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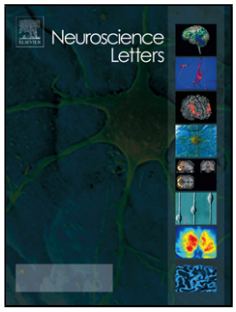

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Temporal changes in the expression of the translocator protein TSPO and the steroidogenic enzyme 5α-reductase in the dorsal spinal cord of animals with neuropathic pain: effects of progesterone administration

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Highlights

- Spinal cord injury (SCI) induces an early increase in TSPO and 5α-RII mRNA levels
- A later decrease in 5α-RI and 5α-RII expression after SCI is observed
- TSPO and 5α-RII up-regulation may represent a protective response against injury
- PG induces a marked increase in spinal TSPO, StAR, 5α-RI and 5α-RII expression
- PG may favor local production of reduced metabolites and prevents allodynia

Abstract

Neuropathic pain is a frequent complication of spinal cord injury (SCI), still refractory to conventional treatment. The presence and biological activity of steroidogenic regulatory proteins and enzymes in the spinal cord suggests that neurosteroids locally generated could modulate pain messages. In this study we explored temporal changes in the spinal expression of the 18kDa translocator protein TSPO, the steroidogenic acute regulatory protein (StAr) and the steroidogenic enzyme 5α-reductase (5α-RI/II) in an experimental model of central chronic pain. Male Sprague-Dawley rats were subjected to a SCI and sacrificed at different time points (1, 14 or 28 days). The development of mechanical and cold allodynia was assessed. Injured animals showed an early increase in the mRNA levels of TSPO and 5α-RII, whereas in the chronic phase a significant decrease in the expression of 5α-RI and 5α-RII was observed, coinciding with the presence of allodynic behaviors. Furthermore, since we have shown that progesterone (PG) administration may offer a promising perspective in pain modulation, we also evaluated the expression of steroidogenic proteins and enzymes in injured animals receiving daily injections of the steroid. PG-treated did not develop allodynia and

showed a marked increase in the mRNA levels of TSPO, StAR, 5α-RI and 5α-RII 28 days after injury. Our results suggest that in the acute phase after SCI, the increased expression of TSPO and 5α-RII may represent a protective endogenous response against tissue injury, which is not maintained in the chronic allodynic phase. PG may favor local steroidogenesis and the production of its reduced metabolites, which could contribute to the antiallodynic effects observed after PG treatment.

Abbreviations

Spinal cord injury (SCI), Central nervous system (CNS), Progesterone (PG), Control animals (CTL), Injured animals (HX), Injured animals that received daily injections of natural progesterone (HX+PG), Polymerase chain reaction (PCR), Cyclophilin (Cyc), Translocator protein (TSPO), Steroidogenic acute regulatory protein (StAr/STARD1), 5α-reductase (5α-RI/II), 5α-dihydroprogesterone (5α–DHP), 3α,5α-tetrahydroprogesterone (3α,5α-THP), allopregnanolone (ALLO).

Keywords

Chronic pain; Steroidogenesis; Spinal cord injury; 18kDa translocator protein TSPO; 5α-Reductase; Progesterone

1. Introduction

In the spinal cord, as well as in other central nervous system structures, steroids can be synthesized either *de novo* from cholesterol or from circulating steroid hormones, that easily cross the blood-brain/spinal barrier and serve as precursors for neurosteroidogenic enzymes. Thus, steroids produced by the nervous system are referred to as “neurosteroids” (Baulieu and Robel 1990), while those acting in the nervous system, including those synthesized locally or in the peripheral glands (ovary,

testis and adrenal glands) and also synthetic steroids, are called "neuroactive steroids" (Paul and Purdy 1992, Schumacher et al. 2015).

Among neurosteroids/neuroactive steroids, progesterone (PG) and its reduced metabolite 3α,5α-tetrahydroprogesterone (3α,5α-THP), also known as allopregnanolone (ALLO), exert a wide range of actions in the central nervous system, acting as physiological regulators of nervous function, as well as protective agents in pathological conditions (De Nicola et al. 2013, Melcangi et al. 2014, Schumacher et al. 2014, Guennoun et al. 2015). In the recent years, an important area of research has been devoted to explore the role of PG and ALLO, either exogenously administered or endogenously synthesized, in the modulation of neuropathic pain (Giatti et al. 2015, Coronel et al. 2016a).

Thus, PG administration has been found to prevent allodynia after injuries to the sciatic nerve (Coronel et al. 2011a, Dableh and Henry 2011), trigeminal nerve root (Kim et al. 2012) or spinal cord (Coronel et al. 2011b), and eradicate allodynic and hyperalgesic symptoms in animals subjected to chemotherapy induced peripheral neuropathy (Meyer et al. 2010). In addition, ALLO administration has been shown to reduce mechanical and thermal hyperalgesia after sciatic nerve ligature (Pathirathna et al. 2005b), counteract diabetes-induced motor impairment and thermal hyperalgesia (Afrazi and Esmaeili-Mahani 2014) and suppress neuropathic symptoms evoked by antineoplastic drugs, such as vincristine (Meyer et al. 2010) or oxaliplatin (Meyer et al. 2011).

In addition, it has been demonstrated that local PG and ALLO synthesis increases in the nervous system of animals with neuropathic pain, probably as an endogenous mechanism triggered to cope with the chronic pain condition (Mensah-Nyagan et al. 2008, Mensah-Nyagan et al. 2009). The expression and/or activity of different steroidogenic enzymes increase in the spinal cord of animals that develop neuropathic pain after peripheral nerve injury, resulting in an up-regulation of the biosynthetic pathways leading to PG and ALLO production (Mensah-Nyagan et al. 2008, Mensah-Nyagan et al. 2009). However, the spinal expression of steroidogenic regulatory proteins and enzymes has not been evaluated during the onset and development of SCI-induced neuropathic pain.

Therefore, in this study we evaluated temporal changes evoked by the SCI in the expression of key components of the enzymatic pathways leading to PG and ALLO synthesis and correlated our findings with the presence of allodynic behaviors. We centered our studies on the 18kDa translocator protein (TSPO), previously known as peripheral benzodiazepine receptor (Papadopoulos et al. 2006) and the steroidogenic acute regulatory protein (StAR/STARD1) (King and Stocco 2011), which mediate and regulate the translocation of cholesterol from intracellular stores to the inner mitochondrial membrane, the rate limiting step for neurosteroidogenesis (Rone et al. 2009), and on both isoenzymes of 5α-reductase (5α-R type I and II), the enzyme regulating the synthesis of PG reduced metabolites (Stoffel-Wagner 2003). Furthermore, since we have previously demonstrated that PG administration prevents both mechanical and thermal allodynia after SCI, we have also analyzed whether PG anti-allodynic effects could be related to changes in the expression of the regulatory proteins / enzymes under study.

2. Methods

2.1 Spinal cord injury

All experimental procedures were reviewed and approved by the local Animal Care and Use Committee (Assurance Certificate Nº A5072-01) and the Ethical Committee from Instituto de Biología y Medicina Experimental (Buenos Aires, Argentina), and followed the Guide for the Care and Use of Laboratory Animals (National Institutes of Health). Care was taken to minimize animal discomfort and to limit the number of animals used. Male Sprague-Dawley rats (200-220 g), bred at the colony of the Instituto de Biología y Medicina Experimental, were deeply anesthetized with ketamine (50 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.). In a group of rats, the spinal cord was exposed and unilaterally hemisected at thoracic T13 level (Labombarda et al. 2008, Coronel et al. 2011b, Coronel et al. 2014, Coronel et al. 2016b), as originally described by Christensen et al (Christensen et al. 1996). In sham-operated animals the spinal cord was exposed but not lesioned. Post-operative care included control of body temperature using an electric heating pad, and prophylactic antibiotic administration (cephalexine 20 mg/kg/day) during 5 days, starting immediately after surgery. Animals were monitored for eventual infections until they were euthanized either 1, 14 or 28 days after injury.

2.2 Progesterone administration

Injured animals received daily subcutaneous injections of natural progesterone (Sigma, Saint Louis, MO, USA; P8783, 16 mg/kg/day; HX+PG) or vehicle (Ricine oil, Ewe, Sanitas, Buenos Aires, Argentina; HX) (Coronel et al. 2011b, Coronel et al. 2014, Coronel et al. 2016b). In PG-treated animals, the steroid was administered immediately after performing the lesion and once a day thereafter until the animals were euthanized, 28 days after injury. We have previously tested this dose of PG in several animal models of nervous system injury (Labombarda et al. 2009, Coronel et al. 2011a, Coronel et al. 2011b, Coronel et al. 2014, Garcia-Ovejero et al. 2014, Coronel et al. 2016b). In particular, this dose of PG has been shown to prevent mechanical and thermal allodynia after spinal cord (Coronel et al. 2011b, Coronel et al. 2014, Coronel et al. 2016b) and sciatic nerve (Coronel et al. 2011a) injuries. Sham-operated animals receiving oil were used as control animals (CTL).

2.3 Assessment of pain behaviors

Behavioral testing was performed by a blinded observer. The animals were tested 1 day before surgery, in order to obtain normal baseline values, and at different time points (days 7, 14, 21 and 28) after SCI or sham-operation, as previously described (Coronel et al. 2011b, Coronel et al. 2014, Coronel et al. 2016b). Only rats showing normal responses to mechanical and thermal stimulation before surgery were included in the experiments. Eight animals were included in each experimental group. They were placed in transparent testing chambers and allowed to acclimate for 15 min before testing.

2.3.1 Mechanical allodynia

Paw mechanical sensitivity was assessed by evaluating the response to normally innocuous mechanical stimuli using a series of 8 calibrated von Frey filaments (1, 2, 4, 6, 8, 10, 15, 26 g, Stoelting, Wood Dale, IL, USA). Each filament was delivered three times with 5 s intervals. The lowest force at which application elicited at least two withdrawal responses (brisk paw withdrawal together with a nocifensive behavior such as attack to the stimulus, escape or vocalization) was taken as the mechanical response threshold. A paw withdrawal reflex obtained with 6 g or less was considered an allodynic response (Coronel et al. 2011b, Coronel et al. 2014, Coronel et al. 2016b).

Values shown in Fig 1a correspond to the mean ± SEM. As previously reported, results were analyzed using the Friedman Repeated Measures of Analysis of Variance followed by Multiple Comparison Test (Coronel et al. 2011b, Coronel et al. 2014, Coronel et al. 2016b).

2.3.2 Cold allodynia

Cold sensitivity of the hindpaw to acetone (Choi test) was quantified by paw withdrawal frequency (Choi et al. 1994). Thus, 100 μl of acetone was applied to the plantar surface of the paw using a plastic tubule connected to a 1 ml syringe. Acetone was applied five times to each paw at intervals of at least 5 min. The number of brisk foot withdrawals accompanied by nocifensive behaviors (mentioned in the previous section) was recorded. If paw withdrawal was observed at least two times after acetone exposure, it was considered an allodynic response (Coronel et al. 2011b, Coronel et al. 2014, Coronel et al. 2016b). Values shown in Fig 1b correspond to the mean ± SEM. As previously reported, results were analyzed using the Friedman Repeated Measures of Analysis of Variance followed by Multiple Comparison Test (Coronel et al. 2011b, Coronel et al. 2014).

2.4 Tissue preparation for Real Time-Polymerase Chain Reaction (PCR)

Either 1, 14 or 28 days after SCI, animals receiving PG or vehicle, as well as CTL animals, were deeply anesthetized with chloral hydrate (800 mg/kg, i.p.) and killed by decapitation. Spinal lumbar segments caudal to the injury site (L4-5) and equivalent regions from CTL animals were immediately removed and the dorsal spinal halves were dissected (Coronel et al. 2011b, Coronel et al. 2014, Coronel et al. 2016b). Tissues were frozen and stored at -70^{o}C until gene expression studies were performed. Samples from the different experimental groups were run at the same time.

2.5 Real Time-PCR

Spinal dorsal halves were collected as described above (n=8 in each group). RNA was extracted using Trizol (Invitrogen, USA), as previously described (Coronel et al. 2011b, Coronel et al. 2014, Coronel et al. 2016b). Nucleotide sequences of forward (F) and reverse (R) primers used for amplification were: TSPO: F: CTT GCA GAA ACC CTC CTG GCA TC, R: CCA AGG GAA CCA TAG CCT CCT CTG (designed using the

Oligo Primer Analysis software version 6.54, Molecular Biology Insights Inc, Cascade, Colorado, USA); StAR: F: CTG CTA GAC CAG CCC ATG GAC, R: TGA TTT CCT TGA CAT TTG GGT TCC (Abatikuw et al. 2011); 5α-RI: F: ACT GGG CAA CCT GCC TAA C, R: ATC AGA ACC GGG AAA ACC A (Munetsuna et al. 2009); 5α-RII: F: CAG GAA GCC TGG AGA AGT CA, R: CAA TAA TCT CGC CCA GGA AA (Munetsuna et al. 2009). Cyclophilin (Cyc) F: GTG GCA AGA TCG AAG TGG AGA AAC, R: TAA AAA TCA GGC CTG TGG AAT GTG; accession number: NM_022536, was chosen as housekeeping gene and designed using the Oligo Primer Analysis software version 6.54 (Molecular Biology Insights Inc, Cascade, Colorado, USA). Relative gene expression was determined using Syber green master mix and the ABI PRISM 7500 sequence detection system (Applied Biosystems, Foster City, California, USA) (Coronel et al. 2011b, Coronel et al. 2014, Coronel et al. 2016b). The change in the target mRNA was calculated using the method describe by Pfaffl (Pfaffl 2001) and expressed as fold-increase relative to control values. Eight animals were included in each experimental group and samples were run in triplicate. Data shown in Figs 2 and 3 correspond to the mean ± SEM of mRNA levels relative to control values (CTL: sham-operated animals receiving oil). Statistical analysis was performed by applying Student t Test (Fig 2, a-c) or One-Way Analysis of Variance followed by Newman-Keuls post-hoc test (Fig 2, d).

3. Results

3.1 Behavioral evaluation of neuropathic pain: development of mechanical and cold allodynia after spinal cord injury, and effect of progesterone administration

As previously observed, animals subjected to a spinal cord hemisection showed guarding behaviors and changes in the posture such as plantar flexion and toe-clenching.

After injury, both the ipsilateral and contralateral hindpaws showed a progressive decrease in mechanical withdrawal threshold (Fig 1a, $p<0.001$ vs CTL at day 14) and allodynic values were detected 21 and 28 days after injury (Fig 1a, $p<0.001$ vs CTL at both time points). Paw withdrawals were accompanied by active attention to the stimulus, abrupt head turning and attack, vocalization, and/or body reposturing,

indicating that noxious stimuli were detected supraspinally. These aversive behaviors and the allodynic responses were still observed at the endpoint of this study (day 28, Fig 1a).

When cold sensitivity was assessed, a similar behavioral pattern was obtained: there was a gradual and clear increase in the number of positive nociceptive responses in both hindpaws starting 14 days after injury (Fig 1b, $p<0.001$ vs CTL), with the highest number of allodynic responses detected at days 21 and 28 (Fig 1b, $p<0.001$ vs CTL in both cases). As described in the previous paragraph, paw withdrawals were accompanied by aversive behaviors, until the end of the study.

In correlation with our previous reports (Coronel et al. 2016b), injured animals receiving PG did not develop mechanical allodynia (Fig 1a, $p<0.001$ vs HX at days 21 and 28) and showed reduced sensitivity to cold stimulation (Fig 1b, $p<0.05$ vs HX at day 14, $p<0.001$ vs HX at day 21 and $p<0.01$ vs HX at day 28).

3.2 Temporal changes in TSPO, StAR, 5α-RI and 5α-RII mRNA levels after spinal cord injury

Injured animals showed an early increase in the expression of TSPO (Fig 2, $p<0.05$ vs CTL at days 1 and 14) and 5α-RII (Fig 2, $p<0.05$ vs CTL at day 1, $p<0.01$ vs CTL at day 14). On the contrary, the mRNA levels corresponding to StAR and 5α-RI were similar to those detected in CTL animals (Fig 2, $p>0.05$ vs CTL for both molecules and at both time points).

In the chronic phase (day 28), coinciding with the presence of allodynic behaviors, a significant decrease in the expression of 5α-RI (Fig 2, $p<0.01$ vs CTL) and 5α-RII (Fig 2, $p<0.05$ vs CTL) was observed, while TSPO and StAR mRNA levels remained similar to CTL values (Fig 2, $p>0.05$ vs CTL for both molecules).

3.3 Effect of progesterone administration on TSPO, StAR, 5α-RI and 5α-RII mRNA levels in the chronic phase after spinal cord injury

Twenty eight days after SCI, animals receiving PG showed a marked increase in the mRNA levels of TSPO (Fig 3, $p<0.001$ vs HX and CTL), StAR (Fig 3, $p<0.05$ vs HX and CTL), 5α-RI (Fig 3, $p<0.01$ vs HX) and 5α-RII (Fig 3, $p<0.001$ vs HX and CTL) in the spinal dorsal cord. Thus, at this time point, PG treatment induced TSPO and StAR up-regulation and counteracted the injury induced decrease in 5α-RI and 5α-RII expression.

4. Discussion

The present study shows that: a) SCI induces an early increase in the spinal expression of TSPO and 5α-RII, and a significant decrease in the mRNA levels of 5α-RI and 5α-RII in the chronic phase after injury, coinciding with the presence of allodynic behaviors; b) PG treatment results in a marked increase in the expression of TSPO, StAR, 5α-RI and 5α-RII in the chronic phase, likely favoring local steroidogenesis and the production of reduced metabolites such as ALLO, and prevents allodynia.

Recent evidence shows that both PG and ALLO, either endogenously synthesized or exogenously administered, exert neuroprotective effects and reduce neuropathic pain-associated behaviors in different animal models of pain. In fact, and as previously mentioned, several groups around the world, including ours, have demonstrated the efficacy of administering PG (Meyer et al. 2010, Coronel et al. 2011a, Coronel et al. 2011b, Dableh and Henry 2011, Kim et al. 2012, Coronel et al. 2014) or ALLO (Pathirathna et al. 2005b, Meyer et al. 2010, 2011, Afrazi and Esmaeili-Mahani 2014) to alleviate neuropathic pain in experimental conditions.

As well as in other steroidogenic tissues, biosynthesis of neurosteroids in the spinal cord begins with the translocation of cholesterol to the inner mitochondrial membrane, mediated by TSPO (Papadopoulos et al. 2006), a high-affinity drug- and cholesterol-binding mitochondrial protein, and StAR (King and Stocco 2011), a hormone-induced mitochondria-targeted protein that initiates cholesterol transfer (Rone et al. 2009).

Cholesterol is then converted into pregnenolone, which is further reduced into PG. By the action of 5α-R, PG can be converted into 5α-dihydroprogesterone (5α-DHP), which is reduced into 3α,5α-THP or ALLO (Stoffel-Wagner 2003). Thus, the reaction catalized by 5α-R is crucial for the production of PG-reduced metabolites, 5α-DHP and ALLO. Two isoforms of 5α-R have been identified; in the spinal cord 5α-RI and 5α-RII display distinct expression patterns (Patte-Mensah et al. 2004b).

Recent studies have also reported that TSPO plays critical roles in various neurological diseases, including inflammatory and neuropathic pain (Rupprecht et al. 2010, Wei et al. 2013, Liu et al. 2014). A recent report suggests that the early TSPO up-regulation in the spinal cord and its subsequent activation reverts allodynia and hyperalgesia in rats with spinal nerve ligation (Wei et al. 2013). Moreover, the activation of this translocator protein and the consequent increase in neurosteroid formation have been partly attributed to the inhibition of chemokine-dependent astrocyte-to-neuron signaling and central sensitization (Liu et al. 2016). Furthermore, a current study has reported that thalamic brain levels of TSPO negatively correlate with clinical pain and circulating levels of the pro-inflammatory cytokine interleukin-6 in patients with chronic back pain, suggesting that TSPO exerts pain-protective/anti-inflammatory effects in humans, as predicted by animal studies (Loggia et al. 2015).

In line with these previous findings, we have now shown that SCI also results in an early increase in the spinal expression of TSPO and 5α-RII, possibly resulting in higher local production of PG and its reduced metabolites. Although in this study we did not evaluate the spinal concentrations of neurosteroids, we have already demonstrated that PG and ALLO levels are increased in the spinal cord 75 hours after injury, without a significant increase in plasma (Labombarda et al. 2006). In addition, rat spinal tissue homogenates have been shown to be capable of converting cholesterol into various metabolites including PG and ALLO (Patte-Mensah et al. 2003, Patte-Mensah et al. 2004a, Patte-Mensah et al. 2004b, Saredi et al. 2005), clearly indicating that the regulatory proteins / enzymes detected correspond to active forms. In relation to StAR expression, we did not detect any changes in its mRNA levels. However, since StAR activity is regulated through post-translational modifications (Duarte et al. 2014), we cannot exclude injury-induced changes in its steroidogenic activity.

An increase in the spinal expression and/or activity of other steroidogenic enzymes, such as P450 side-chain cleavage (Patte-Mensah et al. 2003, Patte-Mensah et al. 2004a), 3β-hydroxysteroid dehydrogenase (Saredi et al. 2005) and 3α-hydroxysteroid oxidoreductase (Meyer et al. 2008), has also been described in other experimental models of neuropathic pain (Mensah-Nyagan et al. 2008, Mensah-Nyagan et al. 2009). In addition, and confirming its local production, the spinal levels of PG (Saredi et al. 2005) and ALLO (Patte-Mensah et al. 2004a) have been shown to be significantly increased after peripheral nerve injury.

Interestingly, endogenous PG has been shown to decrease sensitivity to pain by increasing levels of endorphins and opioid receptors (Dawson-Basoa and Gintzler 1997, Dawson-Basoa and Gintzler 1998). In addition, PG administration is able to modulate the spinal expression of N-methyl-D-aspartate (NMDA) receptor subunits, protein kinase C gamma (PKCγ) (Coronel et al. 2011a, Coronel et al. 2011b), pro-inflammatory enzymes (Coronel et al. 2014) and cytokines (Coronel et al. 2016b), all key players for chronic pain generation, probably through genomic actions mediated by the classical intracellular progesterone receptor (PR). In contrast to PG (and 5α-DHP), ALLO does not bind to PR and acts by modulating neurotransmitter receptors. It is well demonstrated that 3α,5α-reduced steroids like ALLO are potent endogenous positive allosteric modulators of the inhibitory functions of $GABA_A$ receptors (Belelli and Lambert 2005), whose activity is crucial in the regulation of pain. ALLO has also been found to enhance specific $GABA_A$ receptor subtypes (Pathirathna et al. 2005b, Peng et al. 2009) and to block neuronal low-voltage activated (T-type) Ca^{2+} channels (Pathirathna et al. 2005a). Thus, the early increase in the steroidogenic pathway may represent an endogenous protective mechanism tending to control pain development, which cannot be maintained in the chronic phase after SCI. Accordingly, and as shown in the present study, 28 days after injury TSPO mRNA levels return to control values, both isoforms of 5α-R are down-regulated and animals develop mechanical and thermal allodynia.

Interestingly, PG administration induces the up-regulation of TSPO, StAR, 5α-RI and 5α-RII, probably increasing spinal cord steroidogenic activity and favouring the

production of reduced metabolites and thus alleviating pain behaviors. Our findings are in line with previous studies showing that early repeated PG administration increases spinal TSPO expression and activity and reduces pain in animals subjected to a peripheral nerve injury (Liu et al. 2014). At least in this experimental model, the modulation of TSPO expression seems to be mediated by PG reduced metabolites (Liu et al. 2014). Further, PG regulation of 5α-RII expression could be mediated by the interaction with its intracellular receptor PR, since the presence of PR response elements has been previously described in the promoter region of the 5α-RII gene (Matsui et al. 2002). Thus, after PG treatment, the steroid could be transcriptionally regulating 5α-RII expression in order to favor its own conversion into reduced metabolites for efficient pain control.

Since the dorsal horn is fully equipped to metabolize PG to ALLO (Mensah-Nyagan et al. 2008) and, as we have shown here the generation of reduced metabolites seems to be favored after PG treatment (due to an increase in the expression of 5α-RI and 5α-RII), it is likely that when exogenously administered PG reaches the spinal cord it is converted to ALLO, which contributes to pain reduction via the allosteric modulation of $GABA_A$ receptors and the consequent reinforcement of inhibitory circuits.

Thus, by favoring local steroidogenesis and the production of its reduced metabolites, PG could play a potential role in the modulation of neuropathic pain.

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Figure legends

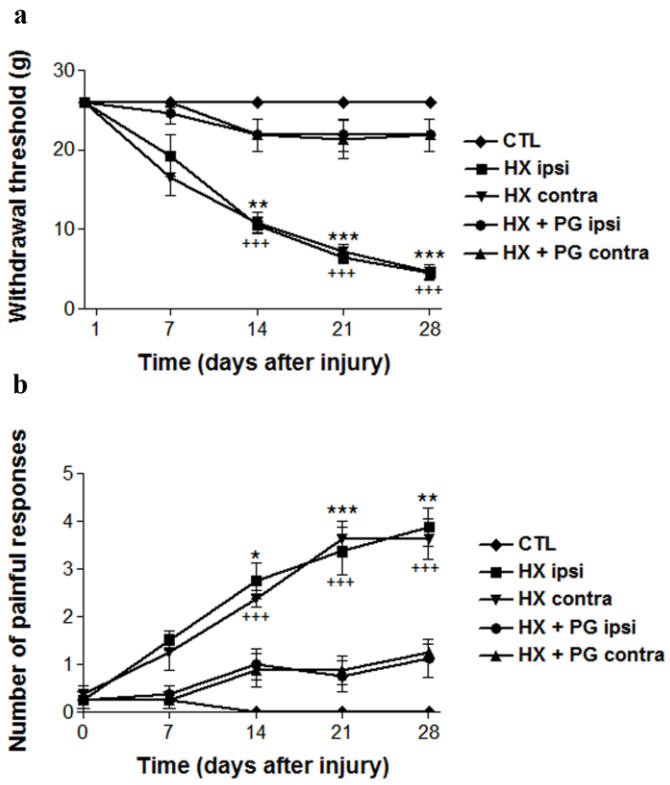

Figure 1: Spinal cord injury induced the development of mechanical (a) and thermal (b) allodynia in both the ipsilateral and contralateral hindpaws. Progesterone administration was able to prevent these pain-related behaviors (a,b). The following symbols were used to represent p values: * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ when comparing HX vs HX+PG, and $^{+}$ $p<0.05$, $^{++}$ $p<0.01$ and $^{+++}$ $p<0.001$ when comparing HX vs CTL.

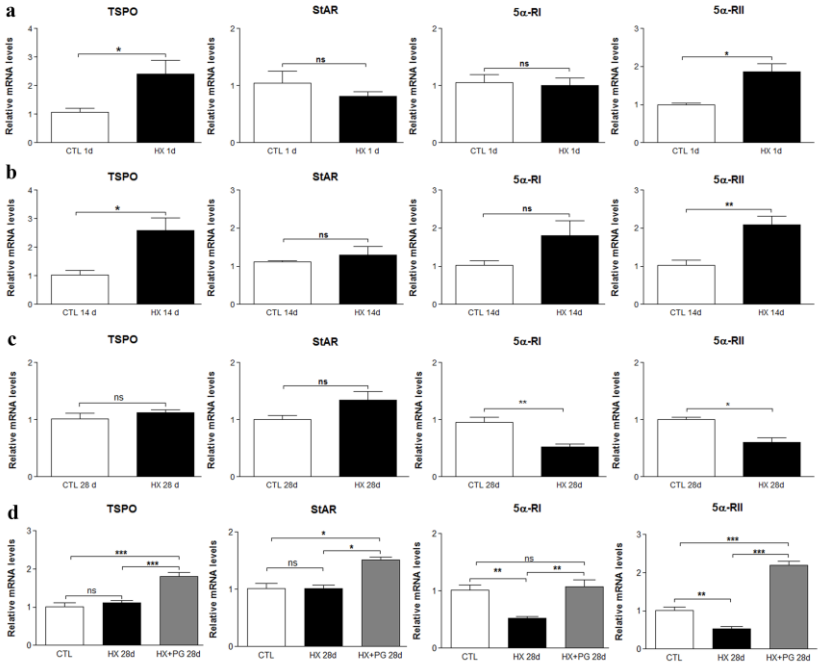

Figure 2: Relative mRNA levels corresponding to TSPO, StAR, 5α-RI and 5α-RII detected in the lumbar dorsal spinal cord 1, 14 and 28 days after spinal cord injury (a-c) or 28 days after spinal cord injury and progesterone administration (d). Note the significant increase in TSPO and 5α-RII expression detected 1 and 14 days after injury (a,b). Interestingly, in the chronic phase, TSPO returns to control values and there is a marked down-regulation of 5α-RI and 5α-RII (c). In animals receiving progesterone treatment, an increase in TSPO, StAR, 5α-RI and 5α-RII expression is observed, when compared to vehicle-treated injured animals. Symbols that represent p values: ns $p>0.05$, * $p<0.05$, ** $p<0.01$ and *** $p<0.001$.