; Jokic et al., 2010). For example, in the hours following injury, the interleukin-6 gene (*IL-6*), the *TNF* gene, and the interleukin-1β gene (*IL-1β*) are up-regulated (Hayashi et al., 2000; Pan et al., 2002). In addition, as injury severity increases, pro-inflammatory cytokine levels increase (Yang et al., 2005). Immunocytochemical, RT-PCR and Western Blot assays on spinal cord tissue collected one to six hours post SCI indicated that IL-1β, IL-6 and TNF-α production is increased in neurons and microglia of the spinal cord following SCI, and that this increase in transcription is significantly greater following a severe compared to a mild injury (Yang et al., 2005). Therefore, immune system activation triggered by SCI may influence psychological well-being, and may do so incrementally as injury severity increases.

To test this hypothesis, the current experiment used an established behavioral ethogram (Luedtke et al., 2014) to phenotype spinally injured rodents based on psychological symptoms, and then examined the relationships between inflammation, depression and anxiety. We used pre-mixed multiplex assays to obtain an overall tableau of pro and anti-inflammatory cytokine expression following injury. We found that changes in cytokine expression are associated with the development of an anxiety- and depression-like profile in SCI rodents. Moreover changes in behavioral symptoms, of depression and anxiety, were not simply due to differences in the recovery of motor function. The incidence of depression, with or without co-morbid anxiety, did not differ across injury severity groups and locomotor recovery (BBB scale) levels were not significantly different across the psychological well-being cohorts. We posit that by activating the immune system, SCI may hinder psychological well-being, increasing the development of depression and anxiety-like signs post injury.

## 2. Methods

### 2.1. Subjects

Male Sprague–Dawley rats (Harlan Houston, TX), approximately 90–110 days old (300–350 g), were individually housed in Plexiglas bins [45.7 (length) × 23.5 (width) × 20.3 (height) cm] with food and water continuously available. The rats were maintained on a 12 h light/dark cycle and all behavioral testing was conducted during the light cycle. Food consumption and subject weights were recorded daily. Following surgery, subjects' bladders were manually expressed in the morning (8–9:30 a.m.) and in the evening (6–7:30 p.m.) until they regained full bladder control (which was operationally defined as three consecutive days with an empty bladder at the time of expression), and were checked daily for signs of autophagia and spasticity.

All of the experiments reported here 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.

### 2.2. Procedures

The timeline for experimental procedures is shown in Fig. 1. Subjects ($N = 47$) received a mild, moderate, or severe contusion or were intact controls ($n = 12$/group). Psychological well-being was assessed at baseline (prior to injury) with a comprehensive battery of tests as well as during Test Phase 1 (days 5 and 10) and 2 (days 20–21) post injury. Intact subjects were tested at ages compatible with injured subjects.

### 2.3. Surgery

An Infinite Horizons (IH) impactor (Precision Systems and Instrumentation) fitted with a 2.5 mm impact probe was used to deliver a contusion injury to the T12 spinal cord. Subjects were anesthetized with 5% isoflurane gas, and once a stable level of anesthesia was reached, the concentration was lowered to a 2–3% maintenance level. An area extending approximately 2.5 cm above and below the injury site was then shaved and disinfected with iodine. A 3–4 cm incision was made in the skin along the midline of the animal, followed by 1.5 cm incisions on either side of the spinous processes at the level of injury. A laminectomy was performed removing the T12 vertebra and exposing the spinal cord. The animal was fixed in the IH impactor and the probe exerted a force onto the cord, remaining in contact with the cord for one second (dwell time). A force of 110 kDynes was used to produce a mild contusion injury, 150 kDynes a moderate injury, and 200 kDynes a severe injury. Immediately after the contusion, the incision was closed with Michel clips and subjects were treated with 100,000 units/kg Pfizerpen (penicillin G potassium), both following surgery and again 2 days later, to help prevent infection. To compensate for fluid loss, subjects received 3.0 ml of filtered saline at the time of surgery and again 2 days later. The rats were kept in a recovery room (maintained at 26.6 °C) for 24 h following the injury.

### 2.4. Assessment of psychological well-being

Anhedonia was assessed with the sucrose preference test (procedure described in detail in Luedtke et al., 2014). Briefly, the subjects were acclimated to the sucrose preference test in three sessions beginning 13 days prior to surgery. Baseline preferences, for sucrose-flavored or plain water, were collected 5 days prior to surgery. Sucrose preference was then measured on Days 10 (Test Phase 1) and 21 (Test Phase 2) post SCI. A decrease in sucrose preference is a sign of anhedonia, or lack of interest in rewarding stimuli.

Psychomotor activity and center field activity was assessed with the open field test (procedure described in detail in Luedtke et al., 2014). Subjects were acclimated to the open field environment [a black plywood box, 100 × 100 × 20 (height) cm] during three sessions beginning 13 days prior surgery. Baseline activity levels were collected 5 days prior to surgery, with post-injury mea-