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Neurotoxic lesions of the pedunculopontine tegmental nucleus impair the elaboration of postictal antinociception

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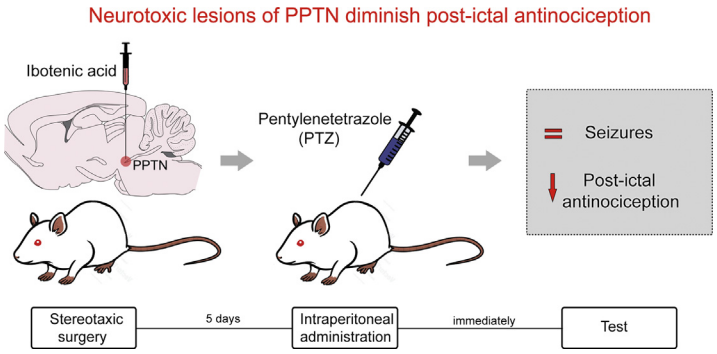
GRAPHICAL ABSTRACT



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ABSTRACT

Generalised tonic-clonic seizures, generated by abnormal neuronal hyper-activity, cause a significant and long-lasting increase in the nociceptive threshold. The pedunculopontine tegmental nucleus (PPTN) plays a crucial role in the regulation of seizures as well as the modulation of pain, but its role in postictal antinociceptive processes remains unclear. In the present study, we aimed to investigate the involvement of PPTN neurons in the postictal antinociception. Wistar rats had their tail-flick baseline recorded and were injected with ibotenic acid (1.0 μg/0.2 μL) into the PPTN, aiming to promote a local neurotoxic lesion. Five days after the neuronal damage, pentylenetetrazole (PTZ; 64 mg/kg) was intraperitoneally administered to induce tonic-clonic seizures. The tail-withdrawal latency was measured immediately after the seizures (0 min) and subsequently at 10-min intervals until 130 min after the seizures were induced pharmacologically. Ibotenic acid microinjected into the PPTN did not reduce the PTZ-induced seizure duration and severity, but it diminished the postictal antinociception from 0

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to 130 min after the end of the PTZ-induced tonic-clonic seizures. These results suggest that the postictal antinociception depends on the PPTN neuronal cells integrity.

1. Introduction

Tonic-clonic seizures cause a loss of sensitivity to nociceptive stimuli in laboratory animals, a phenomenon known as postictal antinociception [1–3]. Hypo-algesia has also been reported to occur in certain types of human epilepsy [4]. However, the neural mechanisms that cause this reduction in responsiveness to pain following epileptic seizures are unclear. To better understand the mechanisms that mediate postictal antinociception, animal models of pharmacologically induced seizures, such as in rodents, where seizures have been induced by the blockade of GABAergic neurotransmission via intraperipheral (i.p.) administration of pentylenetetrazole (PTZ), have been used. Indeed, several studies have shown that in laboratory rats, i.p. injections of PTZ trigger epileptic seizures [5–7]. In addition, epileptic seizures induced by i.p. injections of PTZ elicit a long-lasting opioid and non-opioid antinociceptive response [8–14].

Although little is known about the endogenous mediators that modulate the postictal antinociception, some evidence indicates that the cholinergic system plays an important role in the organisation of tonic-clonic seizure-induced antinociception [1,15–18]. The peripheral administration of a muscarinic cholinergic receptor antagonist decreases tail-flick latencies in seizing rats (i.e., facilitates nociception), suggesting that the endogenous acetylcholine contributes to the postictal antinociceptive responses [1]. Additionally, nicotinic receptors are also candidates to mediate this response, since peripheral administrations of mecamylamine, a nicotinic cholinergic receptor antagonist, attenuate tonic-clonic seizure-induced antinociception [15].

A similar role, played by the cholinergic system on the elaboration of postictal antinociception, is also observed in some brain structures. In this regard, microinjections of muscarinic and nicotinic cholinergic receptor antagonists in the dorsal hippocampus (DH) attenuate antinociception following tonic-clonic seizures [19]. The hippocampus is a key structure of the brain in the pathophysiology of the epileptic state because seizures may be triggered by the loss of hippocampal neuronal cells [20–22]. Sensory inputs to the DH have been identified from structures that comprise the endogenous pain modulatory system, such as the nucleus raphe magnus, the dorsal raphe nucleus and the locus coeruleus [23]. The cholinergic system of this encephalic neural network is suggested to modulate postictal antinociception, since microinjections of muscarinic and nicotinic cholinergic receptors antagonists into the nucleus raphe magnus [24], the dorsal raphe nucleus [17] and the locus coeruleus [18] impair the antinociception that follows tonic-clonic seizures. However, the main cholinergic projections to these brainstem structures come from the pedunculopontine tegmental nucleus (PPTN) [25,26], which is a pontine nucleus known to be rich in cholinergic neurons [27–29], and its inputs are suggested to play a relevant role in nociceptive modulation during convulsive reaction. For example, the synaptic inactivation of the PPTN by local microinjections of cobalt chloride decreases the postictal antinociception, suggesting the involvement of intrinsic PPTN connections in the modulation of the seizure-induced analgesic phenomenon [14]. In addition, studies have shown that using cholinergic agonists, intra-PPTN pharmacological manipulations were able to cause antinociception. This antinociceptive effect caused by the cholinergic activation was reversed by mecamylamine, a nicotinic cholinergic receptors antagonist [30], and hemicholinium-3, an inhibitor of choline reuptake [31]. In fact, the PPTN cholinergic neural network seems to be implicated in the modulation of nociceptive mechanisms, including those related to epileptic seizures.

In addition to PPTN being involved in the control of nociceptive mechanisms, a morphological study using immunohistochemical techniques for labelling the vesicular acetylcholine transporter protein (VAChT) showed that VAChT-stained varicose fibres in the PPTN of epileptic rats are more densely distributed than those of control rats in a model of temporal lobe epilepsy [32].

Due to the participation of the PPTN in the modulation of pain [30,31] and seizures [33] and its projections to the nuclei that comprise the pain endogenous modulatory system [29,34], the goal of the present study was to determine whether the lesions of the PPTN with local microinjections of ibotenic acid can impair tonic-clonic seizures and postictal antinociception induced by the i.p. administration of PTZ in rats. The hypothesis of the present work was that the integrity of the PPTN neurons is critical for the elaboration of seizure-induced hypoalgesia.

2. Material and methods

2.1. Animals

Male Wistar rats (total N = 35; n = 6 or 8 per group; 7 out of 35 (20%) animals used in the behavioural tasks died during the seizures), weighing 230–280 g, from the animal facility of the Ribeirão Preto Medical School of the University of São Paulo (FMRP-USP) were used. The animals were housed in a temperature-controlled (22 ± 1 °C) room and maintained on a 12 h light/12 h dark cycle with lights on at 7:00 a.m. They were housed four per cage in the experimental room for at least 48 h prior to the experiments, with free access to water and food on a 12 h/12 h light/dark cycle (lights on at 7:00 AM) at 22–23 °C. All of the experiments were performed according to the recommendations of the FMRP-USP Commission of Ethics in Animal Experimentation, consistent with the ethical principles for animal research adopted by the National Council for Animal Experimentation control (CONCEA) and were approved by the Commission of Ethics in Animal Research (CEUA-FMRP-USP) (process 174/2005).

2.2. Stereotaxic surgery

The animals were anaesthetised with 0.1 mL of xylazine (Calmium, União Química Farmacêutica Nacional, São Paulo, Brazil) at a dose of 9.2 mg/kg and with 0.2 mL of 10% ketamine (Agener, União Química Farmacêutica Nacional, São Paulo, Brazil) at a dose of 92 mg/kg and were fixed in a stereotaxic frame (David Kopf, Tujunga, California, USA). The infusions of the neurotoxin and its vehicle were performed during the stereotaxic surgery using an infusion pump (Stoelting, Kiel, Wisconsin, USA) through a polyethylene tube (PE10) attached to a dental needle. The dental needle was vertically introduced in the mesopontine tissue using the following coordinates: anteroposterior, 0.2 mm; mediolateral, 2.0 mm; and dorsoventral, 7.0 mm to reach the PPTN. The coordinates were based on Paxinos and Watson's rat brain in stereotaxic coordinates atlas [35], and the interaural line was used as a reference. The dose of ibotenic acid and the injection time were based on previous studies (11). The dental needle was left in place for 2 min after the end of each microinjection to allow for local drug diffusion. After the completion of the microinjection procedure, the dental needle was removed and the skin sutured.

2.3. Drugs

Pentylenetetrazole (α,β-cyclopentamethylenetetrazole; Sigma/Aldrich, St. Louis, MO, USA), at a dose of 64 mg/kg [14], and ibotenic acid (α-amino-2,3-dihydro-3-oxo-5-isoxazoleacetic acid; Sigma), at a

dose of 1.0 μg/0.2 μl, were used. The ibotenic acid neurotoxin was dissolved in saline (0.9% NaCl) shortly before administration. Physiological saline was also used as a control for all the groups [11].

2.4. Nociceptive testing

The nociception thresholds of the experimental animals were compared using the tail-flick test. Each animal was placed in an acrylic-walled restraining apparatus (Tail-Flick Analgesia Instrument; FMRP-USP Precision Workshop Facilities, Ribeirão Preto, SP, Brazil) so that its tail was placed on a heating sensor filament, in which the calorimetric progressive elevation was automatically interrupted at the moment the animal detached its tail from the apparatus. The electric current was the source to raise the temperature of the coil (Ni/Cr alloy; 26.04 cm in length × 0.02 cm in diameter) at the rate of 9 °C/s [36–38], starting from room temperature (approximately 20 °C). The tail withdrawal latency was measured in seconds. A small current intensity adjustment was done, if necessary, at the beginning of the experiment, aiming to obtain three consecutive tail-flick latencies between 2.5 s and 3.5 s. If the animal did not remove its tail from the heating filament within 6 s, the apparatus was turned off in order to prevent damage to the receptors in the skin of experimental animals' tail. Three baseline control tail-flick latencies were taken at 5-min intervals. The tail-flick latencies were also measured 5 min prior to the peripheral administration of PTZ or physiological saline.

2.5. Behavioural seizure test

The rats were evaluated in a circular transparent acrylic-walled arena (60 cm in diameter, 50 cm in height), with a floor that was brightened by a 350 lx-fluorescent lamp, which was located in a separate and calm experimental compartment. The motor tonic-clonic convulsive reactions were the parameters used to evaluate the effect of the PTZ administered intraperitoneally at a dose of 64 mg/kg [18]. The latency of the seizures was defined as the time between the injection of PTZ and the first evidence of anterior paw myoclonia, and it was considered the first sign of the beginning of the seizures. The severity of the convulsive reactions was evaluated through a modified procedure proposed by de Freitas and collaborators [19], which was based on a version of Racine's scores [39] that were later modified by Maggio and Gale [40] (Fig. 1). Considering the total duration of the convulsive reactions induced by PTZ and based on previous works [17,19], a cut-off of 600 s was considered to evaluate all the groups of animals. PTZ-induced convulsive reactions were recorded using a video camera (Sony Handycam, Tokyo, Japan), and the videos were subsequently evaluated for classification, characterisation, and quantification of the convulsive reactions. The evaluation of the effects of the drug administration (PTZ, ibotenic acid and physiologic saline) was made with the rats inside the arena.

2.6. Experimental procedure

All the animals were exposed to the tail-flick test in order to record their baseline latencies. The next day, the rats were submitted to a stereotaxic surgery for the intra-PPTN approach. Independent groups of animals received a single unilateral microinjection of physiological saline (0.2 μl) or ibotenic acid (1.0 μg/ 0.2 μl) in the PPTN (n = 8 and 6 animals per group, respectively). In addition, a group of animals (n = 8) was submitted to the sham procedure (introduction of the injector dental needle in the PPTN without the microinjection). Five days after these procedures, the tail-flick latencies were recorded again, and after 5 min, the rats were intraperitoneally administered PTZ (64 mg/kg). The tail-flick withdrawal response to the nociceptive stimulus was measured immediately after the seizures, including 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, and 130 min after the convulsive reactions. Following the same protocol described above, a PPTN physiological saline + i.p. physiological saline control group (n = 6) was used as an additional control group. A temporal schematic representation of the experimental procedure is provided in Fig. 1.

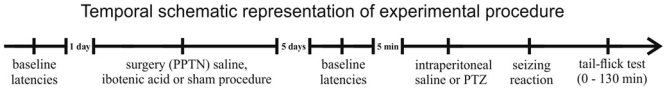

Fig. 1. − Temporal schematic representation of the experimental procedure.

2.7. Histology

Upon completion of the experiments, the animals were anaesthetised with 92 mg/kg ketamine and 10 mg/kg xylazine perfused through the left cardiac ventricle. The blood was washed out, and the brain was fixed in ice-cold 4% (w/v) paraformaldehyde in 0.1 M sodium phosphate buffer at pH 7.3. The brainstem was sectioned, removed and immersed in fresh fixative for 4 h at 4 °C. After immersion in cryoprotectants (for 24 h), the midbrain tissue pieces were prepared for cutting on a cryostat. They were then rinsed in 10% and 20% sucrose dissolved in 0.1 M sodium phosphate buffer (pH 7.4) at 4°C for at least 12 h in each solution. The tissue pieces were immersed in 2-methylbutane (Sigma), frozen on dry ice, embedded in Tissue Tek, and cut with a cryostat (CM 1950, Leica, Mannheim, Germany) at −22 °C. The sections were then mounted on glass slides coated with chrome alum gelatine to prevent detachment and stained with Klüver-Barrera [41] to localise the positions of the guide cannula in a motorised photomicroscope (AxioImager Z1; Zeiss, Oberkochen, Germany). A statistical analysis was performed exclusively with data from the animals that presented signs of microinjections to the PPTN that were successful.

2.8. Statistics

The behavioural data are expressed as the mean ± standard error of the mean (S.E.M.). The statistical analyses were performed as indicated in the results section using GraphPad Prism (version 6.0; GraphPad Software Inc.; San Diego, CA, USA). To investigate the effect of the ibotenic acid microinjection in the PPTN on the duration, latency, frequency and severity of convulsive reactions, the data from the experiments were analysed using a one-way analysis of variance (ANOVA) followed by Tukey's post hoc comparisons to determine significant overall F-values.

The tail-flick latencies are expressed as the mean ± S.E.M. The data from the tail-flick latencies following the tonic-clonic seizures were submitted to a repeated measurement analysis of variance (RM-ANOVA). In the case of a significant treatment versus time interaction, one-way ANOVAs were performed at each time interval, followed by Tukey's post hoc test. Comparisons between the tail-flick baseline values before stereotaxic surgery and the tail-flick baseline 5 days later were analysed by a Student's *t*-test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Histology

Microinjections of ibotenic acid in the PPTN (Fig. 2 A and B) caused neurotoxic damage in the neuronal bodies that showed a pyknotic nucleus, karyorrhexis, and intracytoplasmic vacuolisation (Fig. 2 C and D), surrounded by reactive gliosis, and there was preservation of the fibres of passage (Fig. 2 C and D).

Histologically confirmed sites of the microinjection of ibotenic acid, physiological saline or the sham procedure into the PPTN are shown in Fig. 3A. The parameters described for the ibotenic acid injections resulted in small lesions, which are represented by the black areas in Fig. 3 B.

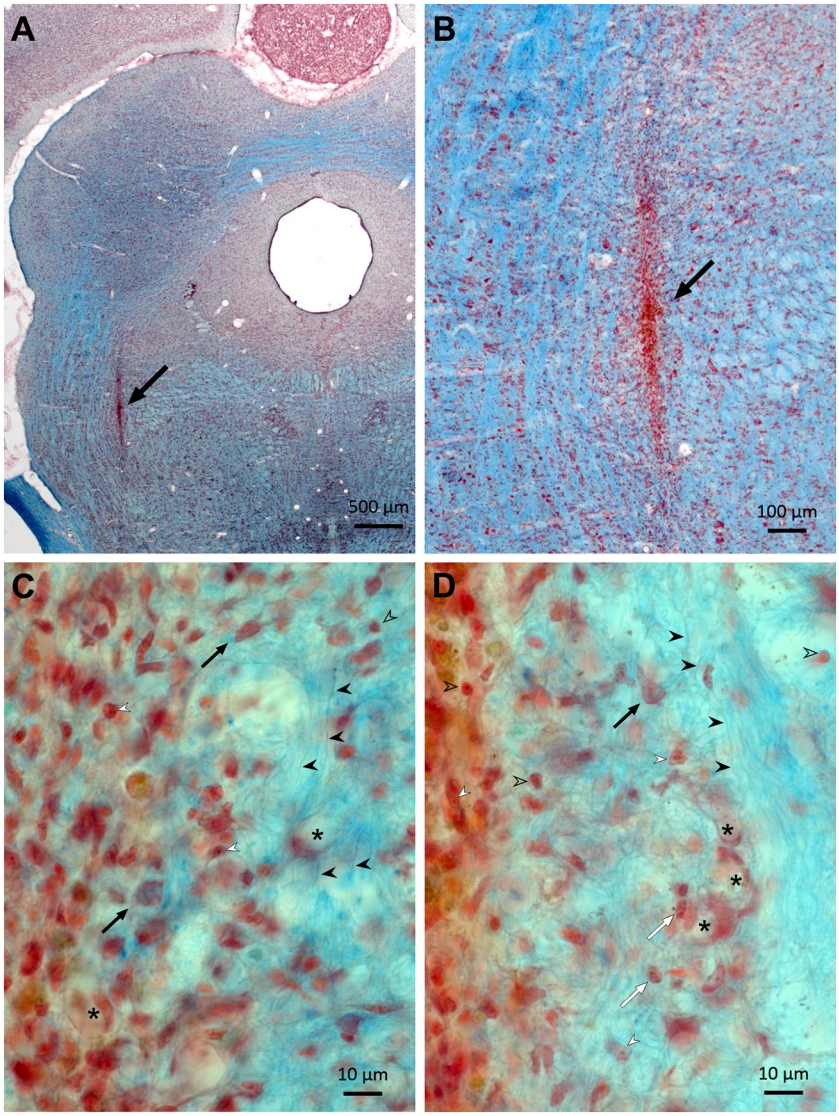

Fig. 2. –Photomicrographs of transverse sections of the midbrain at the level of the peduculopontine tegmental reticular nucleus (PPTN), showing a representative histological site (black arrow in A and B) of a ibotenic acid microinjection (1 µg/0.2 µL) in the PPTN. Note the presence of lesioned neuronal perikarya stained in red (black arrows in C and D), showing pyknotic nucleus (white arrowheads), karyorrhexis (white arrows), and vacuolisation (asterisk), surrounded by the reactive gliosis (open arrowheads), with preserved fibres of passage stained in blue (black arrowheads in C and D). Staining: Klüver-Barrera.

3.2. Behavioural seizures

The blockade of gamma-aminobutyric acid A ($GABA_A$) tonic-clonic seizures induced by the peripheral administration of PTZ at 64 mg/kg was characterised by jaw or facial, paw and head myoclonic reactions and the loss of postural control. Wild running was not displayed by the rats subjected to peripheral administration of PTZ in the present work.

According to the one-way ANOVA followed by Tukey's post hoc test, the systemic administration of PTZ at 64 mg/kg (physiological saline + PTZ-treated group) significantly decreased the latency [$F_{(3,26)} = 20.82$; $p < 0.001$] of the seizure compared with the control group (PPTN physiological saline-IP physiological saline-treated group), which indicated that the time necessary for the animal to start the seizure was significantly smaller than that displayed by the control group (Fig. 4A), and there was an increase of the duration [$F_{(3,26)} = 1.715$; $p < 0.05$; Fig. 4B] of the tonic-clonic seizures. A systemic administration of PTZ at 64 mg/kg (PPTN physiological saline + PTZ-treated group) caused a significant increase in the frequency [$F_{(3,26)} = 8.134$; $p < 0.001$; Fig. 4C] and severity of the seizures [$F_{(3,26)} = 31.76$; $p < 0.001$; Fig. 4D] compared with the control group (PPTN physiological saline + IP physiological saline-treated group). In addition, neither the sham nor the ibotenic acid-treated groups changed these parameters (Tukey's post hoc; $p > 0.05$ in all cases), as shown in Fig. 4.

3.3. Postictal antinociception

The convulsive motor reactions were followed by an increase in tail-flick latencies, a phenomenon known as postictal antinociception, as shown in Fig. 5. According to the RM-ANOVA, there were significant effects of treatment [$F_{(3,26)} = 20.31$; $p < 0.001$], time [$F_{(14,13)} = 19.12$; $p < 0.001$] and the interaction between treatment and time [$F_{(42,35)} = 3.51$; $p < 0.001$]. Both the physiological saline (PPTN) + i.p. PTZ and sham procedure (PPTN) + i.p. PTZ-treated groups had increased tail-flick latencies from 0 to 130 min [$F_{(3,26)}$ varying from 4.14 to 25.88; $p < 0.01$], displaying antinociception after seizure compared with the physiological saline (PPTN) + i.p. physiological saline-treated control group. On the other hand, the pretreatment of the PPTN with ibotenic acid followed by intraperitoneal administration of PTZ (PPTN ibotenic acid + i.p. PTZ-treated group) significantly decreased the postictal antinociception from 0 to 130 min

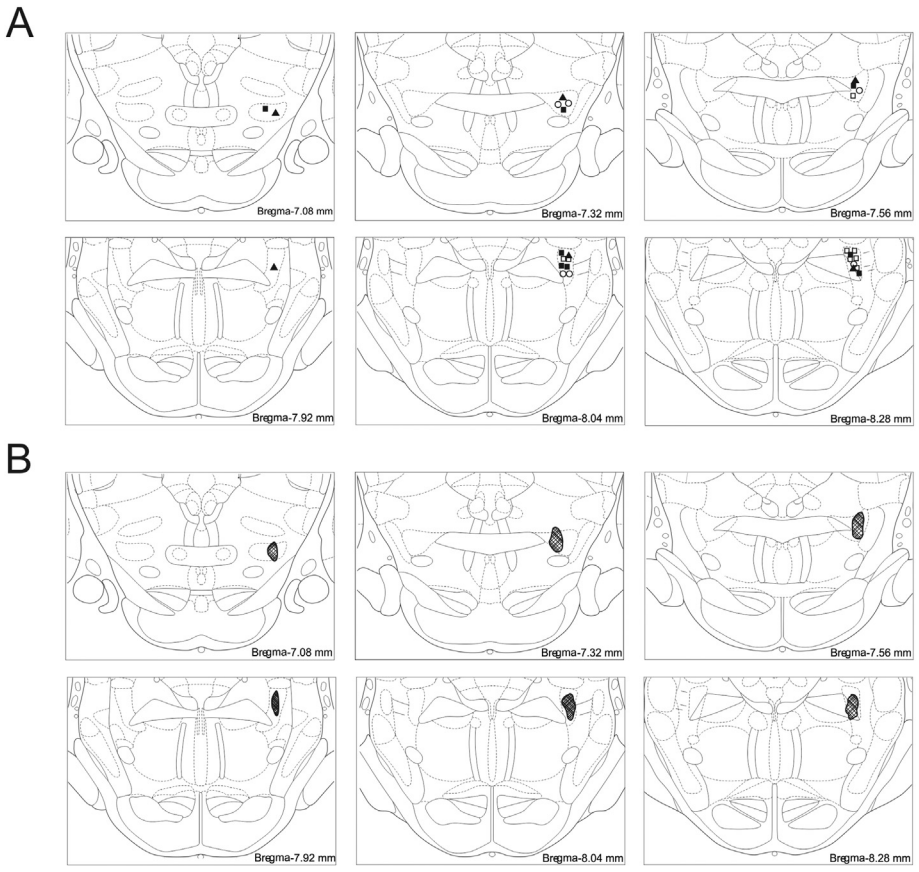

Fig. 3. – (A) Histologically confirmed sites of the physiological saline, ibotenic acid microinjections or sham procedure in the pedunculopontine tegmental nucleus (PPTN). (○) physiological saline (PPTN) + physiological saline (i.p.) (n = 6); (□) physiological saline (PPTN) + PTZ (i.p.) (n = 8); (■) sham procedure (PPTN) + PTZ (i.p.) (n = 8), (▲) ibotenic acid (PPTN) + PTZ (i.p.) (n = 6) depicted in modified anagrams of Paxinos and Watson's atlas (2007). (B) Schematic representation of the extension of the neurochemical lesion (hatched lines) and histologically confirmed sites of ibotenic acid injection (1 μg/0.2 μL) into the pedunculopontine tegmental nucleus (PPTN) (n = 8) followed by intraperitoneal (i.p.) treatment with pentylenetetrazole (PTZ), as depicted in the modified anagrams of Paxinos and Watson's rat brain in the stereotaxic coordinates atlas (2007).

after seizure compared with the PPTN physiological saline + i.p. PTZ-treated group [$F_{(3,26)}$ varying from 4.14 to 25.88; $p < 0.01$] and from 0 to 60 min after seizure compared with the PPTN physiological saline + i.p. PTZ-treated group [$F_{(3,26)}$ varying from 10.28 to 25.88; $p < 0.01$].

There were no significant differences between the basal nociceptive threshold recorded before the central microinjections of physiological

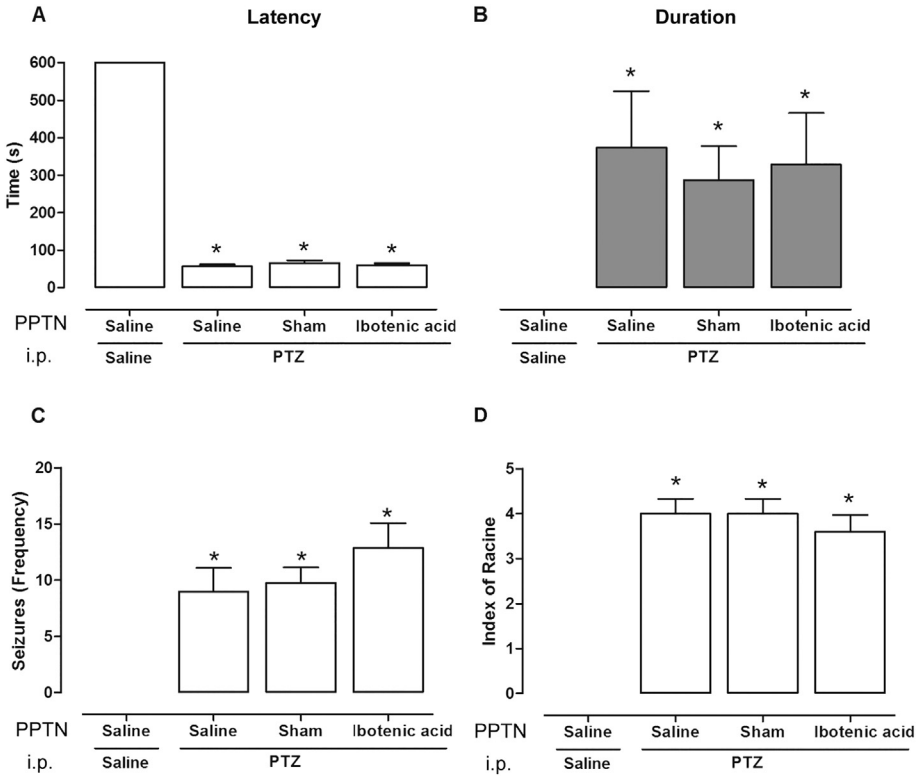

Fig. 4. – Effect of the treatment of the pedunculopontine tegmental nucleus (PPTN) with ibotenic acid (1 μg/0.2 μL), physiological saline or the sham procedure followed by intraperitoneal (i.p.) injections of pentylenetetrazole (PTZ; 64 mg/kg) or saline on the latency (A), duration (B), frequency (C), and severity (D) of the tonic-clonic seizures, according to one-way ANOVA. The data are presented as the mean ± S.E.M. *P < 0.05 compared to the saline (PPTN) + saline (i.p.)-treated group.

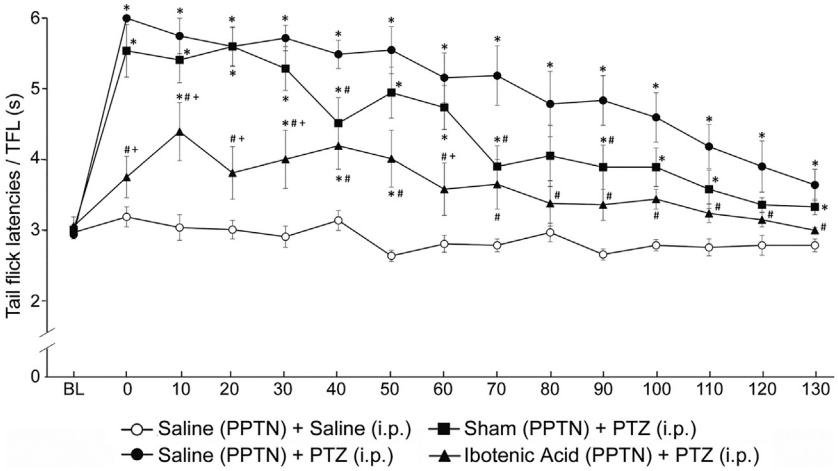

Fig. 5. – Effect of the microinjection of ibotenic acid (1 μg/0.2 μL), physiological saline or the sham procedure into the pedunculopontine tegmental nucleus (PPTN) (n = 6–8) on the postictal antinociception induced by an intraperitoneal (i.p.) treatment with pentylenetetrazole (PTZ; 64 mg/kg). The data are presented as the mean ± S.E.M. * # +, $p < 0.05$ compared to (*) the saline (PPTN) + physiological saline (i.p.)-treated group; (#) the physiological saline (PPTN) + PTZ (i.p.)-treated group; (+) the sham procedure (PPTN) + PTZ (i.p.)-treated group, according to repeated-measures ANOVA followed by Tukey's post hoc test. BL, baseline; s, second.

saline, ibotenic acid or the sham procedure performed during the stereotaxic surgery and 5 min before the i.p. treatment with PTZ performed 5 days after surgery ($p > 0.05$; Fig. 6).

4. Discussion

The neurotoxic lesion caused by ibotenic acid microinjection into the PPTN showed no effect on tonic-clonic seizures evoked by the peripheral administration of PTZ. The present data corroborate other previous findings that demonstrate the relevance of mesopontine reticular structures in experimental epilepsy and the postictal time-window. In fact, Mazzei-Silva et al. (2014) [14] demonstrated that the synaptic neurotransmission inactivation of PPTN neurons decreases postictal antinociception, and Miller et al. (1992) [42] showed that a lesion of the cholinergic neurons of the latero-dorsal tegmental nucleus (LdTN) was not able to change the motor reactions in an animal model of tonic seizures. In addition, there is evidence that neuroexcitotoxic lesions of pedunculopontine and laterodorsal tegmental nuclei neurons do not change the spike-and-wave activity of the electroencephalogram in an animal model of non-convulsive epileptic seizures. On the other hand, when chemically stimulated, both mesopontine nuclei, which are rich in cholinergic neurons, suppress the spike-and-wave discharges [43]. Thus, in agreement with other studies, our data suggest that the PPTN exerts a phasic inhibitory control on the activity of the neural network related with epilepsy.

Interestingly, Mazzei and colleagues [14] demonstrated that although pretreatment of the PPTN with cobalt chloride ($CoCl_2$), a non-selective blocker of synaptic contacts, did not cause anticonvulsive effects in epileptic rats, it was able to diminish the antinociceptive response elicited by the seizures in these same animals, suggesting that the dissociation between tonic-clonic seizures and postictal antinociception could be the result, at least in part, of a decrease in the activity of the cholinergic inputs to this pontomesencephalic structure. Here, although we showed that neurotoxic lesions of the PPTN that received ibotenic acid microinjections caused a similar effect to those observed with intra-PPTN injections of $CoCl_2$, since the ibotenic acid microinjected into the PPTN impaired the hypoalgesic response without influencing the seizures, the present findings do not precisely highlight the relevance of the synaptic inputs to the PPTN but do highlight the role played by the PPTN neurons in the organisation of postictal antinociception. Thus, we can argue that during the tonic-clonic seizures, not only are intrinsic connections within the PPTN recruited to elaborate the postictal antinociception but so are PPTN neurons and PPTN fugal pathways. Interestingly, a recent report showed evidence of the morphological neuroplasticity in mesopotine structures with an increased density of cholinergic varicosities in two different population of neurons in seizing animals [32]. Presumably, the postictal antinociception is under PPTN cholinergic control, and the evidence supported by our findings is highlighted by the relevance of the cholinergic system in pontomesencephalic tegmentum [27–29]. It is known that the PPTN is part of the cholinergic division of the ascending reticular activating system [44,45], and together with other tegmental structures, such as the LdTN, it sends descending projections to the medullary reticular formation [46], a modulatory centre of nociceptive processes [16].

Cholinergic systems of other brainstem nuclei may also be recruited during the inhibitory pain control mechanism and seem to modulate a complex neuronal network responsible for epileptic activity and

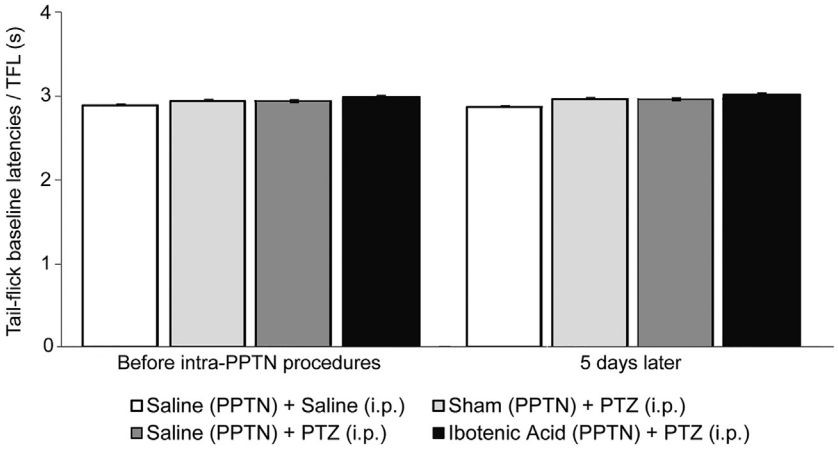

Fig. 6. – Lack of effect of the microinjection of ibotenic acid (1 μg/0.2 μL), physiological saline or the sham procedure into the pedunculopontine tegmental nucleus (PPTN) (n = 6–8) on the basal nociceptive thresholds recorded before and 5 days later, according to a one-way repeated-measure ANOVA. The data are presented as the mean ± S.E.M. s: second.

postictal antinociception [1,2,47,48]. In fact, microinjections of increasing doses of muscarinic and nicotinic cholinergic receptors antagonists into the nucleus raphe magnus [24], dorsal raphe nucleus [17] and locus coeruleus [18] impair the antinociception that follows tonic-clonic seizures. These findings support the hypothesis that ascending cholinergic pathways that reach the dorsal raphe nucleus and descending cholinergic pathways that project to the nucleus raphe magnus and locus coeruleus [29] play an important role in the organisation of the postictal antinociceptive phenomenon. Indeed, the neurotransmitter acetylcholine is released in the synaptic connection of neural networks in the initial stages of the postictal antinociception [15]. However, we cannot rule out the participation of other neurotransmitters, such as serotonin [11,12,16], noradrenaline [8], endogenous opioid peptides [9,10,49–51], and the endocannabinoid neuromodulators [52], in the PPTN in the organisation of postictal antinociception at different time-windows. In fact, there is evidence that serotonergic receptors [53], endogenous opioid peptides [54], and endocanabinoids [55] can interfere with the functions of PPTN neurons.

In the present study, the neurotoxic lesion of the PPTN that recieved ibotenic acid microinjections did not significantly alter the basal nociceptive thresholds, excluding any possible hyperalgesic phenomenon or non-expected intrinsic effect exerted by the neurotoxic lesion on the nociceptive baseline. Due to the reduction of postictal antinociception caused by the ibotenic acid-induced neurotoxical lesions in the PPTN, we suggest that the PPTN may be a key structure of the neural network that organises the postictal antinociception, without a significant effect on the severity of tonic and tonic-clonic seizures.

Curiously, a discrete reduction in the antinociceptive effect was observed after the sham procedure. Studies have shown that the lesions in the PPTN neurons caused by a sham procedure provoke pronociceptive effects in laboratory rats subjected to formalin tests, possibly due to local cholinergic depletion [56]. On the other hand, the non-damaging electrical stimulation of the PPTN increases tail-flick latencies in rodents subjected to a noxious heat stimulus [57]. This analgesic effect is suggested to be a result of the activation of the cholinergic fibres projecting to the nucleus raphe magnus [31]. In fact, the integrity of the PPTN cholinergic pathways is closely related to pain modulation, and it seems that its integrity is needed for the organisation of analgesic responses.

In conclusion, our results suggest that at least part of the postictal antinociception depends on the integrity of the PPTN neurons.

Conflicts of interest

The authors declare that there are no conflicts of interest with respect to the presented work.

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