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INVITED REVIEW

# Does being female provide a neuroprotective advantage following spinal cord injury?

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## Abstract

It has been controversial whether gender has any effect on recovery following spinal cord injury (SCI). Past experimental and clinical research aimed at addressing this subject has led to constrasting findings on whether females hold any advantage in locomotor recovery. Additionally, for studies supporting the notion of a female gender related advantage, a definite cause has not been explained. In a recent study, using large sample sizes for comparative male and female spinal cord injury cohorts, we reported that a significant gender advantage favoring females existed in both tissue preservation and functional recovery after taking into consideration discrepancies in age and weight of the animals across sexes. Prior animal research frequently used sample sizes that were too small to determine significance with certainty and also did not account for two other factors that influence locomotor performance: age and weight. Our finding is important in light of controversy surrounding the effect of gender on outcome and the fact that SCI affects more than ten thousand new individuals annually, a population that is disproportionately male. By deepening our understanding of why a gender advantage exists, potential new therapeutics can be designed to improve recovery for the male population following the initial trauma or putatively augment the neuroprotective privilege in females for enhanced outcomes.

***Key Words:*** *sex; gender; hormone; neuroprotection; estrogen; progesterone; apoptosis; Schwann cell*

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## Introduction

Current experimental modalities for spinal cord injury (SCI) treatment have focused on the utility of neuroprotective and reparative therapeutic agents during and after the secondary injury phase of spinal cord trauma. Improved understanding of the mechanisms that contribute to secondary injury has led to the identification of promising experimental agents that have yet to be translated to evaluation in clinical trials (Samantaray et al., 2010a). Previous research has shown that sex hormones play an important role in limiting tissue damage after injury and can improve functional outcomes across species when delivered therapeutically (Herson et al., 2009; Liu et al., 2010; Chan et al., 2012). Neuroprotective efficacy has been reported with female sex hormones progesterone and estrogen as well as the male sex hormone testosterone (Hammond et al., 2001; Bialek et al., 2004; Schumacher et al., 2007; Samantaray et al., 2010a, b; Sribnick et al., 2010). Clinical and experimental comparisons of genders after SCI have suggested the presence of a gender advantage in functional recovery and/or anatomical preservation, favoring females, similar to findings observed following traumatic brain injury (TBI; Bramlett and Dietrich, 2001; Sipski et al., 2004; Farooque et al., 2006; Bramlett, 2013). It has been postulated that the female advantage is caused by the superior neuroprotective and reparative effects of estrogen and progesterone (**Figure 1**). However, other reports have suggested that the female advantage occurs independently of sex hormone action (**Figure 1**; Roof and Hall, 2000; Hauben et al., 2002; Swartz et al., 2007).

## Female Rats Exhibit Improved Locomotor Recovery due to an Inherent Neuroprotective Advantage

The secondary phase of tissue injury occurs in the initial hours to weeks after SCI. Secondary injury exacerbates tissue loss and neural cell death subsequent to the mechanical insult, resulting in an increase in the degree of neurological dysfunction. These pathophysiological changes are triggered by SCI-induced release of tissue-degrading proteases, oxidative species, cytotoxic metabolites, pro-inflammatory

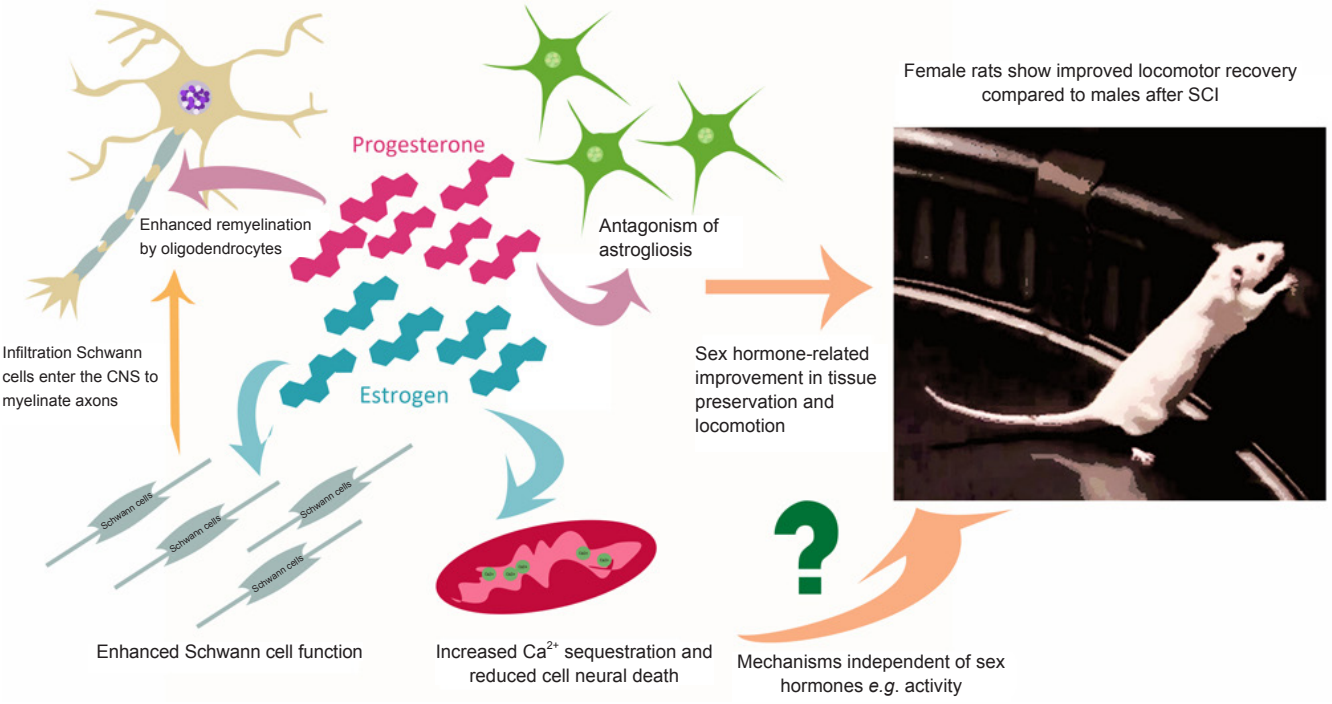


**Figure 1 Potential mechanisms involved in a gender-related improvement in functional recovery after spinal cord injury (SCI) favoring females.**
Improved tissue preservation and locomotor recovery in female rats over males may be due to both hormone-dependent and - independent mechanisms. The female sex hormones, progesterone and estrogen, are known to be involved in a myriad of cellular processes that may contribute to the antagonism of cell death and the promotion of neurorepair after central nervous system (CNS) injury. These include: (1) antagonism of astrogliosis, (2) the sequestration of $Ca^{2+}$ and inhibition of cell death signaling, and (3) enhancing the survival of, and remyelination repair mediated by, oligodendrocytes and Schwann cells. In addition, hormone-independent mechanisms, including differences in activity and associated production of tissue protective growth factors, may also play a role in gender biases in tissue protection and recovery after SCI.

cytokines, and excitotoxic neurotransmitters as well as mitochondrial dysfunction, immune cell activation, and calcium-induced neuron death. It is this phase that is most suitable for neuroprotective interventions (Park et al., 2004) and examinations of whether gender can alter responses to these pathological processes that precipitate continued tissue injury and functional loss. Previous rodent experimental studies that have sought to investigate whether a gender advantage exists in recovery after central nervous system (CNS) injury have not reached a consensus (Hauben et al., 2002; Farooque et al., 2006; Singh et al., 2006; Fee et al., 2007; Swartz et al., 2007; Ung et al., 2007). These investigations used small sample sizes, of 4–11 animals in each group, and produced conflicting results, with some detecting significantly improved locomotor recovery and tissue preservation in females (Hauben et al., 2002; Farooque et al., 2006) and others finding no significant gender-related difference in locomotor behavior following SCI (Singh et al., 2006; Fee et al., 2007; Ung et al., 2007). In the studies that detected a significant gender difference, a correlation between a reduction in tissue damage and improved locomotor recovery suggested that females have a neuroprotective advantage during the sub-acute injury phase. Farooque et al. (2006) found that histopathological analysis of the injured spinal cord revealed a reduction of the number of macrophages and an improved structural preservation of the spinal cord in females. In this report, locomotor performance of the animals as assessed using a Basso, Beattie, and Bresnahan (BBB) test over 14 days, demonstrated that female rats scored significantly higher than the male rats. Hauben et al. (2002) similarly found that female rats displayed greater tissue preservation, tissue organization, and continuity of myelinated fibers 4 months after initial injury. However, both of these investigations did not standardize weight or age among genders or examine whether changes in outcome were due to these variables rather than gender. Other factors that could contribute to discrepancies among laboratories in finding a significant gender advantage include study design differences in the duration of locomotor assessment, the timing post-injury of any histological or stereological analyses, and the study of the effect of hormone treatments (progesterone and estrogen) in addition to that of a gender difference.

## Examining Gender-related Differences in Outcome after SCI Using Larger Group Sizes

In our recently reported investigation, we sought to examine gender-related differences in histopathological and locomotor outcomes temporally using large sample sizes of > 20 rats per gender cohort and found that gender-related differences, favoring females, were present in tissue preservation and locomotor recovery (Datto et al., 2015). The use of a larger sample size provides a more holistic and reliable measure of any gender effects, if present. A strong correlation between the degree of tissue preservation and the improvement in

locomotor performance of the female rats suggested that the difference was caused by a neuroprotective effect rather than other variables, such as a difference in the overall activity of male and female rats during testing sessions. However, activity-related changes in protective molecules, such as growth factors, among genders could have played a role in tissue preservation and functional outcome differences. Another interesting finding is that not all of the locomotor outcome parameters measured produced a significant difference among genders after SCI. While behavioral tests that measured gross walking ability, such as the BBB score and CatWalk gait analysis, did detect significant differences between genders, other measures, such as the Gridwalk, which is related to tract-specific control of foot positioning and more sensitive to dorsal column functional loss (Muradov et al., 2013), did not show a significant difference. This holistic improvement indicates that female rats may have experienced improved locomotor recovery due to a protection of lateral and ventral white matter tracts in the sub-acute injury phase rather than an enhancement of repair and plasticity in descending axon systems, such as the corticospinal tract, which is located within the epicenter of the contusion site, or of dorsal column axons, located immediately peripheral to the injury impact.

## The Effects of Age and Weight on Gender-related Differences in Outcome after SCI

The female rats we used in our study were, on average, older and lighter than the male rats. Due to the significant disparity in growth curves between male and female animals of a given species, matching both age and weight for SCI studies is very difficult without the introduction of additional variables, such as fasting, which are known to impact recovery (Jeong et al., 2011). Age and weight data were included in our study as covariates when conducting an analysis of variance (ANCOVA) analyses on gender, time, and all outcome measures. No prior studies of the effect of gender differences on post-SCI recovery analyzed age and weight disparities between males and females. Singh et al. (2011) had used rats of the same weight, which likely caused a mean age difference between the gender groups that was not accounted for. Age differences have already recently been shown to have an effect on the recovery of locomotor function, lesion pathology and microglia/macrophage responses following SCI (Hooshmand et al., 2014). Age and weight are important to consider, as they may have significant effects on the durability of the spinal cord tissue, contusion size following injury, and other factors affecting locomotor recovery. Using ANCOVA analysis, significant differences in recovery among genders favoring females were demonstrated in the absence of an effect of age or weight (Datto et al., 2015).

## The Role of Sex Hormones in Neuroprotection and Gender-related Disparities in Recovery

Previous studies have indicated that the female sex hormones estrogen and/or progesterone can limit tissue damage and improve function in animal models of CNS injury (Kasturi et al., 2007). Schumacher et al. (2007) suggested that progesterone acts as a glio-active factor by enhancing remyelination and suppressing reactive gliosis. Several studies also show that supra- and normal levels of estrogen are neuroprotective following SCI (Samantaray et al., 2010a, b; Sribnick et al., 2010), though Swartz et al. (2007) showed a lack of significantly improved performance after controlled estrogen circulation in both male and ovariectomized female rats. The mechanism of estrogen's neuroprotection is thought to involve the alteration of $Ca^{2+}$ loading, the maintenance of mitochondrial membrane potential during cellular stress, and positive regulation of anti-apoptotic protein expression and localization (Singh et al., 2006). Estrogen allows neurons to buffer increased concentrations of $Ca^{2+}$, thereby protecting against glutamate excitotoxicity (Nilsen et al., 2003). The finding of Swartz et al. (2007) suggests that the potential neuroprotective and functional locomotor recovery benefits observed in females following SCI are not only due to the presence of estrogen but also its fluctuating levels in the body and its regulated expression.

The neuroprotective effect of estrogen has also been reported in male rats following ischemia (Bagetta et al., 2004). In addition, estrogen has been shown to enhance the survival of Schwann cells (Siriphorn et al., 2010), which are known to infiltrate into the injured spinal cord in large numbers after SCI and which exhibit protective and reparative effects when transplanted into the site of injury in animal SCI models (Takami et al., 2002; Pearse et al., 2004; Schaal et al., 2007). Thus, we postulate that the improved performance in female rats after SCI could be due to the neuroprotective capabilities of female sex hormones estrogen and/or progesterone through a variety of molecular and cellular processes (**Figure 1**). The significant female advantage observed in our study neither refutes or supports the possible neuroprotection of testosterone; however, it may suggest that estrogen and/or progesterone could possess greater neuroprotective capabilities. In experimental TBI, Bramlett and Dietrich (2001) have shown that both proestrous and non-proestrous female rats have significantly smaller cortical contusion sizes after injury compared to males, suggesting that other endogenous circulating hormones or inherent female factors influence neuroprotection. Examining ovariectomized females after SCI in the same paradigm used in Datto et al. (2015) would be important to shed light on the involvement of other inherent female factors in the observed tissue preservation and functional recovery.

## Conclusions

The finding of a female gender advantage in functional recovery and neuroprotection in a pre-clinical SCI model has important implications for advances in SCI therapies. The use of larger sample sizes, analysis of covariates (ANCOVA) (age and weight) and the wide range of analyses of locomotor recovery employed allowed for the specific detection of previously unexplored differences in locomotor performance

between genders. Although the exact factors governing this disparate response are currently unknown, results from previously published studies suggest that it is more complex than the mere presence or absence of estrogen, and it is likely due to a combination of multiple factors in which fluctuations and feedback regulatory mechanisms are important. This work highlights the value of investigating gender-related differences in outcome after CNS injury; understanding the mechanisms responsible for this advantage should allow us to harness this process, leading to improved SCI recovery in males and augmented recovery in females.

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