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Cortical Neurostimulation and N-Methyl-D-Aspartate Glutamatergic Receptor Activation in the Dysgranular Layer of the Posterior Insular Cortex Modulate Chronic Neuropathic Pain

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ABSTRACT

Background and Aims: The dysgranula parts of the posterior insular cortex (PIC) stimulation (PICS) has been investigated as a new putative cortical target for nonpharmacologic therapies in patients with chronic and neuropathic pain (NP). This work investigates the neural bases of insula neurostimulation–induced antinociception and glutamatergic neurochemical mechanisms recruited by the PICS in animals with neuropathy.

Materials and Methods: Male Wistar rats were submitted to the von Frey and acetone tests to assess mechanical and cold allodynia after 21 days of chronic constriction injury (CCI) of the sciatic nerve or Sham procedure ("false operated"). Either the Cascade Blue 3000 MW lysine-fixable dextran (CBD) or the biotinylated dextran amine 3000 MW (BDA) neural tract tracer was microinjected into the PIC. The electrical PICS was performed at a low frequency (20 μA, 100 Hz) for 15 seconds by a deep brain stimulation device. PIC N-methyl-D-aspartate (NMDA) receptors (NMDAR) blockade with the selective antagonist LY235959 (at 2, 4, and 8 nmol/200 nL) followed by PICS was investigated in rats with CCI.

Results: PIC sends projections to the caudal pontine reticular nucleus, alpha part of the parvicellular reticular nucleus, dorsomedial tegmental area, and secondary somatosensory cortex (S_2). PICS decreased both mechanical and cold allodynia in rats with chronic NP. Blockade of NMDAR in the PIC with LY235959 at 8 nmol attenuated PICS-produced antinociception.

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Conclusion: Neuroanatomic projections from the PIC to pontine reticular nuclei and S_2 may contribute to chronic NP signaling. PICS attenuates the chronic NP, and the NMDA glutamatergic system in the PIC may be involved in PICS-induced antinociception in rodents with NP conditions.

Keywords: Chronic constriction injury (CCI) of the sciatic nerve, chronic neuropathic pain, cortical neurostimulation, NMDA glutamatergic receptors, posterior dysgranular insular cortex (PIC)

Conflict of Interest: The authors reported no conflict of interest.

INTRODUCTION

Considered a health crisis owing to its high prevalence and associated physical and emotional disability, chronic pain (CP) is one of the leading causes of disability in many regions of the world.[1,2] Neuropathic pain (NP) is a critical and prevalent chronic condition in the adult population, and its chronicity affects approximately 20% of people.[3]

Several healthy professional efforts have been made to produce clinical practice guidelines for NP treatment through the administration of opioids, anticonvulsants, antidepressants, NMDA antagonists, and nonsteroidal anti-inflammatory drugs. However, owing to methodologic and conceptual reasons, its applicability becomes limited, resulting in low efficacy in pain relief and, consequently, people's poor quality of life.[4] Although these therapies suffice to control pain in many cases, up to 40% of patients become resistant and remain symptomatic.[5]

Concerning the pharmacologic treatment for pain conditions, there is evidence that the prescription of opioid analgesics has only limited effectiveness in pain management. In addition, the National Institute for Health and Care Excellence guideline on chronic pain (NG193) recommends considering an antidepressant to manage chronic pain because these medicines may help with the quality of life, pain, sleep, and psychological distress, even in the absence of a diagnosis of depression.[6]

Considering cases in which the condition is refractory to pharmacologic treatment, neuromodulation is an alternative therapy for the treatment of CP and NP. Since the 1990s, significant advances and attention have been obtained for its effectiveness in treating several pain syndromes, such as cortical epidural stimulation.[7–9] It was shown that electrical stimulation of the motor cortex induced antinociception in naïve rats owing to the inhibition of thalamic nuclei and disinhibition of the periaqueductal gray matter (PAG) mediated by the ventral posteromedial nucleus of the thalamus in rats with chronic constriction injury (CCI) of the nervus ischiadicus (sciatic nerve).[10]

The primary motor cortex (M_1) electrical stimulation (MCS) attenuated cold allodynia in rats with chronic NP 21 days after CCI.[11] In addition, Negrini-Ferrari et al[12] showed that the M_1 cortex glutamatergic system is also involved in the modulation of chronic NP in the model of spinal nerve injury in rats. The antinociceptive effect of MCS may depend on glutamate signaling recruiting the N-methyl-D-aspartate receptor (NMDAR) located on the dorsal column of PAG neurons in rodents with chronic NP.

There is a growing interest in understanding the mechanisms involved in neuromodulation. Therefore, considering these non-motor areas as potential research targets, exploration of the activity of new cortical telencephalic areas will expand knowledge about putative preclinical and clinical models for pain attenuation. Evaluating the involvement of the PIC as a possible neural target regarding the procedures for producing analgesia can be crucial.

Studies point to the role of the posterior insular cortex (PIC) in pain control, especially in patients with chronic pain and NP. These patients may have abnormal cortical activity in ipsilateral and contralateral stimuli.[13] They may differentially respond to thermal and mechanical pain perceptions evoked by laser stimulation.[14]

It is known that painful stimuli activate the insular cortex (IC). Most functional imaging studies indicate that the insular activation is bilateral, especially in a region known as the dorsal posterior insula, which is fundamental for nociception and homologous to the human insula. In addition, this dorsal posterior insula region presents itself as a nociceptive cortical region specific for sensory input, characterizing the sensory-discriminatory aspects.[15] However, little is known about the modulatory mechanisms of these responses.[16] Furthermore, brain imaging studies show that the IC is constantly activated under NP conditions.[17]

Glutamatergic neurotransmission recruiting NMDAR interacts in several functions, such as neural development, synaptic plasticity, learning, memory, and especially NP conditions.[18] Increased glutamate concentrations can contribute to pain and central sensitization and increase levels in NP conditions.[19,20] In contrast, increased brain activation is observed in fMRI studies with the administration of the NMDAR agonist d-cycloserine, in the learning context, denoting better behavioral outcomes.[21]

In this sense, studying the effect of the dysgranula parts of PIC stimulation (PICS) (by deep brain stimulation [DBS] device) and the NMDARs selective antagonist LY235959 microinjections effects on PICS-induced antinociception modulation becomes interesting. The current study addresses these issues by studying the effects caused by both PICS and blockade of NMDARs focused on the dysgranular layer of the PIC in laboratory animals submitted to an experimental neuropathy procedure.

MATERIAL AND METHODS

Animals

Male Wistar rats ($N = 83$) were used; they initially weighed approximately 100 g (~40 days) and were supplied by the Central Animal Facility of the University of São Paulo, Ribeirão Preto Campus. They were housed in the vivarium of the Department of Surgery and Anatomy of Ribeirão Preto School of Medicine of the University of São Paulo (FMRP-USP) under a 12/12-hour light/dark cycle, in controlled temperature (22 ± 2 °C) and humidity (55%), with access to water and food ad libitum. The handling of animals followed the Ethical Principles in Animal Experimentation. The project was submitted to the Ethics Committee on the Use of Animals of the FMRP-USP, which follows federal law number 11.794 of October 8, 2008. The experiments were carried out according to

the ethical principles elaborated by the Animal Experimentation Ethics Committee, approved by FMRP-USP (Process: 241/2019). All experiment sessions were performed by a trained researcher who blindly analyzed the experimental data.

Nociceptive Test: Mechanical Allodynia Threshold

The von Frey filament test (North Coast) was used to assess the nociceptive threshold to mechanical stimulation. It consists of a set of nylon monofilaments of various thicknesses that exert different degrees of force when applied to the plantar surface of the paw, thus allowing the evaluation of the amount of force necessary to evoke the withdrawal behavior.

The animals (n = 7–8 per group) were individually placed in acrylic boxes, measuring 23 × 20 × 18 cm, arranged on a table with a nonmalleable steel grid floor, with 5 mm^2 of space between the meshes. The tip of the stimulation rod was applied between the floor meshes on the center of the plantar hind paw of each rat until the animal displayed the response of withdrawal of the stimulated paw. After that, von Frey's filaments were applied in ascending order to the midplantar surface of the injured hind paw through the mesh floor. If the use of the filament three times did not evoke a reaction, the higher-pressure filament was used. The time interval before applying the next filament was at least 5 seconds. The cut-off value force in grams was 100 g.[22–25] Data were expressed as mean ± SEM of the paw withdrawal threshold in grams. This procedure was performed on the injured right paw of each animal with CCI (ipsilateral paw) and the left paw (contralaterally to the CCI or Sham surgery).

The von Frey test baseline 1 (before CCI or Sham surgeries) was recorded 30 minutes after the laboratory animals were acclimatized to the experimental apparatus; a von Frey test baseline 2 was performed 21 days after the CCI or Sham procedure for each pharmacologic treatment or IC stimulation. The open field test was

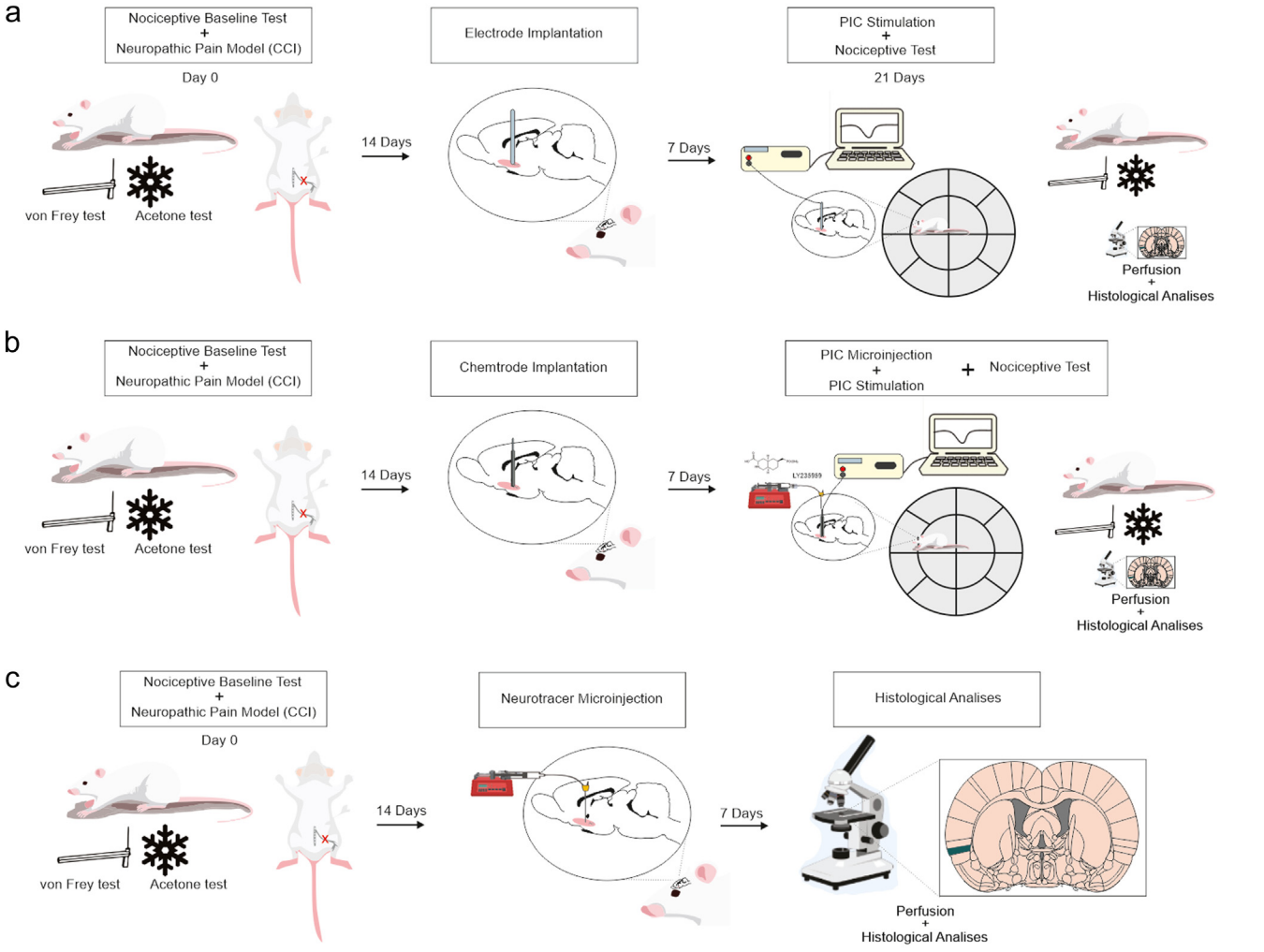

Figure 1. Timeline of the experimental procedure. The animals (n = 8 per group) were divided into groups. The temporal sequence of experiments involving electrical stimulation in the dysgranular region of the PICS using the DBS equipment (a), the glutamatergic neurotransmission blockade of the dysgranular PIC through the microinjection of the NMDAR selective antagonist LY235959, followed by electrical stimulation of PIC in rats with CNP (b) and neural tract tracing (c). The mechanical or thermal stimulus–induced response threshold was measured once before the CCI of the ischiadicus nervus or Sham procedure (day 1). On the 14th day, stereotaxic surgery was performed to implant the electrical stimulation electrode or chemitrode for microinjection of drugs inside the insula. After 21 days of CCI or Sham procedures, the mechanical stimulus–induced response threshold was measured before either the NMDAR blockade or PICS. The mechanical and thermal withdrawal responses threshold was measured at the following time until 30 minutes after the pharmacologic treatment or neurostimulation of the insula, respectively. Subsequently, the animals were perfused, and histologic analyses were conducted. [Color figure can be viewed at www.neuromodulationjournal.org]

performed for 5 minutes before and after the either pharmacological treatment or DBS of the PIC. Furthermore, 5 minutes after the open field test, the von Frey test was performed in rodents for 30 minutes every 10 minutes (Fig. 1).

Nociceptive Test: Acetone Test (Cold Allodynia Model)

The acetone test was used to assess cold stimulus allodynia (n = 7–8 per group). In the test, the rats were positioned on a platform (the same used in the von Frey test) in which the lower surface of the animal's paw was accessed. Next, 0.5 mL of 100% acetone was administered to the plantar surface of the rat's hind paw, starting from the bottom of the grid. The nociception afferent is given by a score that consists of three classes: 0—no movement; 1—rapid and sudden movement of the paw; 2—repeated movement of lifting the paw; 3—paw movement followed by licking.[26] If the animal behavior is sketched in 20 seconds, it is observed by measure plus 20 seconds, the sum of the behaviors sketched in 40 seconds, with repetition in three nociceptive times of the test. If the score in 20 seconds is 0, no more 20-second repetitions are needed. Acetone also was applied to the animal's left paw, contralateral to the CCI or Sham surgery (Fig. 1).

Open Field Test

The open field apparatus consists of a circular crystal acrylic arena (97 cm in diameter with 32.5 cm high walls, open top, and floor divided into 19 similar sections). Each animal (n = 7–8 per group) was placed gently in the center of the enclosure, and the motor behavior was recorded by a handcam (Sony Handycam HDR-SR10, Osaki, Shinagawa, Tokyo, Japan) for 5 minutes immediately after the PIC electrical stimulation. The videos were further analyzed using X-Plo-Rat software. The software was developed by the research team of Dr Morato (Ribeirão Preto School of Philosophy, Science and Literature of the University of São Paulo, Brazil). The test evaluated the environmental exploration and general locomotor activity (the number of crossings surveys).[24]

Surgery for Chronic Constriction Injury of the Sciatic Nerve Procedure

To induce experimental peripheral mononeuropathy, the animals were submitted to the procedure of CCI of the sciatic nerve, as previously described by Bennett and Xie,[27] modified by Sommer and Myers,[28] and adapted by Medeiros et al.[29,30]

Initially, the animals were anesthetized by intramuscular administration in the left hind paw, using a solution in the proportion of 0.1 ml of ketamine at 92 mg/kg (União Química Farmacêutica Nacional, Brazil) for 0.2 ml of 9.2 mg/kg xylazine (Hertape/Calier, Juatuba, Minas Gerais, Brazil). The animals were placed prone on a table, and the right hind paw was held by tape. Trichotomy of this paw and skin disinfection with povidone-iodine was performed. Next, a 15-mm longitudinal incision was made at the height of the thigh, dorsolateral region, at the level of the trochanter/femur. The right sciatic nerve was accessed and exposed through muscular dissection of the greater gluteus and femoral biceps. A simple ligation was performed, an experimental model adapted from Medeiros et al,[29,30] with 4-0 catgut thread in the sciatic nerve of the right paw proximal to the sciatic nerve trifurcation. The tension generated in the ligation was of mild intensity, sufficient to cause mild ischemia without interrupting total blood flow. The skin incisions were sutured with 5-0 mononylon suture, and hydrogen peroxide was passed on the right hind limb of the rodent. The "false operated" control group (Sham) underwent all surgical procedures by the exposure of the right sciatic nerve but without its ligation (without the CCI) (Fig. 1).

Stereotaxic Surgery

Fourteen days after CCI or Sham surgery, the animals (n = 7–8 per group) were taken to the stereotaxic apparatus (Insight, Ribeirão Preto, São Paulo, Brazil), where the upper incisors were used to fix their heads. Before exposure of the skullcap, the skin and subcutaneous tissue were anesthetized with a 2% lidocaine solution (0.1 mL, s.c.). The periosteum was removed, and the skullcap was dried with 10% hydrogen peroxide. The implantation of either the electrode or the chemitrode was into the contralateral hemisphere (left side) relative to the paw submitted to surgery (CCI or Sham—right hind limb). Either an electrode was implanted in the dysgranula parts of PIC for the DBS procedure, or a chemitrode (electrode with a guide cannula) was used for the microinjection of drugs (before the electrical stimulation) directly into the area functionally associated with the hind limb, according to the following coordinates: AP = −0.48 mm; ML = 5.8 mm and DV = 6.9 mm for electrode; and DV = 5.9 mm for chemitrode with guide cannula, according to the atlas of Paxinos and Watson.[31] For intracortical microinjections of drugs, the guide cannula must be 15 mm and the injector needle 16 mm long, and for PICS, a 16 mm electrode was used.

After implantation, either the electrode or the chemitrode was fixed to the calvaria with a self-curing acrylic prosthesis, which, in turn, was anchored by two stainless steel furniture screws (Fig. 1).

Electrical Stimulation of Dysgranular Layer of the PIC (PICS)

One week after stereotaxic surgery (for implantation of the electrode in the dysgranular layer of the PIC), the animals (n = 7–8 per group) were placed in a circular arena (60 cm in diameter and 50 cm in height, with the floor divided into 12 sections), with the experimental compartment illuminated with a 40 W fluorescent lamp (350 lx at arena floor level). Next, the electrode implanted in the PIC was connected to an stimulus generator (STG3008-FA, multichannel system, Thomas Recording, Giessen, Germany) which allowed the application of pulse current (cathode pulse width 100 µs, pulse interval 100, and anodic pulse width of 100 µs, repeating for 15 seconds). Brain stimulation was performed at the intensity in steps of 20 µA for 15 seconds. In fact, the effect of the electrical stimulation from other DBS targets varies depending on the choice of stimulation parameters, such as current amplitude, frequency, and pulse width.[32–34] Next, we chose the intensity used in this work (20 µA/15 s), based on previous finds.[11,12]

After this procedure, the animal was taken to the von Frey apparatus to measure mechanical allodynia and hypersensitivity to cold in animals with and without NP. Each animal was used once and received electrical stimulation once in the PIC. An additional group was performed, with electrode implantation but without PICS. The effect of insular cortex DBS on mechanical allodynia test (von Frey test) and cold sensitivity test (acetone test) in animals with chronic NP was evaluated for up to 30 minutes after PIC neurostimulation. After three days, the animals were stimulated and placed in the open field test for 5 minutes, to assess locomotor and exploratory responses and anxiety-related behavior (Fig. 1a).

Blockade of NMDARs Followed by PICS

After seven days of stereotaxic surgery for implantation of a PIC chemitrode, 200 nL of LY235959 (2, 4, and 8 nmol) were microinjected in the PIC to verify the effect of the PIC NMDAR blockade in rats with NP ($n = 7–8$ per group). For microinjection into the PIC, a thin needle (Mizzi) connected to an l0 µL syringe (Hamilton, Reno, NV) was inserted into the chemitrode. Its end reached 1 mm below the guide cannula. A polyethylene catheter was attached to the needle to monitor the microinjections made through a drug infusion pump (Stoelting, Kiel, WI). Then, after 5 minutes of microinjection, electrostimulation was performed in the same place to analyze the effect of NMDAR blockade in the PIC on rats with NP (Fig. 1b).

Neural Tract Tracing

The rodents (CCI; $n = 5$) were anesthetized with a solution in the proportion of 0.1 ml of 10% ketamine (in a dose of 90 mg/kg, IP) to 0.2 ml of 4% xylazine (in a dose of 10 mg/kg, IP), and fixed in a stereotaxic device (Insight). The bar of the maxillary incisors was positioned 3.3 mm below the interaural line so that the skull was in a horizontal position between bregma and lambda. A micropipette was introduced vertically, targeting the dysgranular layer of the PIC, according to the coordinates AP = −0.48 mm; ML = 5.8 mm; and DV = 6.9 mm for the injection needle.

Through this device, either the Cascade Blue 3000 MW lysine-fixable dextran (CBD) or the biotinylated dextran amine 3000 MW (BDA) neural tract tracer was administered by an injection pump (Stoelting Co, model 250) in the PIC to investigate the connections between PIC and nuclei of the endogenous pain modulation system. The cytoarchitecture and other characteristics of the labeled neural cells and the neural hodology were evaluated by light microscopy (AxioImager Z1 photomicroscope; Zeiss, Oberkochen, Germany).

Fluorescent neurotracing was performed on animals 21 days after the CCI or Sham procedure. The neural tract tracer was deposited in the PIC using a system consisting of a gingival needle (30G) coupled to a polyethylene thread that, in turn, was coupled to a 10 µL syringe (Hamilton) microinjected by an infusion pump with a flow rate of 0.2 µL/min. To protect the trepanation from impurities, the calvaria was sutured with 5-0 mononylon suture thread at time of microinjection.

The neurotracing procedure analysis was performed after seven days of survival following CBD cortical microinjections. The rats were deeply anesthetized with ketamine (90 mg/kg, intraperitoneal [IP]) and xylazine (10 mg/kg, IP). After anesthesia, they were infused intracardially with buffered saline, followed by 4% paraformaldehyde in 0.05 M phosphate buffer, pH 7.3. Next, the brain was removed, cryoprotected to obtain frozen 20 µm sections in a cryostat (AM 1950; Leica, Wetzlar, Germany), and deposited on acrylic plates, according to the free-floating technique. Sections were washed in phosphate buffer, pH 7.3 and observed under microscopy (AxioImager Z1) for subsequent localization of the injection site and the labeled cells. These sections were mounted between slides and coverslipped with diamidinophenylindol.

For the nonfluorescent procedure, after seven days of microinjection, the rats were anesthetized. Each animal was, in turn, anesthetized with a mixture of ketamine (União Química Farmacêutica Nacional, Embu-Guaçu, São Paulo, Brazil) and xylazine (Hertape/Calier, Juatuba, MG, Brazil) (92 mg/kg and 9.2 mg/kg, respectively, i.p.) and perfused through the left ventricle with cold, oxygen-enriched, Ca++-free Tyrode's buffer (40 mL at 4 °C) and ice-cold paraformaldehyde (200 mL, 4% (w/v) in 0.1 M sodium phosphate buffer, pH 7.3) for 15 minutes at a pressure of 50 mmHg with a perfusion pump (Master Flex L/S peristaltic tubing pump, East Bunker Court Vernon Hills, IL). The brainstem was quickly removed, sectioned, and immersed in fresh fixative for four hours at 4 °C. It was then rinsed for at least 12 hours each in 10% and 20% sucrose dissolved in 0.1 M sodium phosphate buffer (pH 7.4) at 4 °C. The tissue pieces were immersed in 2-methylbutane (Sigma-Aldrich, St Louis, MI), frozen on dry ice, embedded in Tissue-Tek OCT, and cut with a cryostat (CM 1950, Leica, Wetzlar, Germany) at −22 °C. Slices of 20 micrometers were cut, and the labeling procedure, the Biodextran labeling, was visualized using the avidin-biotin method (ABC standard Elite kit; Vector Labohamsterries) with nickel-enhanced 3-3′-diaminobenzidine (DAB; Sigma/Aldrich, St. Louis, MO) peroxidase reaction. After incubation, sections were washed thoroughly in 0.1 M phosphate buffer (pH 7.4), mounted on gelatin-coated glass slides, and stained using hematoxylin-eosin by means of an Autostainer (CV 5030 Autostainer XL, Leica, Wetzlar, Germany). The morphological procedure was based on previous studies.[35,36] The positions of the guide cannula tips were determined according to the Paxinos and Watson atlas under a motorized photomicroscope (AxioImager Z1) (Fig. 1c).

Perfusion and Histology

After performing the neurophysiological and neuropharmacologic procedures, the animals were anesthetized through IP administration with 3 mL of 25% urethane solution, perfused through the left cardiac ventricle with a 0.9% sodium chloride solution, followed by a buffered 4% paraformaldehyde solution. The brain was removed and kept refrigerated in fixative (4% paraformaldehyde) for at least 24 hours and then immersed in a 20% sucrose solution, also stored in a refrigerator for cryoprotection for 24 hours. After these processes, the brain was frozen and cut with a microtome (CM 1950, Leica) in 40 µm-thick coronal sections. The sections were mounted on glass slides, gelatinized, air-dried, and stained with methylene blue. Subsequently, the sections were analyzed with the aid of light microscopy (AxioImager Z1), and the positions of the tips of the stimulation electrodes and the microinjection needles on the neuraxis were marked in anagrams of Paxinos and Watson's[31] rat brain in the stereotaxic atlas. The animals with signs of the presence of the end of either the stimulation electrode or the injector needle within the dysgranular layer of the PIC were included in the statistical analysis.

Statistical Analysis

Data are expressed as mean ± SEM. The results were analyzed using a two-way repeated-measures analysis of variance (two-way ANOVA) statistical test, followed by Tukey's post hoc test for intra- and intergroup comparison. Results that presented a value of $p < 0.05$ were considered statistically significant. In this way, the effect of stimulation was determined according to time. For the data related to the open field test, a normality test was performed. The groups that did not show a normal distribution were submitted to the nonparametric Kruskal-Wallis test, followed by the Dunn's post hoc test. The Graph Prism program (version 8.0.2 GraphPad Software, USA) was used for statistical analysis and graphing.

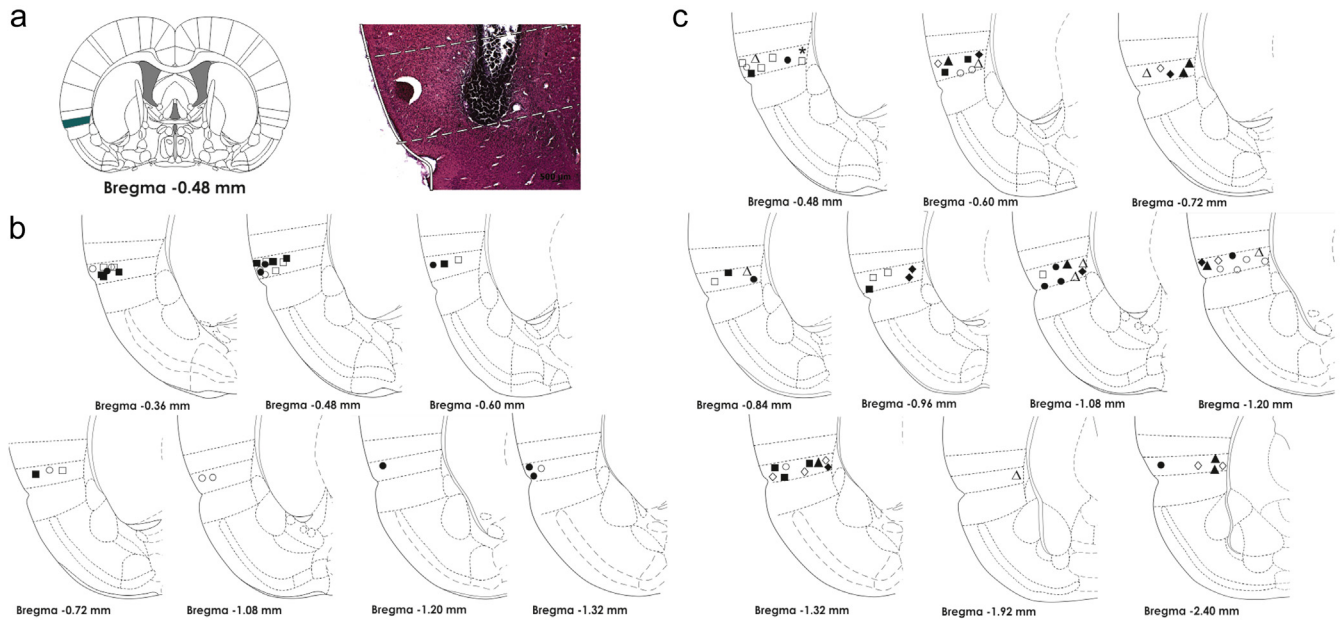

Figure 2. Diagrammatic representation of electrical neurostimulation sites in the dysgranular region of the PICS, according to the atlas by Paxinos and Watson.[31] a. Representation of the stimulation electrode on the PIC. b. Schematic representations of histologically identified electrical stimulation sites (○) PICS at 0 μA/15 s (Sham) ($n = 8$), (●) PICS at 20 μA/15 s (Sham) ($n = 8$), (□) PICS at 0 μA/15 s (CCI) ($n = 7$), (■) PICS at 20 μA/15 s (CCI) ($n = 8$), performed in animals with CNP. c. Representation of sites of microinjections of LY235959 in insula plus the PICS, according to the atlas by Paxinos and Watson.[31] Schematic representations of histologically identified PICS and NMDAR blockade sites (○) LY235959 2 nmol PIC + PICS at 20 μA/15 s (CCI) ($n = 8$), (●) LY235959 4 nmol PIC + PICS at 20 μA/15 s (CCI) ($n = 7$), (Δ) LY235959 8 nmol PIC + PICS at 20 μA/15 s (CCI) ($n = 8$), (▲) LY235959 8 nmol PIC + PICS at 0 μA/15 s (CCI) ($n = 8$) (□) Vehicle PIC + PICS at 20 μA/15 s (CCI) ($n = 8$), (■) Vehicle PIC + PICS at 0 μA/15 s (CCI) ($n = 8$), (◇) LY235959 8 nmol PIC + PICS at 20 μA/15 s (Sham) ($n = 8$), (◆) Vehicle PIC + PICS at 0 μA/15 s (Sham) ($n = 7$), (*) Vehicle PIC + PICS at 20 μA/15 s (Sham) ($n = 8$) performed in animals with CNP. [Color figure can be viewed at www.neuromodulationjournal.org]

RESULTS

Histologic Analysis

Histologically confirmed PICS sites (stimulation and non-stimulation) or drug infusion (physiological saline and LY235959 microinjections) in the PIC in CCI or Sham rats are shown in Figure 2b,c, respectively. Representative photomicrographs of transverse sections of the insula, showing a drug microinjection site in the dysgranular PIC, are shown in Figure 2a. Sites of either deep brain electrical stimulation or microinjection of drugs out of PIC are provided as Supplementary Data. The mechanical and cold allodynia of the out-group were also provided as Supplementary Data.

The PIC Electrical PICS Attenuates the Chronic NP

von Frey Test (Mechanical Allodynia Threshold)

To investigate the participation of the dysgranular subdivision of PIC in CCI-induced chronic peripheral neuropathic pain, PICS at 20 μA for 15 seconds was applied contralaterally to the operated hind limb, followed by mechanical allodynia recording in animals with and without chronic NP. According to the von Frey test, PICS decreased mechanical allodynia in animals on the 21st day after CCI surgery.

Two-way ANOVA revealed that there was a significant effect of the treatment on the mechanical allodynia threshold [$F_{(3,27)} = 22.02$; $p < 0.001$], of time [$F_{(4,108)} = 38.03$; $p < 0.001$], and of treatment vs time interaction [$F_{(12,108)} = 15.17$; $p < 0.001$] in animals submitted to the PICS 21 days after CCI. No PICS/CCI group had greater complementary mechanical allodynia (right paw) than the no PICS/Sham group (Tukey's post hoc test; $p < 0.001$) (Fig. 3a). Furthermore, the PICS (20 μA/15 s)/CCI group decreased mechanical allodynia in the right paw of animals compared with the group without the PICS (0 μA / 15 s)/CCI (Fig. 3a), suggesting that DBS of the dysgranular region of the PIC causes analgesia in animals with chronic neuropathic pain (CNP) 10 minutes after the neurostimulation. In addition, when the mechanical allodynia was measured in the left paw, there was no change in the mechanical nociceptive thresholds of rats in the CCI or Sham groups (Fig. 3b).

Acetone Test (Cold Allodynia Threshold)

To investigate the participation of the dysgranular subdivision of PIC in CCI-induced chronic peripheral neuropathic pain, the PICS was performed at 20 μA for 15 seconds contralaterally to the operated hind limb, followed by the measurement of cold allodynia in animals with or without CNP. PICS decreased allodynia to cold in animals evaluated using the acetone test on the 21st day after CCI surgery.

Considering the cold allodynia threshold, according to the repeated measure two-way ANOVA, there was a significant effect of treatment [$F_{(3,27)} = 29.40$; $p < 0.001$], of time [$F_{(4,108)} = 35.89$; $p < 0.001$], and of treatment vs time interaction [$F_{(12,108)} = 13.41$; $p < 0.001$] in animals that underwent at PICS 21 days after CCI or Sham procedure. No PICS/CCI group had significantly greater allodynia to cold (right paw) than the without PICS/Sham group (Tukey's post hoc test; $p < 0.001$) (Fig. 4a). That is, CCI was effective in producing CNP in animals. However, PICS (20 μA/15 s) in animals with CCI did not significantly decrease cold allodynia in the right paw compared with the group without PICS (0 μA/15 s)/CCI ($p > 0.05$) (Fig. 4a). In addition, when the cold allodynia was measured in the left paw,

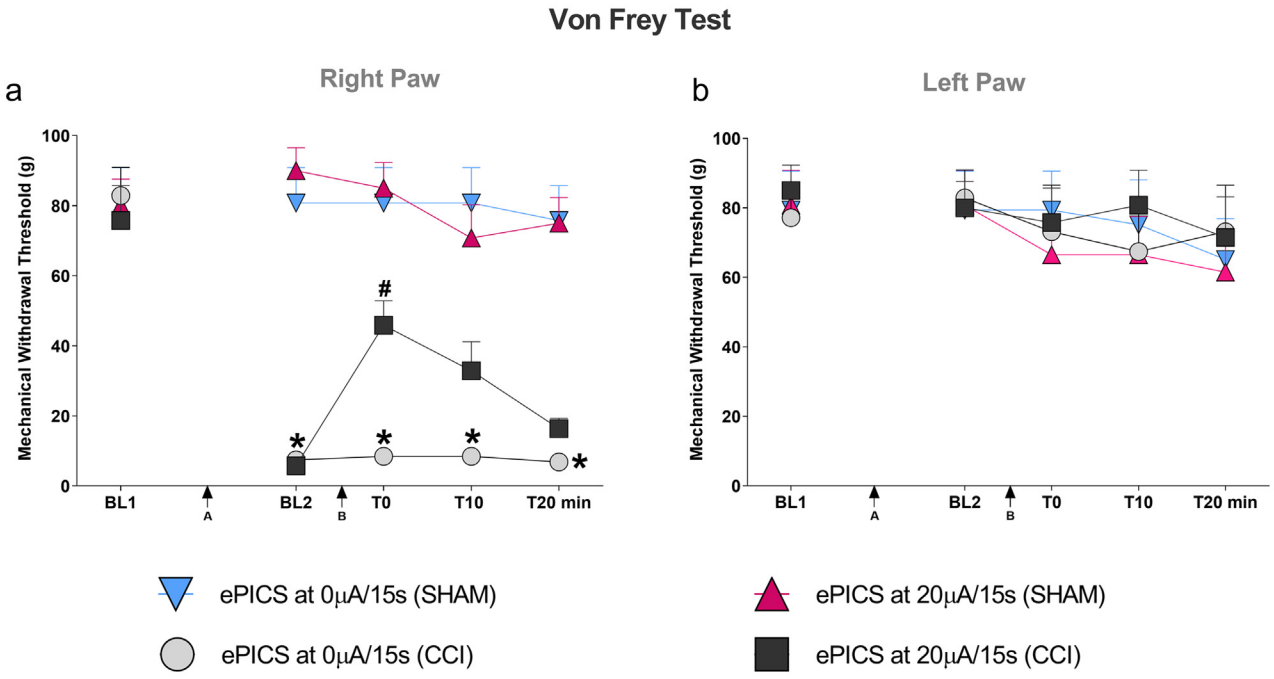

Figure 3. Effect of neurostimulation/DBS of the dysgranular region of the PIC (electrical PICS [ePICS]) on the mechanical allodynia threshold of animals using the von Frey test 21 days after CCI or Sham (n = 8) performed on the right paw (a) and on the left paw (b). *Difference in mechanical allodynia responses between the Sham and CCI groups. #Difference between the CCI groups with (20 μA/15 s) and without (0 μA/15 s) stimulation. There was a decrease in neuropathic pain (increasing the threshold) in the first time immediately after ePICS. BL1, baseline 1, recorded before each procedure; arrow A, CCI or Sham surgery; BL2, baseline 2, after 21 days of CCI or Sham; arrow B, ePICS of the animals followed by the von Frey test performed up to 20 minutes. [Color figure can be viewed at www.neuromodulationjournal.org]

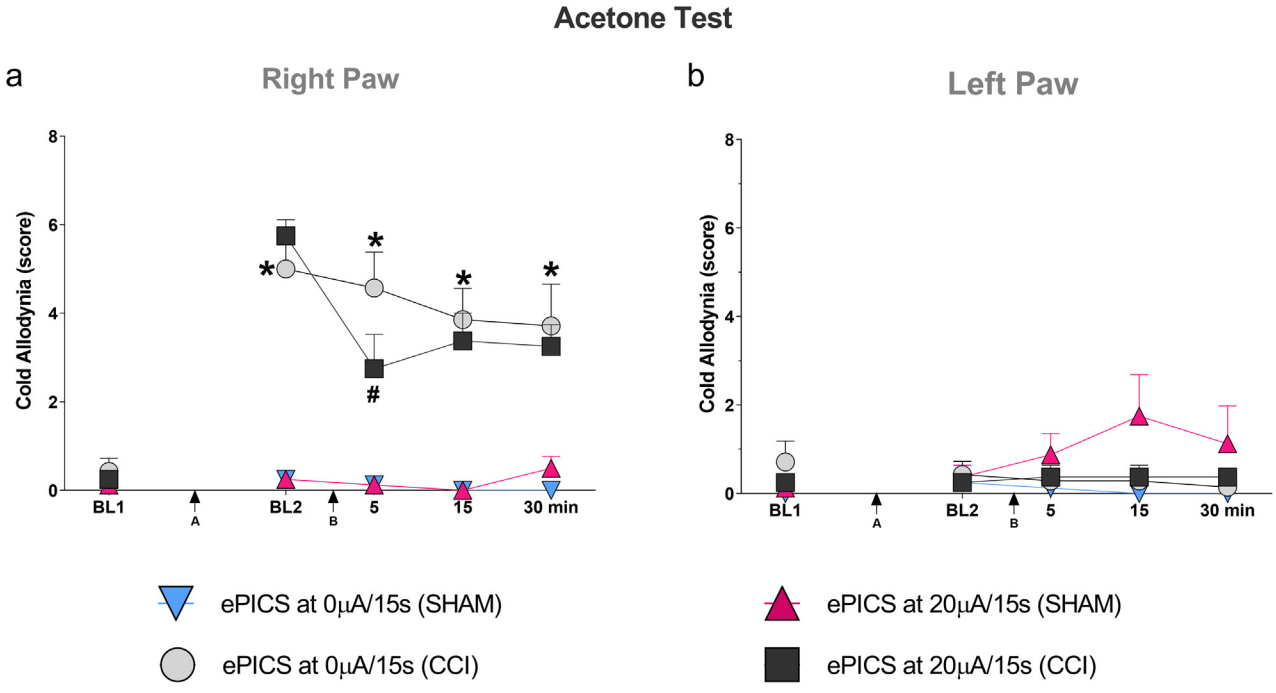

Figure 4. Effect of neurostimulation/DBS of the dysgranular region of the PIC (electrical PICS [ePICS]) on the cold allodynia index of animals through the acetone test 21 days after CCI (n = 8 with ePICS and n = 7 without cPICS) or Sham (n = 8 with ePICS and n = 8 without ePICS) performed on the right paw (a) and on the left paw (b). *CCI animals with ePICS (20 μA/15 s) showing an improvement in cold allodynia threshold immediately after stimulation. #CCI animals without stimulation (0 μA/15 s) maintaining cold allodynia and difference between CCI animals without ePICS and with ePICS. BL1, baseline 1, before procedures; arrow A, CCI or Sham surgery; BL2, baseline 2, recorded after 21 days of either CCI or Sham procedure; arrow B, after BL2 recording, effect of the ePICS on animal behavioral reactions, followed by the acetone test for up to 30 minutes. [Color figure can be viewed at www.neuromodulationjournal.org]

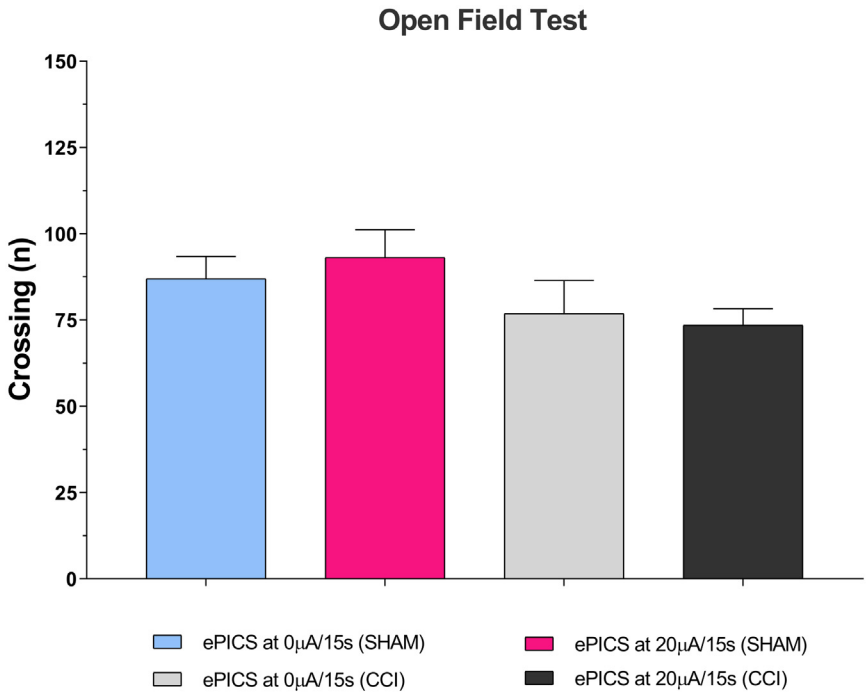

Figure 5. Representation of rat locomotor behavior recorded in the open field test to evaluate the exploratory behavior displayed in a circular arena in Sham and CCI animals (crossing and rearing) after the electrical stimulation of the dysgranular region of the PICS. There was no statistically significant difference between the groups. ePICS, electrical posterior insular cortex stimulation. [Color figure can be viewed at www.neuromodulationjournal.org]

there was no change in the thermal nociceptive thresholds of CCI or Sham animals (Fig. 4b).

Locomotor and Exploratory Activity (Open Field Test)

An open field test was performed to evaluate locomotor and exploratory activities, in which the animals were placed in a circular arena for 5 minutes immediately after the electrical PICS. The crossing behavior between the animals of all groups did not alter in the CCI group with and without electrical stimulation and the Sham group with and without electrical stimulation. The nonparametric Kruskal-Wallis analysis followed by the Dunn's post hoc test showed no statistically significant difference between the CCI and Sham groups with and without stimulation [H = 4,878 $p > 0.05$, respectively], as shown in Figure 5.

Effect of NMDAR Blockade in Dysgranular Insular Cortex on PICS-Induced Antinociception

von Frey Test (Mechanical Allodynia Threshold)

To investigate the participation of NMDAR located in the dysgranular subdivision of the PIC in CCI-induced chronic peripheral NP, the NMDAR were blocked by microinjections of LY235959 at different doses (2, 4, and 8 nmol), followed by PICS at 20 μA for 15 seconds applied contralaterally to the operated hind limb. The mechanical allodynia was recorded in animals with or without chronic NP. The microinjection of LY235959 at a concentration of 8 nmol blocked the analgesic effect produced by PICS, maintaining the low threshold of mechanical allodynia compared with the vehicle/PICS group submitted to the von Frey test 21 days after CCI surgery.

Regarding the mechanical allodynia, according to a repeated measure two-way ANOVA, there was a significant effect of treatment [$F_{(5,41)} = 14.29$; $p < 0.001$], of time [$F_{(4,164)} = 165.6$; $p < 0.001$], and of treatment vs time interaction [$F_{(20,164)} = 3.217$; $p < 0.001$] in animals that underwent NMDAR blockade followed by PICS 21 days after CCI. The pretreatment of PIC with LY235959 at the highest dose (8 nmol) blocked the analgesic effect produced by PICS compared with the vehicle/PICS-treated group (Tukey's post hoc test; $p < 0.001$) for up to 10 minutes. The effect of NMDAR blockade with LY235959 in a dose of 8 nmol followed by PICS was not significantly different from the LY235959 (8 nmol) PIC/without PICS-treated group, suggesting that the NMDAR blockade in PIC per se does not change the mechanical allodynia threshold ($p >$ 0.05 consistently). In addition, the effect of PIC NMDAR blockade with LY235959 at the highest dose was significantly different to the PIC NMDAR blockade with LY235959 at the lowest dose (2 nmol) recorded immediately after PICS (Tukey's post hoc test; $p < 0.001$) (Fig. 6a). Finally, when the mechanical allodynia was investigated in the left paw, there were no significant changes in the mechanical nociceptive thresholds of CCI animals (Fig. 6b).

Acetone Test (Cold Allodynia Threshold)

To investigate the participation of NMDA glutamatergic receptors of the dysgranular subdivision of the PIC in CCI-induced chronic peripheral NP, PIC NMDAR was blocked by microinjections of LY235959 at different concentrations (2, 4, and 8 nmol) followed by electrical neurostimulation of PIC at 20 μA for 15 seconds contralaterally to the operated hind limb, followed by cold allodynia measurement using the acetone test in animals with or without chronic NP. The microinjection of LY235959 at the higher concentration of 8 nmol blocked the analgesic effect produced by PICS, maintaining the low threshold of cold allodynia in the PIC stimulation/PIC vehicle-treated group in animals submitted to the acetone test 21 days after CCI surgery.

According to the repeated measure two-way ANOVA, there was a significant effect of treatment [$F_{(5,41)} = 2.554$; $p < 0.05$], of time

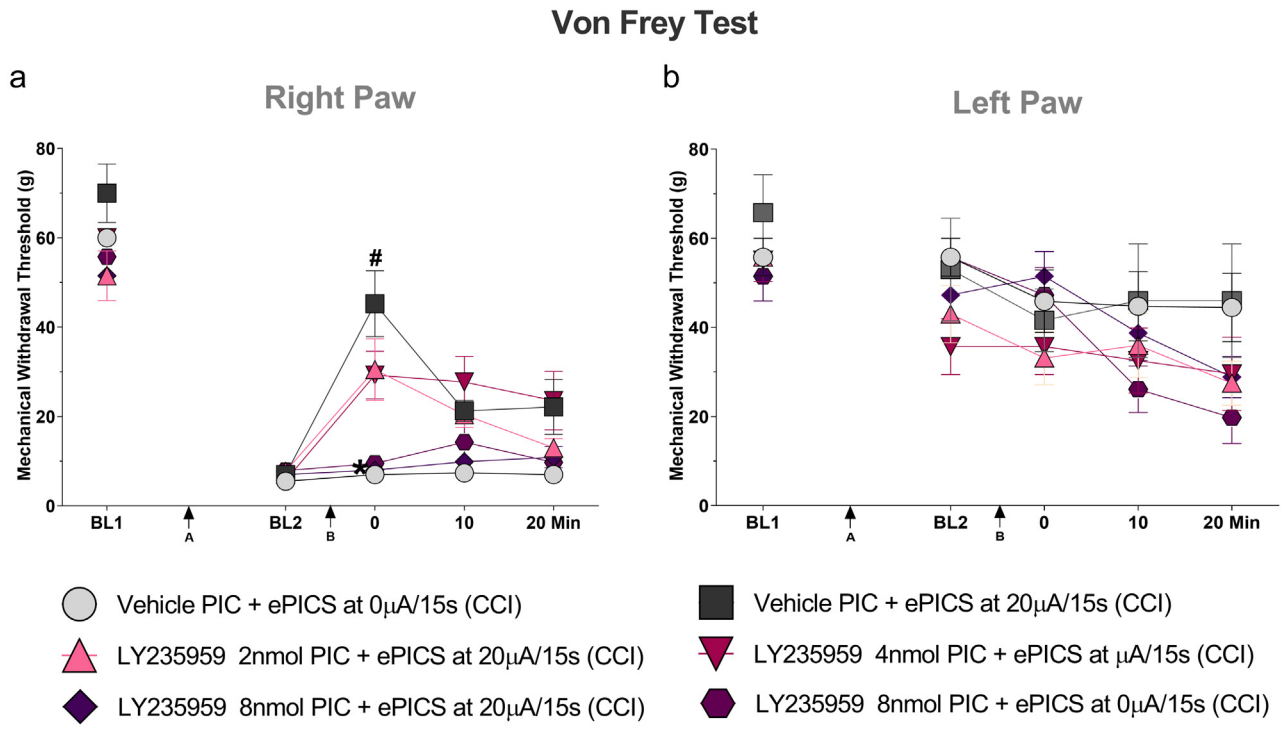

Figure 6. Effect of NMDAR blockade with microinjection of the selective antagonist LY235959 (at 2, 4, and 8 nmol/200 nL) in the dysgranular region of the PIC, followed by electrical neurostimulation (electrical PICS [ePICS]) on the mechanical allodynia threshold of animals, through the von Frey test 21 days after CCI ($n = 8$) performed on the right paw (a) and on the left paw (b). #Significant difference in relation to the vehicle group + without ePICS. *Significant difference in relation to the vehicle + ePICS group. BL1, baseline 1, recorded before each procedure; arrow A, CCI surgery; BL2, new baseline measured after 21 days of either CCI or Sham procedure; arrow B, after BL2, microinjection of LY235959 or vehicle was performed, followed by ePICS or without ePICS, and the von Frey test was performed up to 20 minutes. [Color figure can be viewed at www.neuromodulationjournal.org]

[$F_{(4,164)} = 92.09$; $p < 0.001$], and of treatment vs time interaction [$F_{(20,164)} = 2.885$; $p < 0.01$] in animals submitted to the PIC NMDAR blockade followed by PICS 21 days after CCI. Microinjection of LY235959 at the highest concentration (8 nmol) in PIC blocked the analgesic effect produced by PICS compared with the vehicle PIC/PIC stimulation-treated group immediately after PICS, up to 5 minutes post-DBS (Tukey's post hoc test; $p < 0.05$). The effect of PIC NMDAR blockade with LY235959 at 8 nmol followed by PICS was not significantly different from that recorded in the LY235959 (8 nmol) PIC/PIC nonstimulation-treated group, suggesting that the NMDAR blockade in PIC does not significantly change the mechanical allodynia threshold at any time ($p > 0.05$ in all cases). In addition, the effect of PIC NMDAR blockade with LY235959 at the highest dose was significantly different to the PIC NMDAR blockade with LY235959 at the lowest dose (2 nmol) recorded immediately after PICS (Tukey's post hoc test; $p < 0.05$) (Fig. 7a). Finally, when the mechanical allodynia was investigated in the left paw, there were no significant changes in the mechanical nociceptive thresholds of animals with CCI (Fig. 7b).

Neural Tract-Tracing of Pathways Connected to the PIC

Deposits of either the 3000 MW CB neurotracer (Fig. 8b) or the biotinylated dextran-amine (BDA) neurotracer (Fig. 9b) in the dysgranular region of the PIC (Fig. 8) were performed to study the neuroanatomic connections between PIC neurons and either other neocortical areas or the endogenous pain modulatory system structures situated in the brain stem.

CB neurotracer-labeled neurons were found in the secondary somatosensory cortex (S_2) of Wistar rats connected to the dysgranular region of the PIC, as shown in Figure 8c. BDA neurotracer-labeled neuronal cells, axonal fibers, and terminal buttons were found in the caudal pontine reticular nucleus (PnC), suggesting a reciprocate connection between the dysgranular region of the PIC and the PnC, as shown in Figure 9c,d. Both BDA neurotracer-labeled neuronal perikarya and axonal fibers also were found in the alpha part of the parvicellular reticular nucleus (PCRtA), dorsolaterally situated to the nucleus reticularis pontis caudalis, as shown in Figure 9e,f, suggesting outputs to the dysgranular region of the PIC. Finally, BDA neurotracer-labeled neuronal perikarya and axonal fibers were found in the GABAergic ventral tegmental area inhibitory control center dorsomedial tegmental nucleus (DMTg), suggesting modulatory outputs reaching the dysgranular region of the PIC, as shown in Figure 9g.

DISCUSSION

In this work, we showed that the electrical stimulation of the PIC attenuated both mechanical and thermal allodynia in animals with chronic NP after 21 days of the CCI procedure in Wistar rats. Microinjection of a selective NMDAR antagonist followed by electrical stimulation of this same target insular area blocked PICS-induced antinociception. These findings suggest that the antinociceptive effect produced by PICS involves glutamate-dependent neural networks in the PIC. Connections between the dysgranular region of the PIC and both cortical and brainstem reticular nuclei,

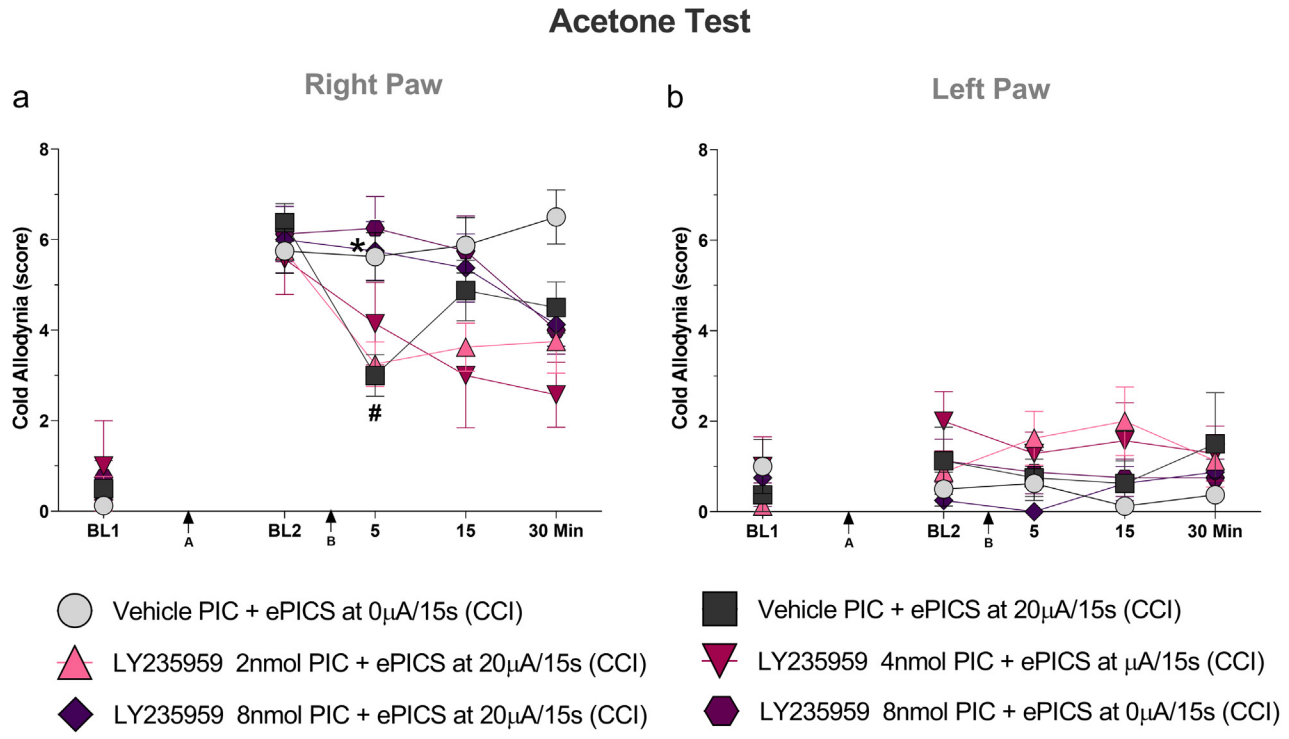

Figure 7. Effect of NMDAR blockade through microinjection of the selective antagonist LY235959 (at 2, 4, and 8 nmol/200 nL) in dysgranular PIC, followed by electrical neurostimulation (electrical PICS [ePICS]) on the cold allodynia threshold of animals recorded by the acetone test 21 days after CCI (n = 8) performed on the right paw (a) and on the left paw (b). #Significant difference in relation to the vehicle group + without ePICS. *Significant difference in relation to the vehicle group + ePICS. BL1, baseline 1, recorded before each procedure; arrow A, CCI surgery; BL2, baseline recorded after 21 days of CCI; arrow B, after BL2, microinjection of LY235959 + ePICS was performed in the animals, and the acetone test was performed up to 30 minutes. [Color figure can be viewed at www.neuromodulationjournal.org]

such as the S_2, the reticular pontine nuclei, the parvicellular reticular nucleus, and the dorsomedial tegmental nucleus, many of them reciprocated connected, consist in a neural network supporting the neuromodulation of chronic NP in animals with CCI. A summary of the findings was shown in Figure 10.

In fact, our findings showed that the electrical neurostimulation at 20 μA for 15 seconds of the insular cortex dysgranular subdivision could influence the contralateral hind paw mechanical and thermal hypersensitivity (cold) (contralateral to the site of stimulation), causing an increase in mechanical and cold allodynia thresholds recorded immediately after PIC stimulation. Another study shows that electrical stimulation of the PIC produces antinociception.[37] To our knowledge, our findings are the first evidence involving the antinociceptive effect by PICS of the dysgranular layer and insular glutamatergic modulation by NMDAR signaling.

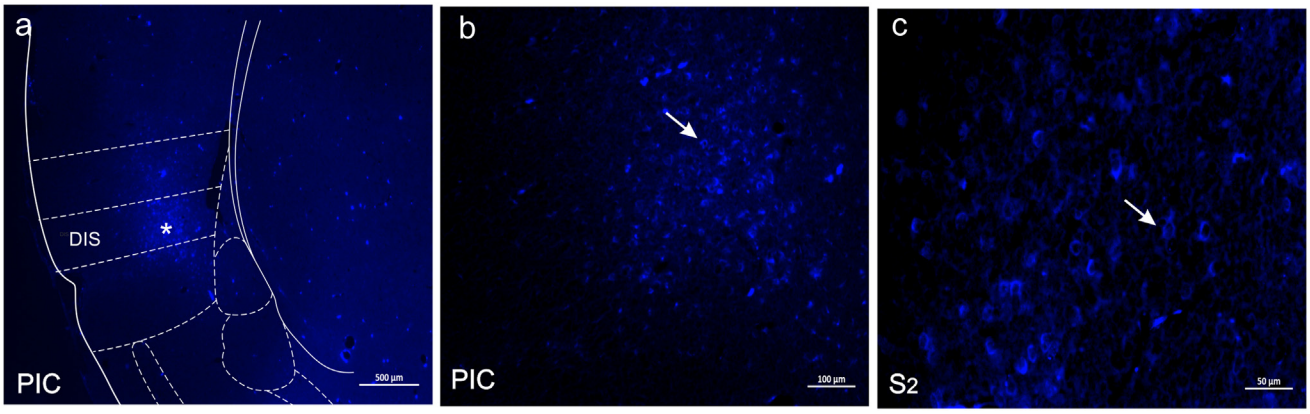

Figure 8. Photomicrographs of transverse sections of dysgranular region of the PIC of Wistar rats. a. Diagrammatic representation of insula transverse section, showing a histologically confirmed microinjection site (*n = 1) of the 3000 MW CB in dysgranular PIC depicted in a modified drawing from Paxinos and Watson's[31] rat brain in stereotaxic coordinates atlas. b. Photomicrograph of a transverse section of insula showing a representative site of CBD deposit in dysgranular PIC (white arrow). c. Photomicrograph of a representative coronal section of the S_2 of a Wistar rat showing CBD-labeled perikarya (white arrow) situated in S_2 cortex, connected to dysgranular PIC. [Color figure can be viewed at www.neuromodulationjournal.org]

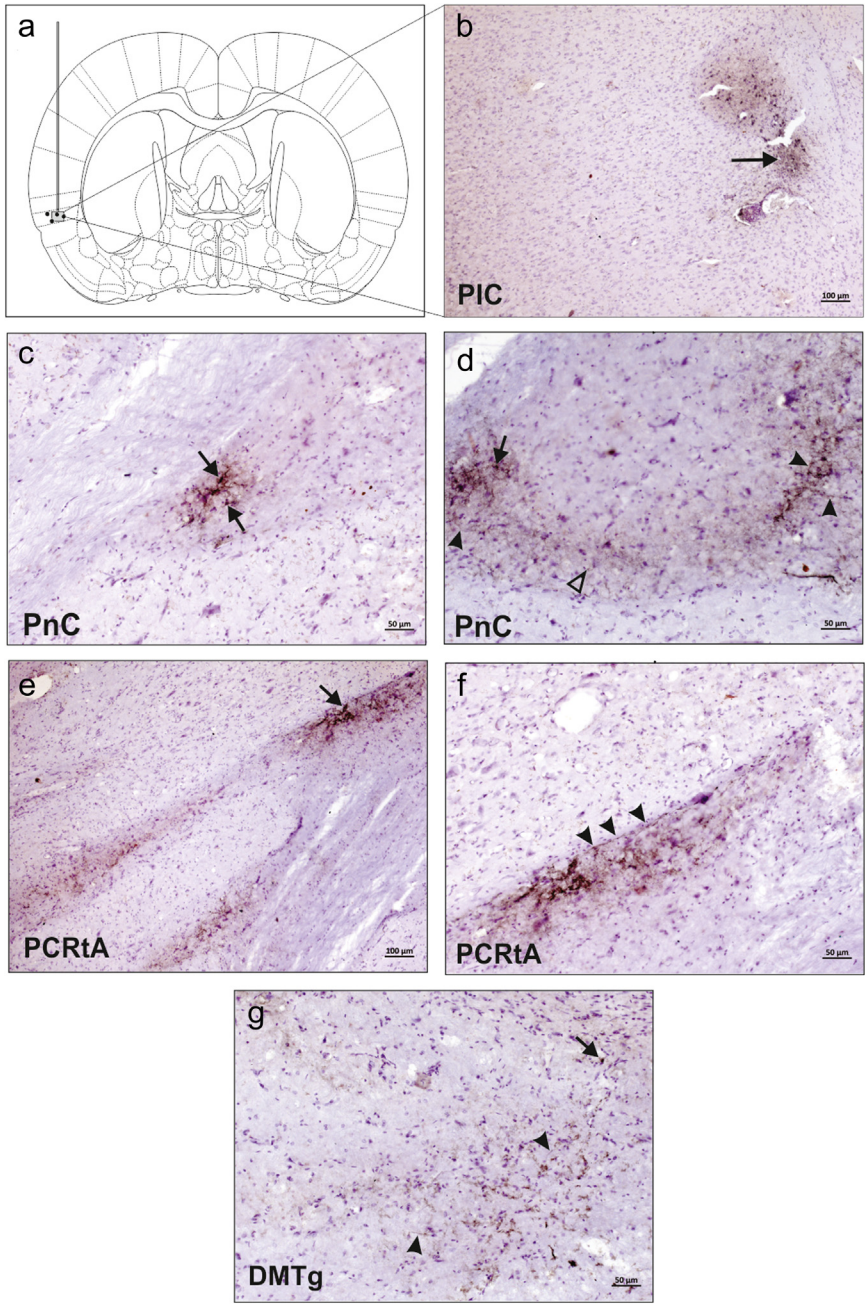

Figure 9. Photomicrographs of transverse sections of the dysgranular region of the PIC of Wistar rats. a. Diagrammatic representation of a transverse section, showing a histologically confirmed microinjection site (black circle $n = 4$) of the 3000 MW BDA deposits in the dysgranular PIC depicted in a modified drawing from Paxinos and Watson's[31] rat brain in stereotaxic coordinates atlas. b. Representative site of BDA microinjection in dysgranular IC (black arrow). c and d. Photomicrograph of a representative coronal section of the pontine nucleus of a Wistar rat showing neural connections between dysgranular PIC neurons and PnC nucleus: BDA-labeled axonal fibers (black arrowheads), terminal buttons (open arrowheads), and cell bodies (black arrow). e and f. Representative photomicrographs of transverse sections of PCRtA nucleus: BDA-labeled cell bodies (black arrow) and axonal fibers (black arrowhead). g. Representative photomicrographs of transverse sections of DMTg nucleus with BDA-labeled axonal fibers (black arrowhead) and cell bodies (black arrow). [Color figure can be viewed at www.neuromodulationjournal.org]

Several studies have shown the efficacy of the glutamatergic system in a variety of functions, such as neural development, synaptic plasticity, learning, memory, and especially in NP conditions.[18] The insular cortex interacts with NMDAR in these several functions and participates in a circuit involving descending modulatory pain systems for antinociceptive effects,[38] and studies involving the administration of d-cycloserine as an NMDA agonist have shown greater brain activation of this region and better behavioral results in learning contexts.[21]

As previously mentioned, the cortical processing network of the chronic NP involves the PIC.[17] According to the neurophysiological study carried out in this work, the microinjection of the selective

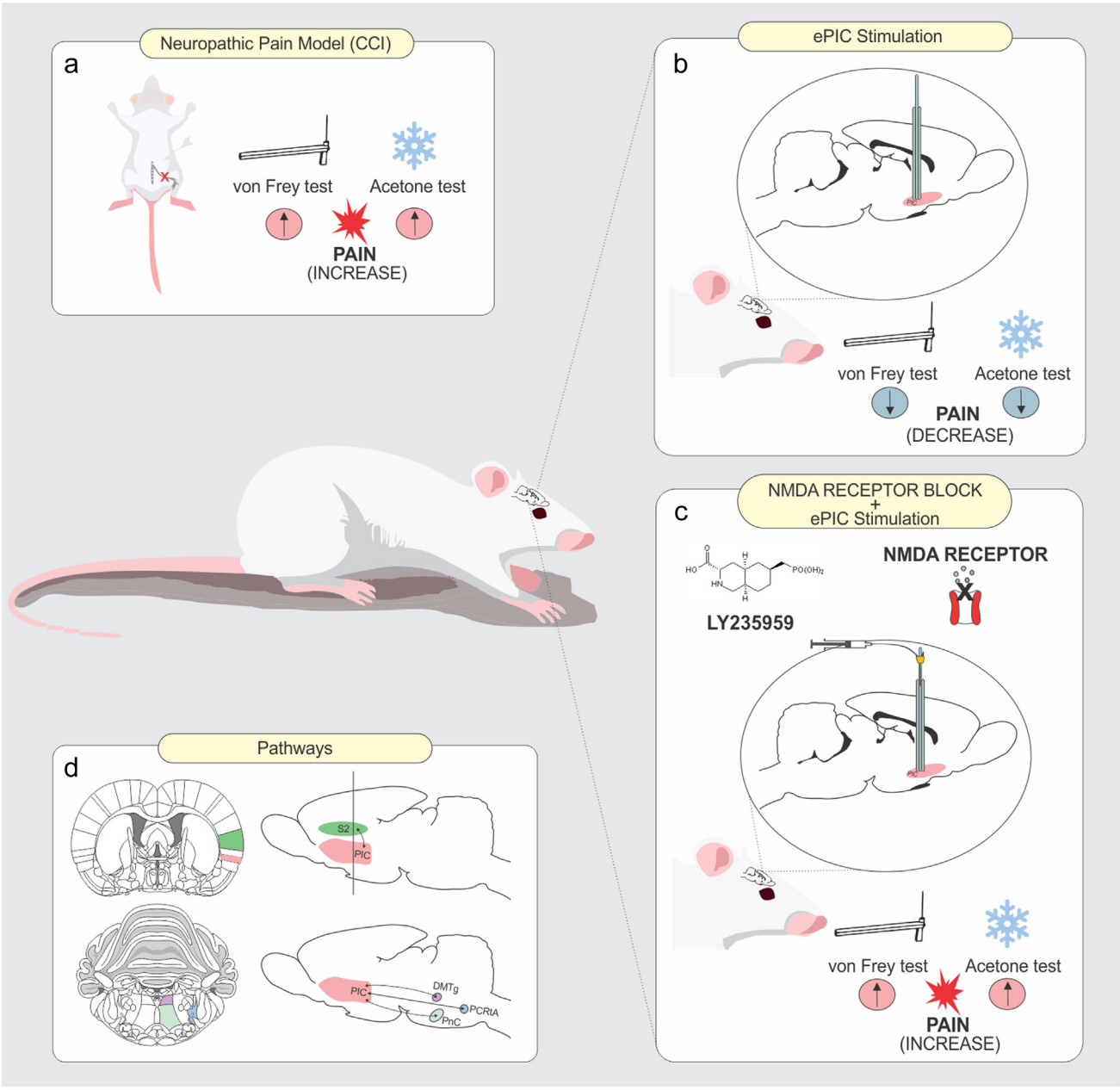

Figure 10. Graphical abstract: effect of electrical PICS (ePICS) and PIC NMDAR blockade followed by PICS on thermal and mechanical allodynia in chronic NP induced by CCI of the sciatic nerve or Sham procedure. a. von Frey and acetone test baselines, and CCI or Sham surgery was performed. b. ePICS was performed in low frequency (20 μA, 100 Hz) for 15 seconds by DBS device after 21 days of CCI or Sham. c. The PIC NMDAR blockade was performed through LY235959 microinjection followed by ePICS after 21 days of CCI. d. Neuroanatomic projections from PIC to pontine and midbrain nuclei (PnC, PCRtA, and DMTg) and S_2 may contribute to the process of NP signaling. PICS attenuates the chronic NP, and the NMDA glutamatergic system in the PIC may be involved in PICS-antinociception in rodents with NP conditions. [Color figure can be viewed at www.neuromodulationjournal.org]

NMDAR antagonist LY235959 at the highest dose was able to block the analgesic effect produced by PIC electrical stimulation, maintaining the low thresholds of mechanical and thermal allodynia recorded immediately after PICS. When the same concentration of LY235959 was microinjected in the PIC without PICS, the result was the same; the drug itself did not cause significant changes in mechanical and thermal allodynia thresholds, only in the presence of the neurostimulation of the insula.

This interesting finding suggests a role of NMDAR and glutamatergic neurotransmission in the dysgranular region of the PIC with analgesic responses in chronic NP stages, precisely after 21 days of CCI in rodents. These results partially confirm our working hypothesis and support the view that DBS-induced analgesia involves glutamate-dependent neural networks and NMDAR on neurons of the insula.

Glutamatergic receptors found in somatosensory, motor, and cingulate cortices have been shown to modulate acute and inflammatory pain. Neutralization of the increased transmission of nociceptive stimuli from the inflamed paw occurred through central mechanisms,[39] and anatomic evidence suggests that IC and the

cingulate cortex neurons probably interact, contributing to the central processing of painful information.[40]

The motor cortex has also been shown to be involved in pain modulation. The recruitment of NMDAR in motor cortex neurons seems critical for the attenuation of mechanical allodynia through direct connections with the dorsomedial column of periaqueductal grey.[12] The suggested mechanisms of glutamatergic neurotransmission involve channels linked to NMDAR that tend to remain open longer, allowing a significant flow of calcium and rapid removal of glutamate from the synapse by its transporters. This mechanism is necessary for normal excitatory neurotransmission and the prevention of glutamate-induced toxicity. It is likely that IC also shares calcium-dependent signaling pathways under conditions of peripheral nervous system injury. However, it may trigger an upregulation of NMDAR for long-term potentiation.[40]

In addition, it has been shown that the PIC is a structure that is constantly activated during electrical stimulation of the motor cortex, resulting in an upregulation of the pain threshold, exerting a direct influence on the posterior thalamus and descending pain inhibitory system structures (for example, PAG).[38] There are also reports demonstrating of changes in c-FOS protein expression around electrodes inserted in the insula, showing an increase in neuronal activity observed after electrical stimulation of the PIC activating the descending inhibitory pain pathways through connections with the pain matrix.[41] Together, these data provide solid evidence that the PIC, intimately connected with several cortical and subcortical areas, participates in the modulatory processes involving analgesia.[37,41–46]

Several pieces of evidence have shown functional differences in the participation of IC subregions in painful and behavioral conditions. The inhibition of the increase in NMDA or GluN2B receptors in the anterior IC by antagonists can prevent or treat the NP, and unilateral microinjection of ionotropic glutamate receptor antagonists restores nociceptive behaviors to preinjury values. Furthermore, increased endogenous GABA levels or increased signaling at inhibitory glycinergic receptors had similar effects to glutamate receptor antagonists.[47]

In contrast, studies involving NMDA excitotoxic lesions of the most caudal portion of the PIC caused modulation of allodynic manifestations.[48] The stimulation sites in our histology showed some positions varying along the anteroposterior axis. However, they were not concentrated in this more caudal portion of the insula, which may provide more information about functional differences of the insular cortex subdivisions regarding pain modulation because there may be different populations of neurons along the rostroposterior axis that allow these specific features. They ultimately provided a basis for explaining our findings.[49]

Shreds of evidence also suggest a specific role of the operculum-insular cortical area in thermal nociception. Studies with transcranial magnetic stimulation of these regions show an impairment of the sensitivity of discrimination of pain stimuli intensity, reduced chronic visceral pain, and an increased nociceptive threshold,[50] reducing thermal pain perception.[51] These findings may corroborate a bidirectional projection with the S_2 confirmed in our neurotracing study, thus opening new possibilities for understanding the analgesic mechanisms found here, given the insular and somatosensory cortices are critical for the sensation of both sensory-discriminative and subjective/emotional pain.

Other evidence found by our study suggests the interaction of IC with reticular nuclei located in the brainstem. The caudal pontine reticular nucleus (PnC), a critical nucleus located in the pontine reticular formation, participates in nociceptive modulation.[52] Anatomic approaches showed that this nucleus receives pathways from the pedunculopontine tegmental nucleus, and their glutamatergic neurons are inhibited by projections from superior and inferior colliculi.[53] The alpha part of the PCRtA, relatively little studied in painful conditions, integrates sensory and motor functions to establish connections with the PnC, forming an autonomic/limbic/nociceptive circuit with a high degree of interconnectivity with the IC.[54]

According to Sukhotinsky et al,[55] a region described as an area of mesopontine tegmental anesthesia located in the brainstem contains several nuclei involved with nociceptive perceptions. These areas have multiple descending projections, the most prominent being the rostromedial ventral medulla, and are less dense in the locus coeruleus (LC) region and its surroundings. Among these adjacent areas are the nuclei found in our study, the PnC, associated with pain modulation. Therefore, these connections can exert antinociceptive actions through a relay of the bulbospinal pathway and the activation of nuclei of the endogenous pain modulation system, such as the PAG, the NDR, and the LC.[56]

Little is explored in the neuroanatomic research concerning the dorsomedial tegmental nucleus and cerebral cortical areas, such as the insula, but DMTg plays a relevant role in GABAergic control of dopaminergic neurons in the ventral tegmental area. The outputs from DMTg to insula can provide an inhibitory control on the dysgranular region of the PIC during motivational behavior impairment in chronic NP conditions.

CONCLUSIONS

In summary, the electrical stimulation of the PIC caused the antiallodynic effect, and the local microinjection of the selective NMDAR antagonist LY235959 blocked the analgesic effect produced by PICS in CCI rats. The possible analgesic effect of increased activity of insula neurons may depend on the recruitment of NMDAR in the PIC. In addition, we found some reciprocated projections between PIC and nuclei located in the pontine reticular formation and S_2 that may partially explain the effects obtained in this study. Future investigations are needed to understand deeply these cortical pain control mechanisms. However, the PIC is a cortical area receiving attention from preclinical and clinical research with the inherent ability to integrate a comprehensive set of sensory, affective, and emotional information and efferent connections to modulate chronic and neuropathic pain.

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Authorship Statements

Renata Cristina Martins Pereira performed and analyzed the experiments, wrote and revised the manuscript. Priscila Medeiros performed the experiments, analyzed and revised the manuscript. Norberto Cysne Coimbra contributed with morphological analyses, corrected and revised the manuscript. Hélio Rubens Machado corrected and revised the manuscript. Renato Leonardo de Freitas designee the project, corrected and revised the manuscript.

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Neuromodulation 2023; 26: 1622–1636.

SUPPLEMENTARY DATA

To access the supplementary material accompanying this article, visit the online version of *Neuromodulation: Technology at the Neural Interface* at www.neuromodulationjournal.org and at https://doi.org/10.1016/j.neurom.2022.05.009.

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COMMENTS

This is an eloquent study by Pereira et al looking at the effects of posterior insula stimulation on neuropathic pain and the mechanisms of action, investigating specifically NMDAR. Recently, there has been increasing interest in the posterior insula and its role in the perception of pain, as well as the adjacent claustrum. This study sheds further light on the neurophysiological aspects of this area and how it may be important in pain perception. It also provides direct evidence that deep brain stimulation of this area may be helpful in pain control.

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This is a carefully constructed and carried out laboratory evaluation of the role of the posterior insula in pain perception as evaluated in a rodent model of neuropathic pain created by sciatic nerve chronic compression. The authors showed significant alterations in pain responsiveness upon stimulation of the posterior insular cortex that could be inhibited by the blockade of NMDAR. They conclude that the posterior insular cortex may have an important ability to integrate sensory, affective, and emotional information that modulates neuropathic pain.

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