Journal of Trace Elements in Medicine and Biology 54 (2019) 126–133

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

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

Pharmacology

3-(4-Chlorophenylselanyl)-1-methyl-1*H*-indole promotes recovery of neuropathic pain and depressive-like behavior induced by partial constriction of the sciatic nerve in mice

Paloma T. Birmann[a], Fernanda S.S. Sousa[b], Micaela Domingues[a], César A. Brüning[b], Beatriz M. Vieira[c], Eder J. Lenardão[c], Lucielli Savegnago[a,c,*]

[a] Technologic Development Center, Biotechnology Unit, Neurobiotechnology Research Group, Federal University of Pelotas, Pelotas, RS, Brazil
[b] Center of Chemical, Pharmaceutical and Food Sciences, Neurobiotechnology Research Group, Federal University of Pelotas, Pelotas, RS, Brazil
[c] Center of Chemical, Pharmaceutical and Food Sciences, Laboratory of Clean Organic Synthesis, Federal University of Pelotas, Pelotas, RS, Brazil

ARTICLE INFO

Keywords:
Comorbidity
Pain
Depression
Indole
Selenium

ABSTRACT

3-(4-Chlorophenylselanyl)-1-methyl-1*H*-indole (CMI) is an organoselenium compound that presents antioxidant activity, antinociceptive, anti-inflammatory and antidepressive-like effect in mice in previous studies conducted by our research group. In this study, we evaluate the anti-allodynic, anti-hyperalgesic and antidepressant-like effects of CMI on partial sciatic nerve ligation (PSNL) in male adult *Swiss* mice (25–35 g) as well as the involvement of oxidative stress in these effects. Mice underwent PSNL surgery and after 4 weeks they were treated with CMI (10 mg/kg, intragastric route [i.g.]) or vehicle. The treatment with CMI (10 mg/kg, i.g.) reversed the increased the percentage of response to Von-Frey Hair (VFH) stimulation, decreased the latency time to nociceptive response in the hot-plate test, increased immobility time in the forced swimming test (FST) and decreased groomings activity in the splash test, all induced by PSNL. Additionally, CMI also reversed increased the levels of reactive oxygen species (ROS) and lipid peroxidation in cortex and hippocampus and plasmatic levels of corticosterone in mice, induced by PSNL. Results demonstrate that CMI reversed behavioral and biochemical alterations in the dyad pain-depression induced by PSNL and possibly modulation of oxidative system.

1. Introduction

3-(4-chlorophenylsilanyl)-1-methyl-1*H*-indole (CMI), an organoselenium compound containing the indole moiety, represents a heterocyclic compound with promising biological properties already reported. Recently, a study performed by our research group showed that CMI significantly attenuated lipopolysaccharide (LPS)-induced depressive-like behavior in mice. CMI attenuated LPS-induced neuroinflammation by reducing the levels of interleukin(IL)-1β IL-4 and IL-6 in the hippocampus and prefrontal cortex, as well as markers of oxidative damage [1]. In another study, our research group demonstrated that the compound shows high reactivity against damage induced by peroxynitrous acid (ONOOH), suggesting that this compound may limit the loss of cell adhesion and matrix damage induced by ONOOH both in vitro and in vivo and may modulate oxidative and nitrosative damage at sites of inflammation, contributing to a reduction in tissue dysfunction and atherogenesis [2]. Also, in another study performed by our research group, demonstrated antinociceptive and anti-inflammatory effects of CMI in animal models. In addition, the antinociceptive effect of CMI was related to monoaminergic, opioidergic and adenosinergic modulations [3].

From these promising results reported of CMI our research group interested in continuing to investigate the biological properties this molecule in an animal model of comorbidity pain-depression, once this dyad occurs in up to 80% of patients causing negative socioeconomic impacts worldwide [4]. Moreover, epidemiological studies have demonstrated that some psychiatric disorders, such as depression, are more common among people suffering from chronic pain than in the general population [5].

The first choice in the treatment of chronic pain and depression is the pharmacological treatment with antidepressants, such as imipramine, together with anti-inflammatory drugs, such as non-steroidal anti-inflammatory drugs, for example, celecoxib [5,6]. Although these drugs present potential benefits, the response ratio to them is still low, due to their adverse effects and discontinuous treatment prematurely [7]. In this regarding, there is a gradual increase in the search for new

* Corresponding author at: Universidade Federal de Pelotas- UFPel, CEP: 96010-900, Pelotas, RS, Brazil.
E-mail address: luciellisavegnago@yahoo.com.br (L. Savegnago).

https://doi.org/10.1016/j.jtemb.2019.04.014
Received 28 November 2018; Received in revised form 18 April 2019; Accepted 23 April 2019
0946-672X/

compounds that have beneficial effects in order to minimize the effects on comorbid pain and depression.

In this context, taking into consideration the pharmacological properties of CMI, together with the low success rate of antidepressants and analgesics for this condition, it is important to develop more effective treatments against pain-depression dyad. The pain-depression dyad can be originated from damage to the neuronal tissue, involving the process of neuroinflammation and oxidative/nitrosative stress. Based on these observations, rodent models of inflammation-associated pain and depression have been developed, such as partial sciatic nerve ligation (PSNL) model, which is a potent activator of the immune system, consequently promoting the release of reactive species (RS) [8,9]. Peripheral nerve injury causes the production of local inflammatory mediators contributing to the establishment of a peripheral inflammatory response [10]. This peripheral inflammatory response is intensified by increased production of RS at the injury site, and this seems to be one of the mechanisms responsible for the intensification of nociceptive stimulation promoting hyperalgesia by exacerbating tissue damage at the lesion site [10,11]. However, the participation of oxidative mechanisms is not limited to the affected region, it is known that the mechanisms of hyperalgesia are not limited to the affected peripheral region, but are mainly determined by changes in the excitability of spinal dorsal horn cells causing a cellular modification at the central level leading to the development of a central inflammatory process [8,12]. These events are conducive to the development of psychiatric disorders, such as depression [13].

Thus, this study aimed to evaluate anti-hyperalgesic, anti-allodynic and antidepressive-like effect of CMI in partial sciatic nerve ligation (PSNL)-induced pain-depression dyad as well as the involvement of oxidative stress in these effects.

2. Material and methods

2.1. Animals

Male *swiss* mice (25–35 g) were used in this study. They were maintained at 22°C with fresh water and food *ad libitum*, under a 12:12 h light/dark cycle. All manipulations were performed between 8:00 a.m. and 5:00 p.m. The animal's manipulation was according to the guidelines of the Committee on Care and Use of Experimental Animal Resources at the Federal University of Pelotas, Brazil (CEEA 2226-2018).

2.2. Drugs

CMI (10 mg/kg) (Fig. 1) was prepared and characterized as previously described by Vieira et al. [14]. Analysis of the ^{1}H NMR and ^{13}C NMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure. CMI was dissolved in canola oil and administered by intragastric route (i.g.). Imipramine (10 mg/kg) was dissolved in saline solution (0.9%) and was purchased from a commercial pharmacy (*NOVARTIS*). All other chemicals were obtained at the highest available commercial grade.

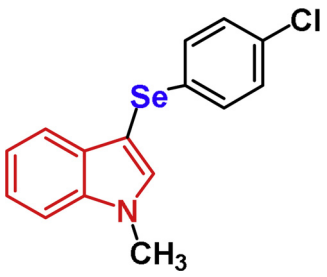

Fig. 1. Chemical structure of 3-(4-chlorophenylselanyl)-1-methyl-1*H*-indole (CMI).

2.3. Experimental design

The PSNL surgery was used as a model of induction of neuropathic pain and depressive-like behavior, performed according to the method described by Seltzer et al. [15] with modifications. The mice were randomly allocated into 6 groups as shown in Table 1. where on day 0 they underwent PSNL surgery, after the procedures the animals were kept in their respective cages for 4 weeks. At day 28 after PSNL surgery the animals have treated a single administration with CMI (10 mg/kg, i.g.), IMI (10 mg/kg, i.g.) or canola oil and after 30 min the animals were subjected to behavioral tests. After behavioral tests, animals were euthanized using isoflurane for biochemical analyses (Fig. 2).

2.3.1. Surgical procedure

Mice were anesthetized with ketamine (150 mg/kg) and xylazine (10 mg/kg) intraperitoneally (i.p.). The right sciatic nerve was exposed and isolated from the surrounding tissues. One ligature (8/0 Ethicon GmbH, Norderstedt, Germany) was made around approximately one-third to one-half of the diameter of the sciatic nerve. Finally, the muscular and skin layers were immediately sutured. A similar procedure was performed without ligation on mice in the sham-operated. After 4 weeks of surgery, the animals were submitted to behavioral tests and euthanized in order to perform biochemical analyses [16].

3. Behavioral tests

3.1. Open field test (OFT)

The OFT evaluates locomotor and exploratory activity in order to rule out non-specific effects as a psychostimulatory activity. The animals were placed individually in the center of a box (30 × 30 x 15 cm) divided into nine quadrants. The number of segments crossed with the four paws (locomotor activity) and the number of time the mice stood on rear limbs (exploratory activity) were observed for 5 min [17].

3.2. Mechanical allodynia

The mechanical allodynia threshold was assessed by the paw withdrawal frequency using von Frey filaments (VFH) (Soelting, Chicaco, IL) [18]. The results were expressed as a percentage of response frequency, which was determined after ten manual applications of a calibrated nylon VFH filament of 1 g (duration of 1–2 s each) perpendicular in the ipsilateral and contralateral hind paws (right and left hind paws, respectively).

3.3. Thermal hyperalgesia

In order to evaluate hyperalgesia, mice were placed on the hot plate with the temperature adjusted to 52 ± 1°C. The latency to the first hind paw licking or withdrawal was taken as an index of nociceptive threshold. The cut off time of 45 s was used to avoid damage to the paw [19].

3.4. Forced swimming test (FST)

The FST was performed as described by Porsolt et al. [20]. Mice were individually placed in a cylinder (15 cm diameter, 25 cm high) containing 20 cm of water maintained at 25 ± 1 °C. The water in the cylinder was changed after each swim session. The adaptation time was 2 min and after adaptation, the immobility time (in seconds) was recorded for 4 min A decrease in the duration of immobility is indicative of an antidepressant-like effect.

Table 1
Experimental design.

Experimental groups	Doses (mg/kg, i.g.)	Experimental condition
Sham + Vehicle	–	Without ligature to the nerve and no treatment
Sham + Imipramine	10	Without ligature to the nerve and treated with imipramine
Sham + CMI	10	Without ligature to the nerve and treated with CMI
PSNL + Vehicle	–	Subjected to PSNL and no treatment
PSNL + Imipramine	10	Subjected to PSNL and treated with imipramine
PSNL + CMI	10	Subjected to PSNL and treated with CMI

Fig. 2. Experimental timeline. Abbreviations: CMI: 3-(4-chlorophenylselanyl)-1-methyl-1H-indole; IMI: imipramine; i.g.: intragastrically.

3.5. Splash test

A 10% sucrose solution was squirted on the dorsal coat of each mice and the grooming activity (including nose/face grooming, head washing and body grooming) was recorded for 5 min. The grooming behavior of mice was observed as a measurement of motivational and self-care difficulties, which are considered to reflect some symptoms of depressive patients [21].

4. Biochemical analyses

4.1. Sample preparation

The cortex and hippocampus were collected e homogenized in 50 mM Tris-HCl, pH 7.4 (1:10, w/v). The homogenate was centrifuged at 2500 × g for 10 min at 4 °C, and the supernatant fraction (S1) was used for the determination of reactive oxygen species (ROS) quantification and thiobarbituric acid reactive species assay. These structures (cortex and hippocampus) were chosen because play an important role in the modulation of the limbic and brainstem structures, which are physiologically involved in mediating emotional behavior; thus, dysfunctions in these circuits must participate in the pathogenesis of depressive symptoms. In addition, the cerebral cortex It is often implicated in the pathophysiology of depression: it is found with increased blood flow in depressed patients and with reduced gray matter, in principle mediates the conscious aspects related to pain (physical), anxiety and depressive ruminations, modulates the emotional responses, participates in cognitive pathways and work memory, in addition, mediates apathy [22].

Plasma was obtained by centrifugation at 2500 × g for 10 min and utilized for biochemical assays.

4.2. Thiobarbituric acid reactive species (TBARS) assay

The evaluation of lipid peroxidation levels through the TBARS assays was performed as described by Ohkawa et al. [23]. An aliquot of the S1 (10 µL) was incubated with 8.1% sodium dodecyl sulfate (SDS), 0.8% TBA and acetic acid/HCl (pH3.4) at 95 °C during 60 min. Levels of TBARS were measured spectrophotometrically at 532 nm. The results were expressed as nmol TBARS/g tissue.

4.3. Reactive oxygen species (ROS) quantification

Quantification of ROS was determinate by a spectrofluorometric method, using 2′-7′-dichlorofluorescein diacetate (DCHF-DA) descript by Loetchutinat et al. [24]. Briefly, an aliquot of S1 (10 µL) was incubated with 1 mM dichloro-dihydro-fluorescein diacetate (DCHF-DA) and 10 mM Tris-HCl pH 7.4. The oxidation of DCFH-DA to fluorescent dichlorofluorescein (DCF) is measured for the detection of intracellular RS. The DCF fluorescence intensity emission was recorded at 520 nm (with 480 nm excitation). RS levels were expressed as units (U) of fluorescence.

4.4. Nitric oxide (NO) levels

NO is an unstable compound being rapidly oxidized into nitrate and nitrite after its production. After centrifugation of the homogenate at 12,000 × g for 20 min at 4 °C the nitrate was converted into nitrite by nitrate reductase and measured using the colorimetric Griess reaction in a microplate reader at a wavelength of 462 nm. The values obtained from this assay represent the amount of nitrite and nitrate derived from NO and were expressed as nitrite (mmol/mg of tissue) [25].

4.5. Plasma corticosterone level estimation

This method is based on the estimation of the plasma corticosterone concentration via fluorimetric assay as described by Zenker et al. [26]. The suitably diluted samples were treated with freshly prepared chloroform: sulfuric acid-ethanol mixture (7:3, v/v) and then extracted directly with the chloroform. The solvent was removed by aspiration and the acid-alcohol reagent transferred to a small cuvette for fluorescence determination in a fluorimeter at 247 nm excitation and 540 nm emission wavelengths and levels of plasma corticosterone were expressed in ng/mL.

5. Statistical analyses

All experimental results are expressed as the mean ± standard error of the mean (S.E.M.). Comparisons between experimental and control groups were performed by two-way ANOVA (STZ vehicle × CMI *vs* vehicle). When ANOVA revealed a significant, the Bonferroni *post-hoc* test was used for between-group comparisons. A value of $P < 0.05$ was considered to be significant. Main effects are presented only when the first order interaction was non-significant. The statistical analysis was accomplished using GraphPad Prism version 7.0 for Windows, Graph Pad Software (San Diego,CA, USA).

6. Results

6.1. Locomotor activity of mice

PSNL and/or the treatment with CMI or IMI did not cause significant

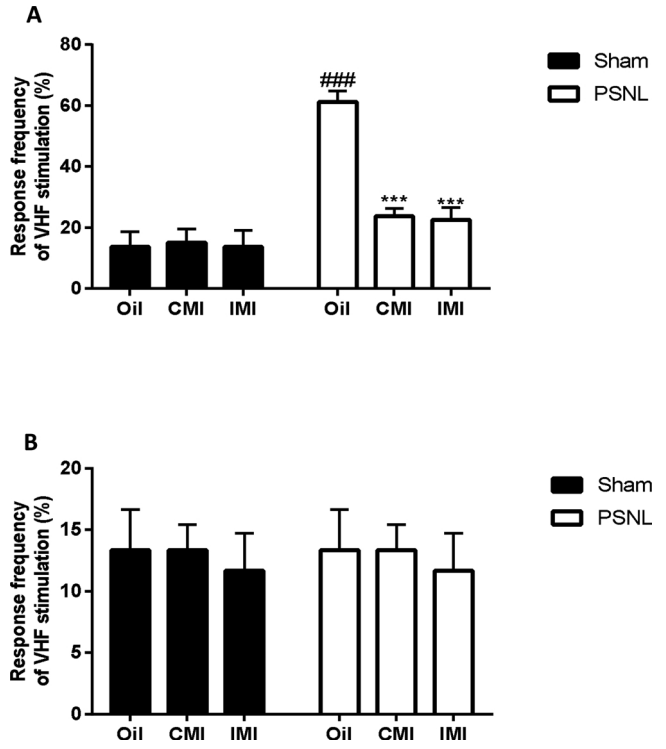

Fig. 3. Effect of CMI on the response frequency of VFH stimulation in the ipsilateral paw (A) and contralateral paw (B). Data are presented as the mean ± S.E.M. (n = 6–8). $^{###}p < 0.001$ when compared to the sham-vehicle group. $^{***}p < 0.001$ when compared to the PSNL-vehicle group. PSNL: partial sciatic nerve ligation; IMI: imipramine; CMI: 3-(4-chlorophenylselanyl)-1-methyl-1*H*-indole (Two-way ANOVA followed by *post hoc* Bonferroni's test).

changes in the number of crossings and rearings in the open-field test, discarding alterations in locomotor activity of mice (data not shown). A two-way ANOVA revealed no significant between groups interaction [$F_{(2,\ 36)} = 0.08$, $p = 0.91$] for the number of crossings and for the number of rearing [$F_{(2,\ 36)} = 0.48$, $p = 0.62$], but revealed a significant main-effect of CMI and IMI crossings [$F_{(}2,36_{)} = 6.11$, $P = 0.005$] and rearings [$F_{(}2,36_{)} = 5.41$, $P = 0.009$].

6.2. Effects of CMI on mechanical allodynia and thermal hyperalgesia induced by PSNL in mice

The results of the CMI in the mechanical allodynia are depicted in Fig. 4. A two-way ANOVA showed a statistically significant PSNL × treatments interaction for the increased the percentage of response to VFH stimulation [$F_{(}2,42_{)} = 13.56$, $P < 0.001$] in the ipsilateral paw (Fig. 3A). In the contralateral paw, there were no differences among groups in the percentage of response to VFH stimulation [$F_{(}2,42_{)} = 0.05$, $P = 0.95$] (Fig. 3B).

The two-way ANOVA of latency time to nociceptive response showed a significant PSNL × treatment interaction [$F_{(}2,30_{)} = 5.33$, $P = 0.001$]. As depicted in Fig. 4, CMI reduced the latency time to nociceptive response when compared to that of the PSNL group.

6.3. Effects of CMI on depressive-like behavior induced by PSNL in mice

The PSNL induced depressive-like behavior in mice. A two-way ANOVA showed a statistically significant PSNL × treatment interaction for the increased immobility time [$F_{(}2,36_{)} = 23.13$, $P < 0.001$] and decreased latency time to the first episode of immobility [$F_{(}2,36_{)} = 17.7$, $P < 0.001$] in the FST (Fig. 5A and 5B, respectively) and by decreased grooming time [$F_{(}2,36_{)} = 8.29$, $P < 0.001$] in the splash test (Fig. 6). This depressive-like behavior PSNL-induced was reversed

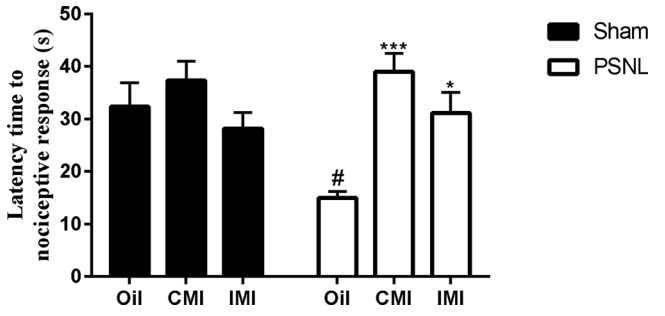

Fig. 4. Effect of CMI on the thermal hyperalgesia. Data are presented as the mean ± S.E.M. (n = 6). $^{#}p < 0.05$ when compared to the sham-vehicle group. $^{*}p < 0.05$ and $^{***}p < 0.001$ when compared to the PSNL-vehicle group. PSNL: partial sciatic nerve ligation; IMI: imipramine; CMI: 3-(4-chlorophenylselanyl)-1-methyl-1*H*-indole (Two-way ANOVA followed by *post hoc* Bonferroni's test).

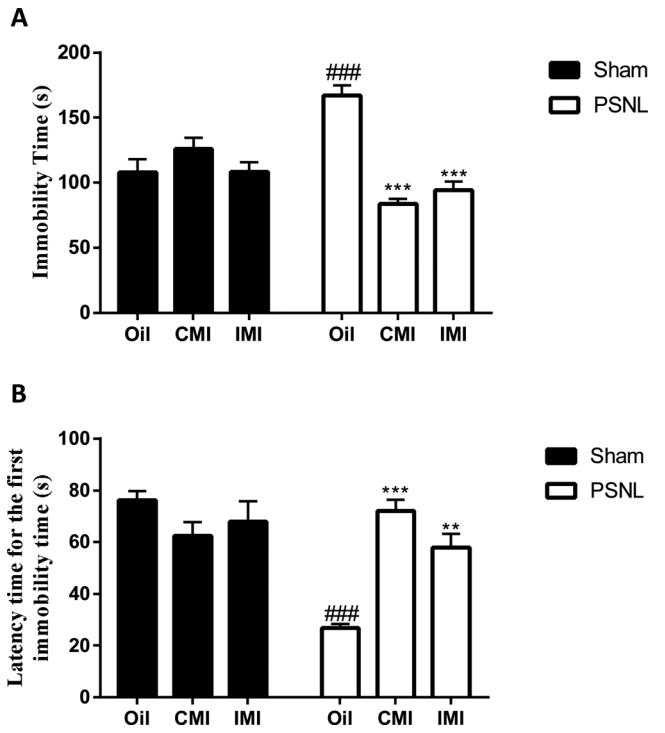

Fig. 5. Effect of CMI on immobility time (A) and latency time (B) in the TST. Data are presented as the mean ± S.E.M. (n = 7). $^{###}p < 0.001$ when compared to the sham-vehicle group. $^{**}p < 0.01$ and $^{***}p < 0.001$ when compared to the PSNL-vehicle group. PSNL: partial sciatic nerve ligation; IMI: imipramine; CMI: 3-(4-chlorophenylselanyl)-1-methyl-1*H*-indole (Two-way ANOVA followed by *post hoc* Bonferroni's test).

by the treatment with CMI (Fig. 7).

6.4. Effect of CMI on oxidative stress induced by PSNL in mice

The two-way ANOVA of ROS levels demonstrated a significant PSNL × treatment interaction in cortex (Fig. 7 A) [$F_{(}2,30_{)} = 9.03$, $P = 0.006$] and hippocampus (Fig. 7B) [$F_{(}2,30_{)} = 12.6$, $P < 0.001$]. The levels of ROS in both structures increased PSNL group (Fig. 8A). *Post-hoc* analysis demonstrated that CMI decreased ROS levels when compared to those of the sham group.

The two-way ANOVA of TBARS levels showed a significant PSNL × treatment interaction in the cerebral cortex [$F_{(}2,30_{)} = 12.38$, $P < 0.001$] (Fig. 8A) and hippocampus [$F_{(}2,30_{)} = 17.75$, $P < 0.001$] (Fig. 8B). Post-hoc analysis revealed that CMI decreased TBARS levels

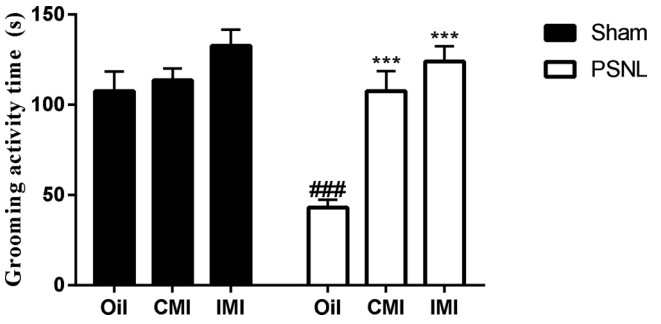

Fig. 6. Effect of CMI on duration of the grooming time in the splash test. Data are presented as the mean ± S.E.M. (n = 6). $^{###}p < 0.001$ when compared to the sham-vehicle group. $^{***}p < 0.001$ when compared to the PSNL-vehicle group. PSNL: partial sciatic nerve ligation; IMI: imipramine; CMI: 3-(4-chlorophenylselanyl)-1-methyl-1*H*-indole (Two-way ANOVA followed by *post hoc* Bonferroni's test).

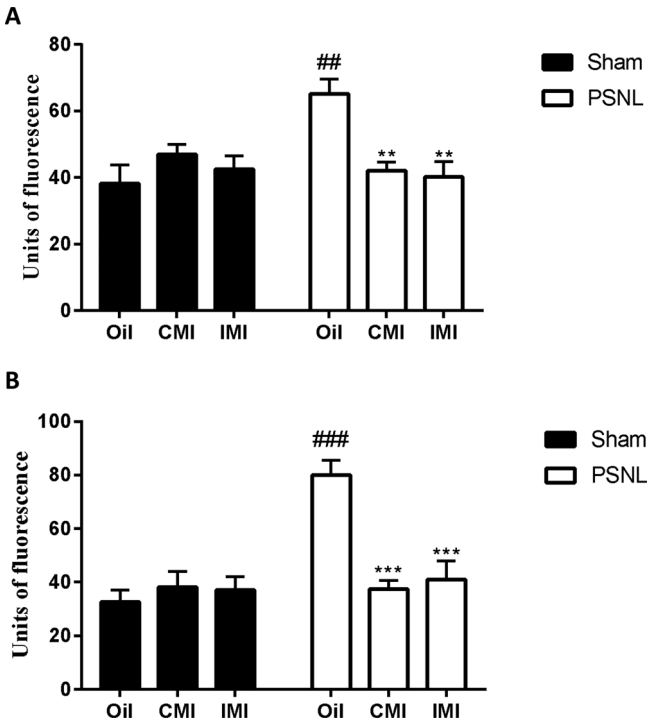

Fig. 7. Effect of CMI on RS levels in cortex (A) and hippocampus (B) of mice. Data are presented as the mean ± S.E.M. (n = 6). $^{##}p < 0.01$ and $^{###}p < 0.001$ when compared to the sham-vehicle group. $^{**}p < 0.01$ and $^{***}p < 0.001$ when compared to the PSNL-vehicle group. PSNL: partial sciatic nerve ligation; IMI: imipramine; CMI: 3-(4-chlorophenylselanyl)-1-methyl-1*H*-indole (Two-way ANOVA followed by *post hoc* Bonferroni's test).

when compared to those of the sham group.

The NO levels were increased by PSNL in the cerebral cortex as compared to sham group (Fig. 9A and 9B). A two-way ANOVA showed a statistically significant PSNL × treatments interaction for the increased NO levels in the [$F_{(2,30)} = 8.56$, $P < 0.001$] (Fig. 9A) and hippocampus [$F_{(2,30)} = 6.07$, $P = 0.006$] (Fig. 9A) of mice. Post-hoc analysis revealed that CMI reversed the increase of NO levels in both cerebral structures.

6.5. CMI treatment reverses increased plasma corticosterone levels induced by PSNL in mice

After 4 weeks of PSNL, mice exhibited increased plasmatic corticosterone levels. A two-way ANOVA demonstrated a statistically

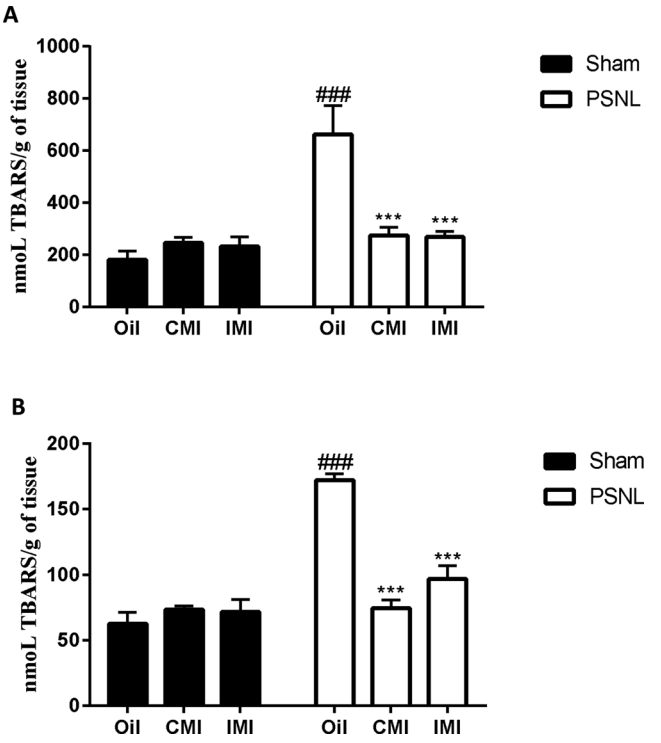

Fig. 8. Effect of CMI on lipid peroxidation in cortex (A) and hippocampus (B) of mice. Data are presented as the mean ± S.E.M. (n = 6). $^{###}p < 0.001$ when compared to the sham-vehicle group. $^{***}p < 0.001$ when compared to the PSNL-vehicle group. PSNL: partial sciatic nerve ligation; IMI: imipramine; CMI: 3-(4-chlorophenylselanyl)-1-methyl-1*H*-indole (Two-way ANOVA followed by *post hoc* Bonferroni's test).

significant PSNL × treatments interaction for the increased plasmatic corticosterone levels [$F_{(2,30)} = 26.71$, $P < 0.001$]. Post-hoc analysis revealed that CMI reversed the increase of plasmatic corticosterone levels (Fig. 10).

7. Discussion

In this study, we demonstrated the restorative effect of the treatment with CMI on the comorbidity of pain and depression induced by PSNL model. This effect was accompanied by reduction of lipid peroxidation, RS and NO levels induced by PSNL in cortex and hippocampus, since both structures are closely related to the neuropathology of depression and pain. Accordingly, neuroimaging studies have shown that there is a 4–10% reduction in hippocampal volume in depressed patients, as well as lower activation of the cortex in patients who have a depressed mood, or loss of interest or pleasure continuing for more than 2 weeks [27,28]. Another major finding was that CMI reversed PSNL-induced increased plasma corticosterone levels since hyperactivity of the hypothalamic-pituitary-adrenal axis (HPA) system is one of the consistent neurobiological findings in patients suffering from depression disorders [29,30]. In addition, CMI did not cause locomotor changes in the number of crossing and rearing in the OFT, excluding the possibility that previous results were affected by changes in locomotor and exploratory activity.

PSNL is a model that mimics the relationship of chronic pain and depression in humans by inducing allodynia (hypersensitivity to innocuous stimuli) and hyperalgesia (exacerbation of the sensitivity of the pain), which can be accompanied by a depressive-like behavior in mice [31,15]. This occurs because the peripheral nerve injury triggers complex anatomical and biochemical changes in the peripheral and central nerves [32]. After the damage to nerves, weakened nerve endings and neurons signal to glial cells via purinergic receptors,

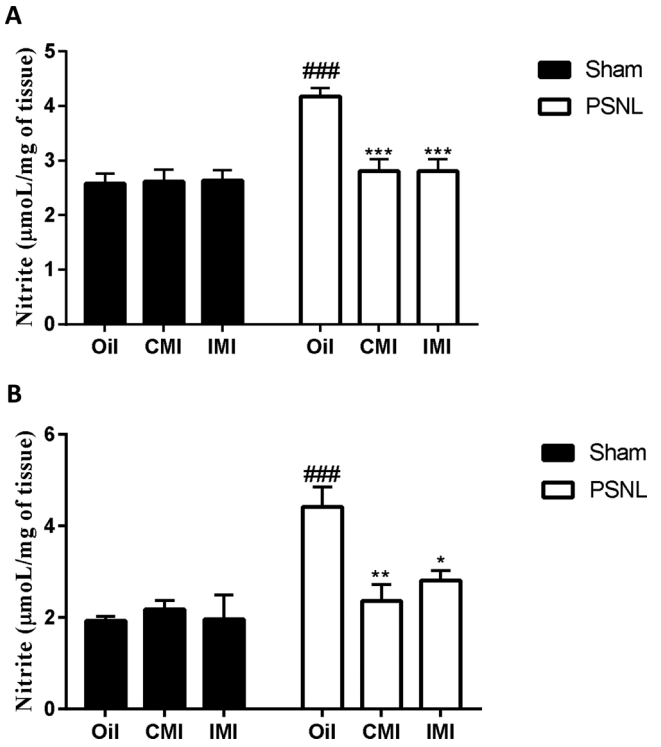

Fig. 9. Effect of CMI on nitrite levels in cortex (A) and hippocampus (B) of mice. Data are presented as the mean ± S.E.M. (n = 7-6). $^{###}$ $p < 0.001$ when compared to the sham-vehicle group. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ when compared to the PSNL-vehicle group. PSNL: partial sciatic nerve ligation; IMI: imipramine; CMI: 3-(4-chlorophenylselanyl)-1-methyl-1*H*-indole (Two-way ANOVA followed by *post hoc* Bonferroni's test).

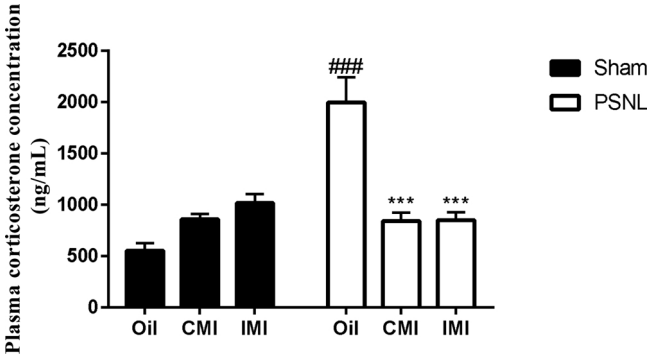

Fig. 10. Effect of CMI on corticosterone levels in plasma of mice. Data are presented as the mean ± S.E.M. (n = 6). $^{###}$ $p < 0.001$ when compared to the sham-vehicle group. *** $p < 0.001$ when compared to the PSNL-vehicle group. PSNL: partial sciatic nerve ligation; IMI: imipramine; CMI: 3-(4-chlorophenylselanyl)-1-methyl-1*H*-indole (Two-way ANOVA followed by *post hoc* Bonferroni's test).

chemokines, and protein kinase activation (p38 MAPK), promoting neuroinflammation through activation of microglia [33]. Activated microglia releases a variety of mediators, including pro-inflammatory cytokines and chemokines. In addition, occurs induction of cyclooxygenase-2 (COX-2), nitric oxide synthase (iNOS) and RS production that induces hyperexcitability in neurons, contributing to signaling and pathological increase of pain, as well as the development of disorders such as major depression. Thus, studies have pointed out that the development of the depression-pain dyad may be closely related to inflammatory events as well as oxidative stress [8,9].

The results of this study demonstrated that mice submitted to PSNL developed a depressive-like behavior indicated by a decrease in latency for the first episode of immobility and an increase in the total time of immobility in FST together with a decrease in self-care time in the splash test, and these changes were reversed with CMI treatment. These results corroborate with a previous study that has already demonstrated the antidepressant-like effect of CMI by attenuating the immobility time in the tail suspension test (TST) and FST increased by lipopolysaccharide (LPS) [1]. Moreover, the present results indicated that PSNL produced a significant increase in the response frequency of VFH stimulation in the ipsilateral hind paw and a decrease latency time to nociceptive response in hot plate test in the hind paw, and CMI administration reversed these effects. These findings are also corroborated by a previous study, which demonstrated antinociceptive effect of CMI in chemical and thermal tests [3]. The results presented here showed that 4 weeks after PSNL the animals developed behavior similar to depression as well as neuropathic pain and that the single administration of CMI reversed these changes.

PSNL increased levels of ROS, NO and lipid peroxidation in cortex and hippocampus of mice, and CMI showed to be effective in reversing these alterations. After the constriction of the sciatic nerve, RS such as peroxynitrite and NO are released by macrophages, Schwann cells and activated microglia [12,34]. The presence of RS triggers the intracellular cascade of second messengers and these events are then responsible for increased neuron sensitization, increasing the feeling of pain [35]. It is also known that RS oxidizes tetrahydrobiopterin (BH4), which is the enzymatic cofactor of tyrosine and tryptophan hydroxylase that participates in the synthesis of monoamines leading to the reduction of these neurotransmitters in the synaptic cleft. Moreover, RS are key mediators for activation of the neuroinflammation [36]. Through the NFκB pathway, RS leads to increased expression of proinflammatory cytokines, iNOS activation, biomolecule damage, and neuron death. All these events are closely associated with the neuroprogression of depression [37]. Our data reinforce findings in previous studies in which the CMI demonstrated antioxidant effect in prefrontal cortex and hippocampus in LPS-challenged mice, moreover, it is worth mentioning that in this same study the CMI ameliorated LPS-induced neuroinflammation by reducing the levels of interleukin (IL)-1β, IL-4 and IL-6 in the hippocampus and prefrontal cortex of the mice [1]. Thus, it can be presumed that the antidepressive-like and antinociceptive effects of the CMI may be associated to the modulation of oxidative stress and neuroinflammation.

In addition, it is known that through MAPK and NF-kB signaling pathways, proinflammatory cytokines activate the indoleamine 2,3-dioxygenase (IDO) enzyme and this activation contributes to the metabolism of tryptophan to kynurenine [38,39]. It is also known that the activation of p38 MAPK may decrease the expression of monoamine vesicle transporters ($VMAT_2$) and increase the neuronal serotonin transporters (SERT) [40,41]. These factors corroborate to a reduction in the serotonin levels in the synaptic cleft and thus, it is believed that they increase the senses of pain and depressive symptoms [42], since serotonin is an important neurotransmitter in emotional status and also in the nociceptive control by the descending inhibition pathway [43,44]. Remarkable, our previous study demonstrated the possible involvement of serotonergic receptors ($5\text{-}HT_{1A}$, $5\text{-}HT_3$, $5\text{-}HT_{2A/2C}$) in the antinociceptive action of CMI in mice [3]. These data may contribute to the findings of this present study.

Another important finding of this study was the increase in plasma levels of corticosterone in mice submitted to PSNL. As previously described, the HPA axis may be another mechanism involved in the processing of pain and depression, since nerve damage triggers the production of proinflammatory cytokines. These cytokines, in turn, activate the HPA axis, increasing the release of corticosterone (main steroid hormone in rodents) that will act through the glucocorticoid receptors (GR). High levels of glucocorticoids and pro-inflammatory cytokines induce resistance of GR, especially in the hippocampus, where there is a higher concentration of these receptors. This impairs the negative feedback mechanism of the HPA axis and also induces

atrophy in the hippocampal apical dendrites contributing to the development of the pain-depression dyad [29,30,13]. It has been postulated, that the abnormalities in the serotonergic system and hyperactivation of the HPA axis are linked and that this link is an important etiological mechanism in depression and pain, it has been suggested that increased corticosterone reduces 5-HT_{1A} receptor function and increases 5-HT_2 receptor function and of these changes may then act to dysregulate HPA axis function further [45]. Therefore, finding a compound that interacts with these two systems is of great importance for the treatment of pain-depression dyad. Moreover, in view of our results, we can suggest that CMI reverses plasma corticosterone levels, which makes it a more attractive molecule to be investigated.

Based on data from the present study and literature data, it is possible to suggest that the CMI promotes retrieval of neuropathic pain and depressive-like behavior. It is possible that this effect is mediated by the reduction of the RS and consequently less lipid peroxidation, suggesting the antioxidant activity of the compound. This antioxidant activity may be interrelated with the restoration of hyperalgesia, allodynia and depressive-like behavior induced by PSLN. These mechanisms differ from the mechanism of action of imipramine, a drug used as a positive control in this study. Imipramine is a tricyclic antidepressant, noradrenaline and serotonin reuptake inhibitor, used to treat neurogenic pain, rheumatologic pain, and depression [46].

In conclusion, the present study demonstrated the antidepressant-like, anti-allodynic and anti-hyperalgesic effects of CMI, at the dose of 10 mg/kg, in the comorbid pain-depression induced by PSNL, and these effects may be related to its antioxidant capacity and restoration of plasma levels of corticosterone altered by PSNL. These properties indicate CMI as a promising molecule for the treatment of pain and depression comorbidity. However, more studies are needed to elucidate the mechanisms of action of this organoselenium compound.

Declaration of interest

No conflict of interest.

Acknowledgements

The authors are grateful to UFPel and especially to the Programa de Pós Graduação em Biotecnologia (UFPel) for providing support to carry out this work. We would like to thank CNPq, CAPES and FAPERGS (PRONEM 16/2551-0000240-1) for the financial support. L.S. and E.J.L., are recipients of CNPq fellowship. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

References

[1] A.M. Casaril, M. Domingues, M. Fronza, B. Vieira, K. Begnini, E.J. Lenardão, F.K. Seixas, T. Collares, C.W. Nogueira, L. Savegnago, Antidepressant-like effect of a new selenium-containing compound is accompanied by a reduction of neuroinflammation and oxidative stress in lipopolysaccharide-challenged mice, J. Psychopharmacol. (Oxford) 31 (2017) 1263–1273, https://doi.org/10.1177/0269881117711713.

[2] A.M. Casaril, M.T. Ignasiak, C.Y. Chuang, B. Vieira, N.B. Padilha, L. Carroll, E.J. Lenardão, L. Savegnago, M.J. Davies, Selenium-containing indolyl compounds: kinetics of reaction with inflammation-associated oxidants and protective effect against oxidation of extracellular matrix proteins, Free Radic. Biol. Med. 113 (2017) 395–405, https://doi.org/10.1016/j.freeradbiomed.2017.10.344.

[3] P.T. Birmann, F.S.S. Sousa, D.H. de Oliveira, M. Domingues, B.M. Vieira, E.J. Lenardão, L. Savegnago, 3-(4-Chlorophenylselanyl)-1-methyl-1H-indole, a new selenium compound elicits an antinociceptive and anti-inflammatory effect in mice, Eur. J. Pharmacol. 827 (2018) 71–79, https://doi.org/10.1016/j.ejphar.2018.03.005.

[4] A. Graff-Guerrero, F. Pellicer, Y. Mendoza-Espinosa, P. Martínez-Medina, J. Romero-Romo, C. de la Fuente-Sandoval, Cerebral blood flow changes associated with experimental pain stimulation in patients with major depression, J. Affect. Disord. 107 (2008) 161–168, https://doi.org/10.1016/j.jad.2007.08.021.

[5] H. Kim, L. Chen, G. Lim, B. Sung, S. Wang, M.F. Mccabe, Contributes To the Comorbidity of Pain and Depression 122 (2012), pp. 2940–2954, https://doi.org/10.1172/JCI61884DS1.

[6] R. Baron, A. Binder, G. Wasner, Neuropathic pain: diagnosis, pathophysiological mechanisms, and treatment, Lancet Neurol. 9 (2010) 807–819, https://doi.org/10.1016/S1474-4422(10)70143-5.

[7] N. Attal, G. Cruccu, R. Baron, M. Haanpää, P. Hansson, T.S. Jensen, T. Nurmikko, EFNS guidelines on the pharmacological treatment of neuropathic pain: 2010 revision, Eur. J. Neurol. 17 (2010) 1113–1123, https://doi.org/10.1111/j.1468-1331.2010.02999.x.

[8] C. Sommer, M. Kress, Recent findings on how proinflammatory cytokines cause pain: peripheral mechanisms in inflammatory and neuropathic hyperalgesia, Neurosci. Lett. 361 (2004) 184–187, https://doi.org/10.1016/j.neulet.2003.12.007.

[9] A. Dellarole, P. Morton, R. Brambilla, W. Walters, S. Summers, D. Bernardes, M. Grilli, J.R. Bethea, Neuropathic pain-induced depressive-like behavior and hippocampal neurogenesis and plasticity are dependent on TNFR1 signaling, Brain Behav. Immun. 41 (2014) 65–81, https://doi.org/10.1016/j.bbi.2014.04.003.

[10] R.P. Guedes, L. Dal Bosco, C.M. Teixeira, A.S.R. Araújo, S. Llesuy, A. Belló-Klein, M.F.M. Ribeiro, W.A. Partata, Neuropathic pain modifies antioxidant activity in rat spinal cord, Neurochem. Res. 31 (2006) 603–609, https://doi.org/10.1007/s11064-006-9058-2.

[11] H.K. Kim, S.K. Park, J.L. Zhou, G. Taglialatela, K. Chung, R.E. Coggeshall, J.M. Chung, Reactive oxygen species (ROS) play an important role in a rat model of neuropathic pain, Pain 111 (2004) 116–124, https://doi.org/10.1016/j.pain.2004.06.008.

[12] V. Fasick, R.N. Spengler, S. Samankan, N.D. Nader, T.A. Ignatowski, The hippocampus and TNF: common links between chronic pain and depression, Neurosci. Biobehav. Rev. 53 (2015) 139–159, https://doi.org/10.1016/j.neubiorev.2015.03.014.

[13] A.K. Walker, A. Kavelaars, C.J. Heijnen, R. Dantzer, Neuroinflammation and comorbidity of pain and depression, Pharmacol. Rev. 66 (2013) 80–101, https://doi.org/10.1124/pr.113.008144.

[14] B.M. Vieira, S. Thurow, J.S. Brito, G. Perin, D. Alves, R.G. Jacob, C. Santi, E.J. Lenardão, Sonochemistry: an efficient alternative to the synthesis of 3-selanylindoles using CuI as catalyst, Ultrason. Sonochem. 27 (2015) 192–199, https://doi.org/10.1016/j.ultsonch.2015.05.012.

[15] Z. Seltzer, R. Dubner, Y. Shir, A novel behavioral model of neuropathic pain disorders produced in rats by partial sciatic nerve injury, Pain. 43 (1990) 205–218, https://doi.org/10.1016/0304-3959(90)91074-S.

[16] B. Malmberg, I. Basbaum, Partial sciatic nerve injury in the mouse as a model of neuropathic pain: behavioral and neuroanatomical correlates, Pain 76 (1998) 215–222 doi:S0304-3959(98)00045-1 [pii].

[17] R.N. Walsh, R.A. Cummins, The open-field test: a critical review, Psychol. Bull. 83 (1976) 482–504, https://doi.org/10.1037/0033-2909.83.3.482.

[18] L.B. Bortalanza, J. Ferreira, S.C. Hess, F. Delle Monache, R.A. Yunes, J.B. Calixto, Anti-allodynic action of the tormentic acid, a triterpene isolated from plant, against neuropathic and inflammatory persistent pain in mice, Eur. J. Pharmacol. 453 (2002) 203–208, https://doi.org/10.1016/S0014-2999(02)02428-7.

[19] I. Yalcin, A. Charlet, M.J. Freund-Mercier, M. Barrot, P. Poisbeau, Differentiating thermal allodynia and hyperalgesia using dynamic hot and cold plate in rodents, J. Pain 10 (2009) 767–773, https://doi.org/10.1016/j.jpain.2009.01.325.

[20] R.D. Porsolt, M. Le Pichon, M. Jalfre, Depression: A new animal model sensitive to antidepressant treatments [27], Nature 266 (1977) 730–732, https://doi.org/10.1038/266730a0.

[21] A. Haj-Mirzaian, S. Amiri, N. Kordjazy, M. Rahimi-Balaei, A. Haj-Mirzaian, H. Marzban, A. Aminzadeh, A.R. Dehpour, S.E. Mehr, Blockade of NMDA receptors reverses the depressant, but not anxiogenic effect of adolescence social isolation in mice, Eur. J. Pharmacol. 750 (2015) 160–166, https://doi.org/10.1016/j.ejphar.2015.01.006.

[22] V. Maletic, Neurobiology of depression, fibromyalgia and neuropathic pain, Front. Biosci. 14 (2009) 5291, https://doi.org/10.2741/3598.

[23] H. Ohkawa, N. Ohishi, K. Yagi, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, Anal. Biochem. 95 (1979) 351–358, https://doi.org/10.1016/0003-2697(79)90738-3.

[24] C. Loetchutinat, S. Kothan, S. Dechsupa, J. Meesungnoen, J.P. Jay-Gerin, S. Mankhetkorn, Spectrofluorometric determination of intracellular levels of reactive oxygen species in drug-sensitive and drug-resistant cancer cells using the 2′,7′-dichlorofluorescein diacetate assay, Radiat. Phys. Chem. 72 (2005) 323–331, https://doi.org/10.1016/j.radphyschem.2004.06.011.

[25] J.F. Cryan, a Markou, I. Lucki, Assessing antidepressant activity in rodents: recent developments and future needs, Trends Pharmacol. Sci. 23 (2002) 238–245, https://doi.org/10.1016/S0165-6147(02)02017-5.

[26] N, B.D. Zenker, NICOLAS DONALD and medicine, J. Biol. Chem. (1957).

[27] T. Akiyama, M. Koeda, Y. Okubo, M. Kimura, Hypofunction of left dorsolateral prefrontal cortex in depression during verbal fluency task: a multi-channel near-infrared spectroscopy study, Elsevier B.V. (2018), https://doi.org/10.1016/j.jad.2018.01.010.

[28] P.C. Koolschijn, N.E. van Haren, G.J. Lensvelt-Mulders, H.E. Hulshoff Pol, R.S. Kahn, Brain volume abnormalities in major depressive disorder: a meta-analysis of magnetic resonance imaging studies, Hum. Brain Mapp. 30 (2009) 3719–3735, https://doi.org/10.1002/hbm.20801.

[29] S. Esmaeili-Mahani, S. Vahedi, F. Motamedi, A. Pourshanazari, M. Khaksari, A. Ahmadiani, Involvement of hypothalamic pituitary adrenal axis on the analgesic cross-tolerance between morphine and nifedipine, Pharmacol. Biochem. Behav. 86 (2007) 806–812, https://doi.org/10.1016/j.pbb.2007.03.011.

[30] T. Demuyser, E. Bentea, L. Deneyer, G. Albertini, A. Massie, I. Smolders, Disruption of the HPA-axis through corticosterone-release pellets induces robust depressive-like behavior and reduced BDNF levels in mice, Neurosci. Lett. 626 (2016)

119–125, https://doi.org/10.1016/j.neulet.2016.05.026.
[31] F.T. Nickel, F. Seifert, S. Lanz, C. Maihöfner, Mechanisms of neuropathic pain, Eur. Neuropsychopharmacol. 22 (2012) 81–91, https://doi.org/10.1016/j.euroneuro.2011.05.005.
[32] J. Scholz, C.J. Woolf, The neuropathic pain triad: neurons, immune cells and glia, Nat. Neurosci. 10 (2007) 1361–1368, https://doi.org/10.1038/nn1992.
[33] D. Julius, Molecular mechanisms of nociception, Nature 413 (2001) 203–210, https://doi.org/10.1038/35093019.
[34] A.H. Miller, C.L. Raison, Cytokines, p38 MAP kinase and the pathophysiology of depression, Neuropsychopharmacology 31 (2006) 2089–2090, https://doi.org/10.1038/sj.npp.1301032.
[35] L.R. Watkins, E.D. Milligan, S.F. Maier, Glial activation: a driving force for pathological pain, Trends Neurosci. 24 (2001) 450–455, https://doi.org/10.1016/S0166-2236(00)01854-3.
[36] C. Cunnington, K.M. Channon, Tetrahydrobiopterin: pleiotropic roles in cardiovascular pathophysiology, Heart 96 (2010) 1872–1877, https://doi.org/10.1136/hrt.2009.180430.
[37] A. Slyepchenko, M. Maes, C.A. Köhler, G. Anderson, J. Quevedo, G.S. Alves, M. Berk, B.S. Fernandes, A.F. Carvalho, T helper 17 cells may drive neuroprogression in major depressive disorder: proposal of an integrative model, Neurosci. Biobehav. Rev. 64 (2016) 83–100, https://doi.org/10.1016/j.neubiorev.2016.02.002.
[38] F. Andreetta, N.M. Barnes, P.B. Wren, L. Carboni, P38 MAP kinase activation does not stimulate serotonin transport in rat brain: implications for sickness behaviour mechanisms, Life Sci. 93 (2013) 30–37, https://doi.org/10.1016/j.lfs.2013.05.014.
[39] A.M. Myint, Y.K. Kim, Cytokine-serotonin interaction through IDO: a neurodegeneration hypothesis of depression, Med. Hypotheses 61 (2003) 519–525, https://doi.org/10.1016/S0306-9877(03)00207-X.
[40] C. Bin Zhu, R.D. Blakely, W.A. Hewlett, The proinflammatory cytokines interleukin-1beta and tumor necrosis factor-alpha activate serotonin transporters, Neuropsychopharmacology. 31 (2006) 2121–2131, https://doi.org/10.1038/sj.npp.1301029.
[41] S. Malynn, A. Campos-Torres, P. Moynagh, J. Haase, The pro-inflammatory cytokine TNF-α regulates the activity and expression of the serotonin transporter (SERT) in astrocytes, Neurochem. Res. 38 (2013) 694–704, https://doi.org/10.1007/s11064-012-0967-y.
[42] X. Zhao, Y. Zhang, W. Qin, J. Cao, Y. Zhang, J. Ni, Y. Sun, X. Jiang, J. Tao, Serotonin type-1D receptor stimulation of A-type K+ channel decreases membrane excitability through the protein kinase A- and B-Raf-dependent p38 MAPK pathways in mouse trigeminal ganglion neurons, Cell. Signal. 28 (2016) 979–988, https://doi.org/10.1016/j.cellsig.2016.05.004.
[43] Y. Xu, H. Sheng, Z. Tang, J. Lu, X. Ni, Inflammation and increased IDO in hippocampus contribute to depression-like behavior induced by estrogen deficiency, Behav. Brain Res. 288 (2015) 71–78, https://doi.org/10.1016/j.bbr.2015.04.017.
[44] D.J. Haleem, Serotonin-1A receptor dependent modulation of pain and reward for improving therapy of chronic pain, Pharmacol. Res. 134 (2018) 212–219, https://doi.org/10.1016/j.phrs.2018.06.030.
[45] R.J. Porter, P. Gallagher, S. Watson, A.H. Young, Corticosteroid-serotonin interactions in depression: a review of the human evidence, Psychopharmacology (Berl.) 173 (2004) 1–17, https://doi.org/10.1007/s00213-004-1774-1.
[46] R. Muscat, D. Sampson, P. Willner, Dopaminergic mechanism of imipramine action in an animal model of depression, Biol. Psychiatry 28 (1990) 223–230, https://doi.org/10.1016/0006-3223(90)90577-O.