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Restricted lesions of the ventrolateral or dorsal columns of the periaqueductal gray promotes distinct effects on tonic immobility and defensive analgesia in guinea pigs

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ABSTRACT

Tonic immobility (TI) is an innate defensive response exhibited by prey when physical contact with a predator is prolonged and inescapable. This defensive response is able to activate analgesia mechanisms; this activation has adaptive value because, during an attack by a predator, the manifestation of recuperative behaviors can affect the appropriate behavioral defense strategy. Some studies have suggested that similar structures of the central nervous system can regulate the response of both TI and nociception. Thus, this study evaluated the effect of chemical lesion through the administration of ibotenic acid in restricted brain areas of the periaqueductal gray matter (PAG) in guinea pig on the TI response and nociception evaluated in the hot plate test before and after emission of TI. The data showed that an irreversible chemical lesion in the ventrolateral PAG reduced of the TI response as well as defensive antinociception. However, a lesion in the dorsal PAG blocked the defensive antinociception induced by TI but did not alter TI duration. In summary, one could hypothesize that the neural substrates responsible for defensive behavior and antinociception represent similar systems that are distinct in modulation. Thus, the ventrolateral PAG has been associated with the modulation of TI and the defensive antinociception induced by TI. In contrast, the integrity of the dorsal PAG should be necessary for defensive antinociception to occur but not to elicit TI behavior in guinea pigs.

1. Introduction

In the natural environment, animals are confronted with threatening situations of extreme biological relevance, and the detection of these threats is of great importance so that the appropriate behavioral response can be performed quickly. One of the most important factors that triggers defensive behaviors is fear, which was defined as a motivational state activated by unconditioned and conditioned stimuli [1]. In fact, fear is an emotion that has one of the longest evolutionary histories, and it is found in various species of many phylogenetic classes. Additionally, in a dangerous situation, the choice of the behavioral defense response takes into account the distance between the prey and its predator [2] and the degree of threat offered by the situation [3]. These behaviors can be expressed in the form of behavioral inhibition, alertness [4,5], freezing [6], escape or flight [7], vocalizations, fight [8], and, finally, tonic immobility (TI), which occurs when physical contact with the predator is prolonged and inescapable [2,9]. It is important to note that defensive response can be followed by the simultaneous activation of the antinociceptive system, which has been demonstrated to be essential for the animal to accomplish the defense behavioral response appropriately. For example, in the case of injury during the confrontation between prey and predator, attending to pain can interfere in the defensive response, not allowing for an appropriate escape and, thus, decreasing the chance of survival. Nociceptive modulation can be considered an integral part of the set of defensive strategies [1]. In this context, a previous study showed that, immediately after the end of the TI episodes in guinea pigs, there was a significant reduction in the number of flinches and lickings in the formalin test and an increase in the index of analgesia in the hot plate test, suggesting that the endogenous antinociceptive systems were activated [10]. Again, Miranda-Páez et al. [11] have shown that melatonin microinjection into the ventrolateral (vl) periaqueductal gray matter (PAG) promoted an increase in the TI response, but did not modify the TI-induced antinociception in rats. In contrast, the same treatment into the

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dorsolateral (dl) PAG blocked TI-induced antinociception as well reduced the TI behavior. In fact, there has been some support for neurotransmitter systems that, together, modulate the defensive behavior and antinociception [12–14], and other reports have suggested that the defensive systems and the antinociceptive response are independent [11,15].

Some studies have suggested that the PAG, a region of the midbrain that surrounds the cerebral aqueduct, plays a major role in the integration of somatic and autonomic responses that are characteristic of defensive reactions, as well it is a major structure of a descending pain inhibitory system [16,17]. In this context, the PAG is known to control a large variety of physiological functions in mammals, including pain modulation [17], autonomic responses [18]; vocalization [19]; micturition and defecation; respiration [20] different patterns of behavior such as freezing, fight, and flight [21,22]; and TI [14,23]. With respect to TI in particular, different regions of the PAG can modulate this defensive response in distinct manners [24]. Cholinergic stimulation of the dorsal (d) PAG decreases the duration of TI episodes, while stimulation of the ventrolateral region increases it [25]. Previous functional study [26] in guinea pigs demonstrated an increase in the FOS protein imunoreactivity in the different functional columns of the PAG, with a greater activation of the ventrolateral (vl) column when compared with the activation of the dorsal areas of the PAG. Based on these observations, the present study was design to investigate whether a chemical lesion with the microinjection of ibotenic acid into the vlPAG or dPAG would be able to alter the TI response or the defensive antinociception induced by TI in the guinea pig.

2. Materials and Methods

2.1. Animals

Adult male guinea pigs (*Cavia porcellus*, University of São Paulo, *Campus* of Ribeirão Preto, Brazil) weighing 400–500 g ($n = 73$) were kept in Plexiglas wall cages (56 × 17 × 39 cm, five guinea pigs per cage) in a room maintained at 24° ± 1 °C, on a 12 h light cycle, with free access to water and food throughout the experimental period. The experiments were conducted during the light phase of the cycle. The experimental protocol was analyzed and approved by the Committee for Animal Care and Use of the University of São Paulo, *Campus* of Ribeirão Preto (n° 06.1.220.53.9).

2.2. Tonic Immobility Recording

This protocol was based on previous reports [10,23]. Briefly, the guinea pigs were submitted to five maneuvers of TI induction, and the duration of the episodes was recorded. The induction of TI was attempted by holding the guinea pig around the thorax with the hands, quickly inverting it and pressing it down into a shaped plywood trough (25 cm long × 15 cm high) (Supplementary Video 1). The pressure applied by the hands of the experimenter was proportional to the resistance offered by the guinea pig in response to the restraining maneuver. When the guinea pig stopped moving, the experimenter slowly withdrew his hand, and a chronometer was activated to measure the duration (in seconds) of the response, which ended when the guinea pig resumed the upright position. If the guinea pig did not become motionless within 60 s, the episode was recorded as having zero duration. For group analysis, the means of five episodes per guinea pig was considered.

2.3. Hot plate test

The hot plate consisted of an aluminum plate that is maintained at a temperature of 52° ± 0.5 °C. A Plexiglas cage (26 cm high × 16 cm long × 18 cm wide) fitted with a removable lid restrained the guinea pig on the surface of the plate. The hot plate test was performed by placing the guinea pig on the heated plate, and the time (latency) that the animal took to lick its genitals was recorded. Latency was considered to be the time taken by the animal to present this response, and the cut-off time was set at 70 s to avoid possible tissue injury. This test was standardized for guinea pigs by Leite-Panissi et al. [10]. The baseline comprised three measurements of the latency time in the hot plate test. After determination of the baseline, the next day, the animals were submitted to TI and, after that, to the hot plate test at 10 min intervals for 1 h. Each hot plate latency was normalized using an index of analgesia (AI) according to the following formula: (hot plate test – hot plate baseline)/(cut off time – hot plate baseline), where the hot plate baseline is the average of three basal latency and the hot plate test is the mean latency recorded for each group.

2.4. Drugs and microinjection procedure

The chemical lesions were made using ibotenic acid (IBO; 0.4 μg/0.2 μL) obtained from Sigma (St. Louis, MO) and dissolved in phosphate-buffered saline (PBS 0.01 M, pH 7.4). This acid is an excitotoxin that destroys neuronal bodies but spares fibers of passage. The dose used was based on previous studies [27]. The microinjections were performed with a dental needle (22 G) connected through a PE-10 polyethylene tube to a 10 μL Hamilton syringe. The microinjections were made during the surgical procedure. The control group was microinjected with 0.2 μL phosphate-buffered saline (PBS 0.01 M, pH 7.4). The total volume (0.2 μL) of ibotenic acid or PBS was injected over 1 min. After that, to avoid drug reflux, the needle was kept in position for > 4 min before removing it.

2.5. Experimental procedure

To investigate the specific role played by the dPAG and vlPAG in the TI, each guinea pig was submitted to control episode of TI induction and the duration of the episodes was recorded. One day after the control TI episode, the guinea pigs were submitted to bilateral ibotenic acid lesions were aimed at the dPAG ($n = 7$) or vlPAG ($n = 8$), and the other groups were microinjected in the vlPAG ($n = 6$) or the dPAG ($n = 6$) with PBS. After six days, the guinea pigs were submitted to TI session

Regarding fear-induced antinociception by tonic immobility, guinea pigs were initially exposed 3 times to the hot plate (basal latency). On the second day, the animals were subjected to the induction TI following the hot plate for every 10 min for 1 h. After this control of fear-induced antinociception by tonic immobility, bilateral ibotenic acid lesions were aimed at the vlPAG ($n = 6$) or the dPAG ($n = 7$), and the other groups were microinjected in the vlPAG ($n = 8$) or the dPAG ($n = 8$) with PBS. Additionally, in the control groups (without TI response induction), the animals received the guide cannula in the vlPAG ($n = 6$) or dPAG ($n = 6$) without microinjection of ibotenic acid or PBS. After six days, the guinea pigs were reexposed to the hot plate test (basal latency). The next day, the animals were subjected to the TI behavior (PBS and IBO groups) and after the hot plate test was performed over a 1 h period.

2.6. Surgical procedures

In this way, each animal was deeply anesthetized by an intramuscular injection of ketamine (100 mg/Kg) plus xylazine (14 mg/Kg) and placed in a stereotaxic apparatus (David-Kopf Instruments, USA) with the buccal piece 21.4 mm below the interauricular line, and two guide cannula (14 mm in length and 0.6 mm in outer diameter, prepared from a hypodermic needle) were implanted. According to the Rössner [28] atlas for the guinea pig, the stereotaxic coordinates for the placement of the guide cannula in the vlPAG were 10.0 mm caudal to the bregma, 0.6 mm lateral to the midline, and 2.8 mm above intraural line; those for the dPAG were 10.0 mm caudal to the bregma, 0.4 mm lateral to the midline, and 3.0 mm above intraural line. The guide

cannula were lowered to a depth of 1 mm above the target regions and fixed to the skull by means of a self-polymerizing resin and an additional anchoring screw. After recovery from anesthesia under a heat lamp, the animals were returned to the housing area. In addition, the guinea pigs received a subcutaneous injection of the anti-inflammatory and analgesic Banamine (Schering-Plought, flunixin meglumine, 2.5 mg/kg, 10 mg/mL, 0.2 mL). For each studied area, there were two experimental groups: one that received a bilateral microinjection of the IBO (IBO group), a second group that received a bilateral microinjection of phosphate-buffered saline (PBS group). Furthermore, in the fear-induced antinociception by tonic immobility protocol, an additional control group was made only with the guide cannula implanted.

2.7. Histological analysis of IBO chemical lesions

The Nissl method of cresyl violet staining was used to histologically examine serial 40 μm sections that encompassed the PAG. A microscopic analysis of the location and extent of lesions was performed with a light microscope, and lesion boundaries were observed under a microscope to determine the locations of the stimulated sites according to the Rössner atlas [28]. To describe the pattern of lesions in the guinea pig PAG, we employed the designations that were proposed in the Paxinos and Watson atlas [29]; from the rostral to the caudal levels, this atlas classifies regions into the dorsomedial (dm), dorsolateral (dl), lateral (l) and ventrolateral (vl) columns. In all of the guinea pigs, an area of neuronal cell loss and gliosis surrounded an area of central cavitation. Only the guinea pigs that had received microinjections that reached the target structure were used for the data analysis. Images were registered using an image analysis system (Image J), available at (http://www.rsb.info.nih.gov/nih-image/).

2.8. Statistical analysis

The data concerning the hot plate tests are reported as the means ± the standard error of the means (SEM) of AI and were analyzed by means of a two-way repeated measures analysis of variance (ANOVA) using experimental time and treatment as factors. Newman-Keuls *post-hoc* tests were performed when appropriate. The significance level was set at $P < 0.05$. TI behavior data are reported as the means ± the SEM of the mean duration of five episodes of TI. The data were analyzed with a paired *t*-test to determine the difference between periods (before or after PBS or IBO) with the level of significance set at $P < 0.05$.

3. Results

Fig. 1 shows the tonic immobility response (TI) in the PBS or IBO groups in distinct regions of the PAG in guinea pigs before (Control period) and after surgery. The statistical analyses indicated that only ventrolateral PAG (vlPAG) lesions induced by ibotenic acid promoted a reduction on TI duration in the animals, compared to the control period [$t_{(5)} = 2.818$; $P < 0.05$ paired *t*-test, Fig. 1A]. In contrast, lesions in the dPAG did not alter TI durations when compared with TI sessions in control before surgery [$t_{(6)} = 2.291$; $P > 0.05$; paired *t*-test, Fig. 1B]. In addition, microinjections of PBS into vlPAG and dPAG did not alter TI duration (Fig. 1A and B) compared to the TI session control.

Regarding the vlPAG, statistical analyses (Two-way RM ANOVA) revealed significant effects of the treatment [$F_{(2,17)} = 7.57$; $p = 0.004$], time [$F_{(14,238)} = 2.45$; $p = 0.003$] and interaction treatment *versus* time [$F_{(28,238)} = 2.48$; $p = 0.0001$]. The Newman-Keuls post-hoc test evidenced that fear-induced antinociception altered the AI ($P < 0.05$) in the PBS and IBO groups before surgery when compared to AI in the Control group from 10 min to 50 min after the TI response (Fig. 2). Furthermore, PBS and IBO groups did not differ before surgery. After surgery, the fear-induced antinociception promoted an increase in the AI only in the PBS group ($P < 0.05$, Newman-Keuls test) during the entire experiment, and the IBO group in the period 10 to 20 min and 40 to 60 min (Fig. 2) after the TI response. Moreover, the Control and IBO groups did not differ after surgery.

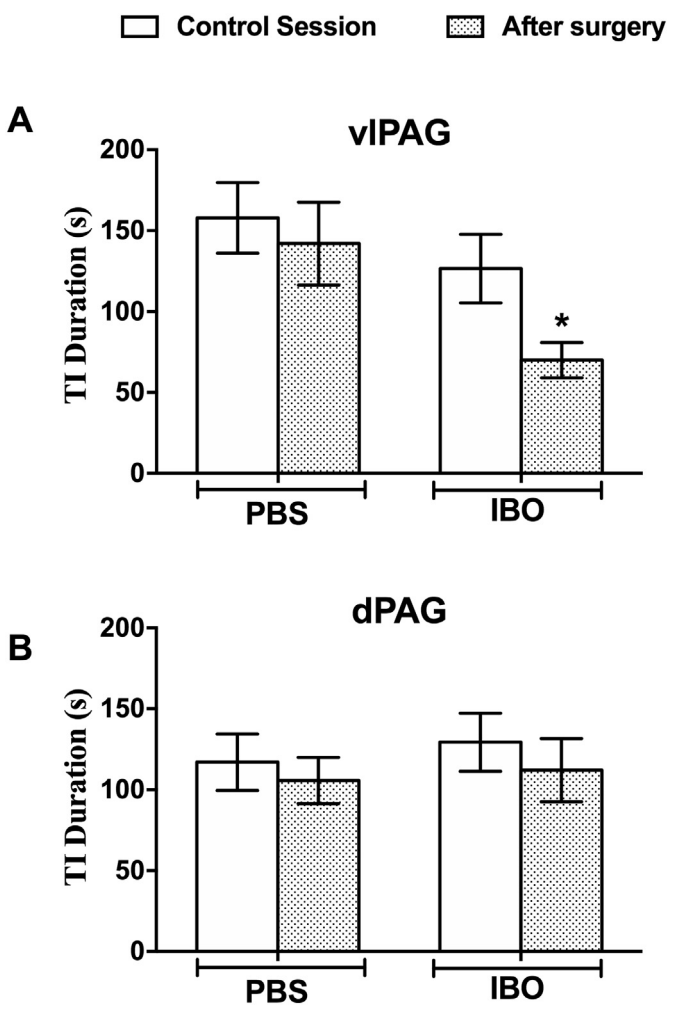

Fig. 1. Duration of tonic immobility episodes. Mean ± S.E.M. values of TI duration before surgery (Control Session) and after the administration (After Surgery) of phosphate buffered saline (PBS) or ibotenic acid (IBO) into the ventrolateral periaqueductal gray matter (vlPAG, A) or the dorsal periaqueductal gray matter (dPAG, B). *$P < 0.05$ by paired *t*-test when compared to respective TI duration before surgery (Control Session). $n = 7–9$ each group.

The Two-way RM ANOVA applied on the AI in the dPAG groups evidenced a significant effect of the treatment [$F_{(2,18)} = 18.91$; $p < 0.0001$], time [$F_{(14,252)} = 2.86$; $p < 0.0001$] and interaction treatment *versus* time [$F_{(28,252)} = 3.34$; $p < 0.0001$]. The Newman-Keuls post-hoc test evidenced that fear-induced antinociception altered the AI ($P < 0.05$) in the PBS (from 0 min to 50 min) and IBO (from 10 to 50 min) groups before surgery, compared to AI in the Control group, after the TI response (Fig. 2B). Furthermore, PBS and IBO groups did not differ before surgery. After surgery, the fear-induced antinociception promoted an increase in the AI only in the PBS group ($P < 0.05$, Newman-Keuls test) during the entire experiment, and IBO group in the period 10 to 50 min (Fig. 2B) after the TI response. Moreover, Control and IBO groups did not differ after surgery.

Fig. 3A shows the whole area of the microinjections of IBO that were placed in distinct coronal sections of the brainstem. Representative photomicrographs of the chemical lesions are shown in Fig. 3B. In the IBO groups, it is possible to identify neuronal body rarefaction, edema and gliosis in damaged areas. Diagrams of transverse sections of the brainstem indicate that lesions promoted by ibotenic acid microinjections in the vlPAG and dPAG extended approximately 1100 μm

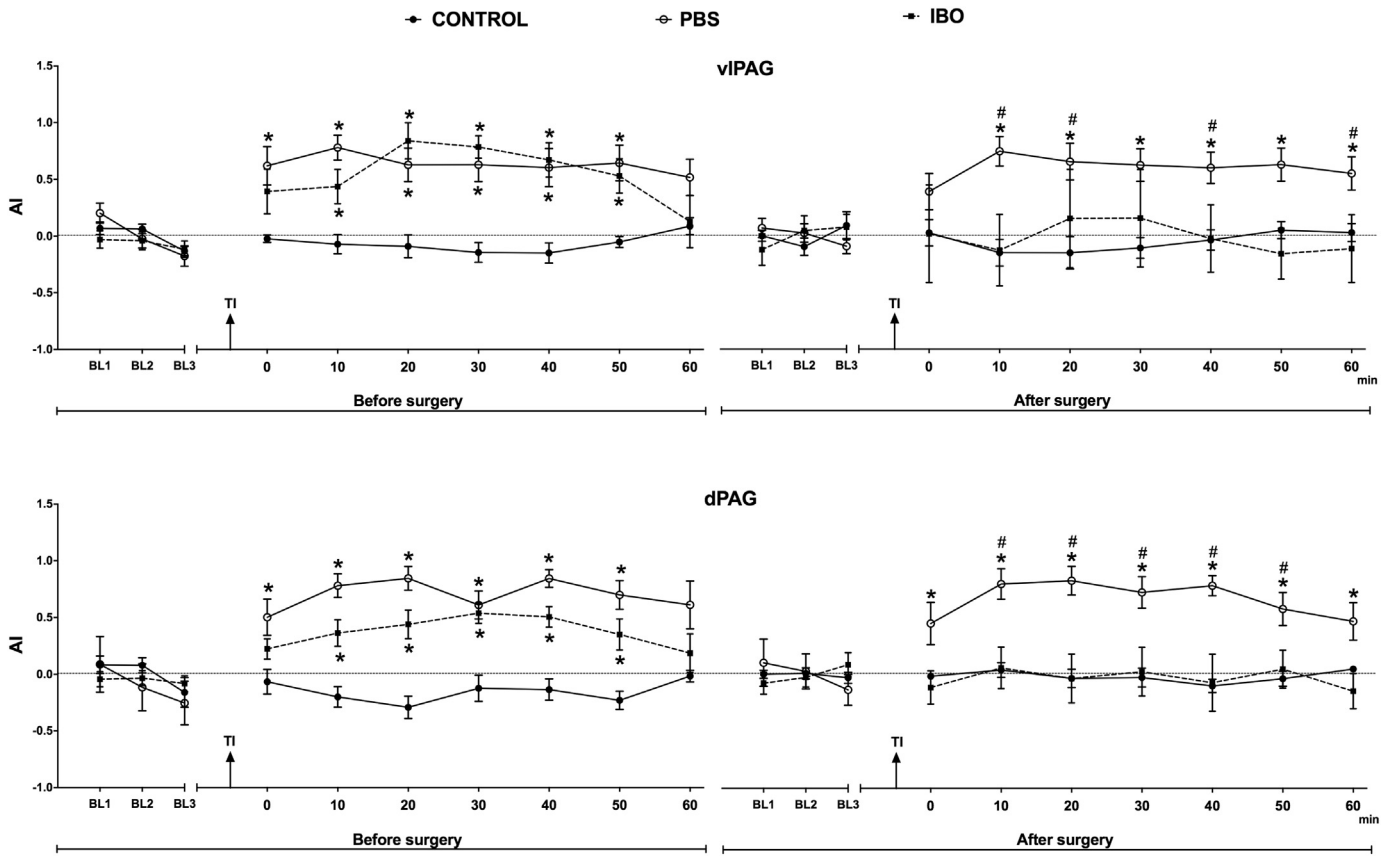

Fig. 2. Evaluation of analgesia index (AI) in the hot plate test in guinea pigs before surgery and after surgery in Control group (without TI induction) and in the groups that received phosphate-buffered saline (PBS) or ibotenic acid (IBO) administered into ventrolateral periaqueductal gray matter (vlPAG, A) or into dorsal PAG (dPAG, B). *P < 0.05 by Newman-Keuls test compared to Control group; #P < 0.05 by Newman-Keuls test compared to PBS group. Data are presented as the mean ± S.E.M. BL: Baseline hot plate latencies; TI: Tonic immobility response. n = 6–8 each group.

rostrocaudally.

4. Discussion

The results of the present study indicate that irreversible chemical lesions of the ventrolateral PAG promoted a reduction of the TI response and blocked defensive antinociception. Additionally, bilateral lesions of the dPAG reduced the defensive antinociception induced by TI, but did not alter TI duration. These data suggest that the vlPAG is a key structure for modulating TI in guinea pigs, and that its integrity is essential for defensive antinociception. However, although the dPAG can be important in modulating defensive antinociception, these areas are not crucial to maintaining the TI response. Since TI response is characterized by inhibition of the righting reflex [9], and vlPAG has direct projections to premotor interneurons in laminae VII and VIII which are involved in the control of neck and back movements [30], the lesion of this region can interfere in the emission of this defensive behavior.

Similar to the present results, a previous report has demonstrated that muscimol microinjection into the ventrolateral PAG drastically decreases the duration of TI episodes [24]. Moreover, the magnitude of TI reduction in the present study was lower than in a previously published report [24]. Interestingly, a study has demonstrated that the decrease in immobility scores (as measured by a forced swim test), produced by the muscimol inactivation of the medial prefrontal cortex, was similar to the decrease in immobility scores that was produced by a radiofrequency lesion of this region, and lower in magnitude than the decrease that was generated by IBO lesions of the same area [31]. In fact, it has been demonstrated that a neural reorganization of the lesioned area occurs after an IBO lesion [32,33], and this reorganization could have contributed to the smaller reduction of TI duration that was observed in our study relative to previously published results. Furthermore, ibotenic acid lesions can trigger a vigorous sprouting of 5-HT fibers; this effect is evident only 3 days after these lesions [34,35] have been produced in the striatum and hippocampus. Therefore, one possible hypothesis is that this reorganization of 5-HT fibers could have occurred in other areas, such as the regions that have been investigated in the present study. In addition, previous results have consistently indicated that the serotoninergic system [36], particularly that of the PAG [37], is involved in the modulation of the TI response. However, a previous report has determined that the reversible unilateral inactivation of the ventrolateral PAG does not alter TI responses in guinea pigs [23]. This result implies that because TI behavior plays an important role in the survival of the animal [38], this response is likely altered only when the lesions of the vlPAG are more widespread, such as in the case of the irreversible bilateral lesions used in the present study or after inactivation with muscimol [24].

With respect to the distinct involvement of the PAG in the modulation of the TI response in guinea pigs, a previous study indicated that the cholinergic stimulation of the dorsal PAG decreases the duration of TI episodes, whereas the stimulation of the ventrolateral region increases this duration [25]. Once again, it could be that the modulation of the TI in the vlPAG can occur through a circuit that is similar to the mechanism that has been proposed for the modulation of the antinociceptive response that involving opioidergic and gabaergic mechanisms [17,24]. These previously published findings are consistent with the results of the current study, which demonstrate that an irreversible lesion in the vlPAG reduces TI behavior and decreases the defensive antinociceptive response in guinea pigs. In fact, the ventrolateral neuronal columns of the PAG participate in the organization

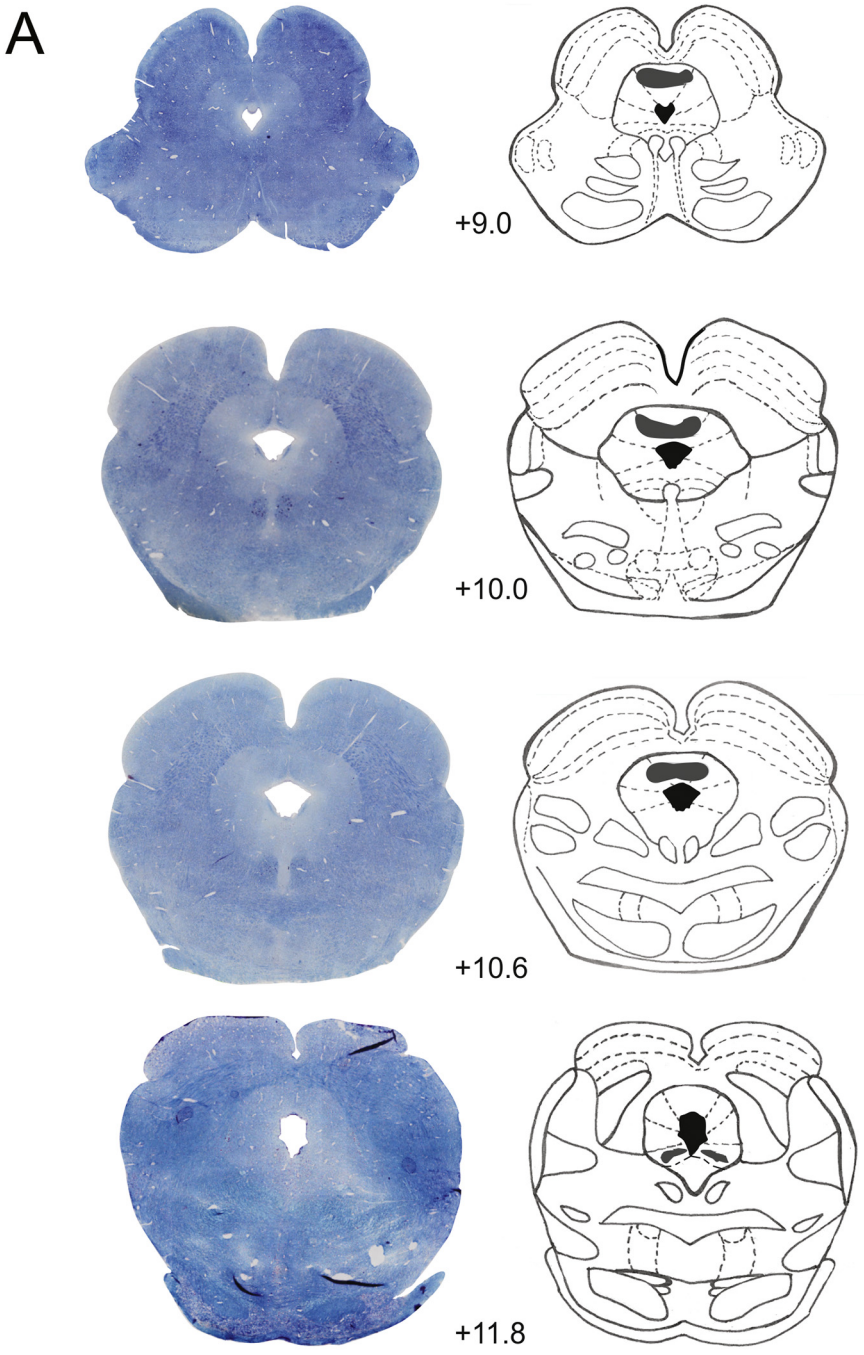

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Fig. 3. Schematic drawings that indicate lesions to the dorsal periaqueductal gray matter (dPAG) or ventrolateral periaqueductal gray matter (vlPAG). A: Photomicrographs and diagrams of coronal sections of the brainstem of the guinea pigs. The shading indicates the extent of neuronal loss. All of the coordinates are relative to the bregma (mm); sections are based on the Paxinos and Watson atlas [29]. B: Representative photomicrographs of a coronal section of the brainstem demonstrating the cell loss that resulted from bilateral injections of ibotenic acid. The scale bar represents 500 μm.

of passive responses that tend to reduce the physiological and emotional impact of an inescapable stimulus or a painful encounter and promote later recovery [39]. A functional study has shown that the electrical stimulation of the dorsal and lateral columns of the PAG produces a complex series of unconditioned behaviors, including tense immobility, exophthalmos, trotting, galloping, jumping, micturition, and defecation [40]. Thus, given that TI behavior is an innate fear response characterized by the profound motor inhibition that occurs after a predatory confrontation, it is possible to hypothesize that the integrity of the vlPAG is more crucial for this defensive strategy than the integrity of the dPAG. Similarly, Morgan et al. [13] demonstrated that the chemical stimulation (kainic acid or morphine sulfate) of the dorsal PAG promoted a fight response, whereas the same treatment in the ventrolateral PAG elicited immobility. Although there is a functional subdivision of the PAG with respect to the coordination of defensive behaviors, this modulation is an integrated functional system, and evidence has indicated that all PAG columns are interconnected by interneurons or projection neurons [41]. These PAG circuits could promote the activation or inhibition of specific PAG columns [42]. With respect to TI in guinea pigs, Monassi and Menescal-de-Oliveira [37] have suggested that during the TI response, ventrolateral PAG neurons may inhibit dorsal PAG neurons. These previous findings support the results of the current study, which indicate that an irreversible lesion of the dorsal PAG does not alter TI duration, whereas an irreversible lesion of the ventrolateral PAG decreases the TI response in guinea pigs. Furthermore, the activation of vlPAG with melatonin potentiated TI duration in a combined trial with a tail-flick test, whereas the same treatment in the dorsal PAG reduced this behavior in rats [11]. Taken together, these results suggested that the TI response could be modulating in a distinct manner by the dorsal or ventrolateral PAG.

Given the integration of defensive and antinociceptive systems, several findings support the notion that these two types of responses are sequentially modulated [1,10,43,44]. It has been demonstrated that different aversive stimuli are able to elicit both a defensive behavioral response and antinociception [43,45]. This simultaneous activation is essential for the animal to properly select the appropriate behavioral response for defense. This behavior occurs in the event of injuries during a prey-predator confrontation because pain-related considerations can interfere with the defensive response by inhibiting proper flight, thereby reducing the chances of survival [43]. In particular, pioneering reports have demonstrated that the TI response in rabbits [46] and in guinea pigs [10] elicited antinociception. This defensive antinociception was blocked by pre-treatment with a systemically administered opioid antagonist (naloxone), suggesting the involvement of at least one opioid synapse in the antinociceptive response [10,14]. These findings corroborate the present results, which demonstrate that the TI response promoted an increase in AI in the hot plate test prior to surgery in all of the experimental groups. However, although several studies have demonstrated that behavioral and antinociceptive responses occur sequentially [1], the dorsal and ventrolateral PAG can also engage in the independent modulation of these responses. For instance, in rats, treating the ventral PAG with an opioid antagonist (naltrexone) reduced fear-induced analgesia but not the occurrence of the freezing response [47]. However, it has been reported that electrolytic lesions of the dorsolateral PAG inhibit the conditioned antinociception and decreased fear responses [48]. Similarly, Miranda-Páez et al. [11] have shown that melatonin microinjection into the dorsal PAG decreased the TI-induced antinociception, as well the TI duration in a combined trial TI and tail-flick test. Therefore, it is possible that the dorsolateral and ventrolateral columns of the PAG can modulate defensive behavior and antinociception in distinct ways. With respect to antinociception modulation, previous report has demonstrated that lateral and ventrolateral PAG projected to ventromedial tegmentum of caudal brainstem, including the nucleus raphe magnus [20], a crucial region involved in nociception modulation [17]. Additionally, electrical stimulation in the dorsal PAG promoted antinociception that was abolished by bilateral lesions in the ventrolateral medulla (i.e. in nucleus paragigantocellularis lateralis) [49]. Again, electrical or chemical stimulation of the dorsal PAG results in non-opioid hypoalgesia, hypertension, and avoidance escape or aggressive responses, while ventrolateral PAG stimulation triggers opioid antinociception, hypotension and defensive freezing [16,18,40,47,49]. Thus, these evidences support the results found in the present study where the lesion of the dorsal or ventrolateral portions of the PAG can modulated antinociception in guinea pigs.

In summary, our results suggest that the neural substrates responsible for defensive behavior and antinociception represent similar systems, but are distinct in modulation. Thus, the ventrolateral PAG has been associated with the modulation of TI and the defensive antinociception induced by TI. In contrast, the integrity of the dorsolateral PAG should be necessary for defensive antinociception to occur, but not for maintenance of TI behavior in guinea pigs.

Conflict of interest statement

The authors have declared that this research does not have any conflict of interest.

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References

[1] R.C. Bolles, M.S. Fanselow, A perceptual-defensive-recuperative model of fear and pain, Behav. Brain Sci. 3 (1980) 291–301.
[2] S.C. Ratner, Comparative aspects of hypnosis, in: J.E. Gordon (Ed.), Handbook of Clinical and Experimental Hypnosis, Macmillian, New York, 1967, pp. 550–587.
[3] D.D. Blanchard, R.J. Blanchard, Ethoexperimental approaches of the biology of emotion, Annu. Rev. Psychol. 39 (1988) 43–68.
[4] N.C. Coimbra, M.L. Brandão, GABAergic nigro-collicular pathways modulate the defensive behavior elicited by midbrain tectum stimulation, Behav. Brain Res. 59 (1993) 131–139.
[5] J.A. Gray, The Neuropsychology of Anxiety: An Enquiry into the Functions of the Septo-Hippocampal System, Oxford University Press, 1982.
[6] S.P. Zhang, R. Bandler, P. Carrive, Flight and immobility evoked by excitatory amino acid microinjection within distinct parts of the sub-tentorial midbrain periaqueductal gray of the cat, Brain Res. 520 (1990) 73–82.
[7] J.A. Panksepp, Critical role for "affective neuroscience" in resolving what is basic about basic emotions, Psychol. Rev. 3 (1990) 315–331.
[8] W.R. Hess, M. Brugger, Das subkortikale zentrum der affectiven abwerreaktion, Helv. Physiol. Pharmacol. Acta. 1 (1943) 33–52.
[9] W.R. Klemm, Neurophysiologic studies of the immobility reflex ("Animal hypnosis"), Neurosci. Res. 4 (1971) 165–212.
[10] C.R.A. Leite-Panissi, C.L. Rodrigues, M.R. Brentegani, L. Menescal-de-Oliveira, Endogenous opiate analgesia induced by tonic immobility in guinea pigs, Braz. J. Med. Biol. Res. 34 (2001) 245–250.
[11] A. Miranda-Páez, S. Zamudio, P. Vázquez-León, V. Sandoval-Herrera, I. Villanueva-Becerril, G. Carli, Effect of melatonin injection into the periaqueductal gray on antinociception and tonic immobility in male rats, Horm. Behav. 89 (2017) 23–29.
[12] L. Menescal-de-Oliveira, A. Hoffmann, The parabrachial region, a possible substrate shared by the systems that modulate pain and tonic immobility, Behav. Brain Res.

56 (1993) 127–132.
[13] M.M. Morgan, P.K. Whitney, M.S. Gold, Immobility and flight associated with antinociception produced by activation of the ventral and lateral/dorsal regions of the rat periaqueductal gray, Brain Res. 804 (1998) 159–166.
[14] A. Miranda-Páez, S. Zamudio, P. Vásquez-León, C. Campos-Rodrígues, E. Ramírez-San Juan, Involvement of opioid and GABA systems in the ventrolateral periaqueductal gray on analgesia associated with tonic immobility, Pharmacol. Biochem. Behav. 142 (2016) 72–78.
[15] C.R.A. Leite-Panissi, M.R. Brentegani, L. Menescal-de-Oliveira, Cholinergic-opioidergic interaction in the central amygdala induces antinociception in the guinea pig, Braz. J. Med. Biol. Res. (10) (2004) 1571–1579.
[16] R. Bandler, P. Carrive, Integrated defense reaction elicited by excitatory amino acid microinjection in the midbrain periaqueductal gray region of the unrestrained cat, Brain Res. 439 (1988) 95–106.
[17] A.I. Basbaum, H.L. Fields, Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry, Annu. Rev. Neurosci. 7 (1984) 309–338.
[18] T.A. Lovick, Interactions between descending pathways from the dorsal and ventrolateral periaqueductal gray matter in the rat, in: A. Depaulis, R. Bandler (Eds.), The Midbrain Periaqueductal Gray Matter Functional, Anatomical and Neurochemical Organization, Plenum Press, New York, 1991, pp. 101–120.
[19] U. Jürgens, R. Pratt, Role of the periaqueductal gray in vocal expression of emotion, Brain Res. 167 (1979) 367–378.
[20] G. Holstege, The periaqueductal gray controls brainstem emotional systems including respiration, Prog. Brain Res. 209 (2014) 379–405.
[21] R. Bandler, A. Depaulis, Midbrain periaqueductal gray control of defensive behavior in the cat and the rat, in: A. Depaulis, R. Bandler (Eds.), The Midbrain Periaqueductal Gray Matter: Functional, Anatomical and Neurochemical Organization, Plenum Press, New York, 1991, pp. 175–198.
[22] M.L. Brandão, V.Z. Anseloni, J.E. Pandóssio, J.E. De Araújo, V.M. Castilho, Neurochemical mechanisms of the defensive behavior in the dorsal midbrain, Neurosci. Biobehav. Rev. 23 (1999) 863–875.
[23] C.R.A. Leite-Panissi, N.C. Coimbra, L. Menescal-de-Oliveira, The cholinergic stimulation of the central amygdala modifying the tonic immobility response and antinociception in guinea pigs depends on the ventrolateral periaqueductal gray, Brain Res. Bull. 60 (2003) 167–178.
[24] C.R. Monassi, C.R.A. Leite-Panissi, L. Menescal-de-Oliveira, Ventrolateral periaqueductal gray matter and the control of tonic immobility, Brain Res. Bull. 150 (1999) 201–208.
[25] C.R. Monassi, A. Hoffmann, L. Menescal-de-Oliveira, Involvement of the cholinergic system and periaqueductal gray matter in the modulation of tonic immobility in guinea pigs, Physiol. Behav. 62 (1997) 53–59.
[26] E.B. Vieira-Rasteli, L. Menescal-de-Oliveira, C.R.A. Leite-Panissi, Functional mapping of the periaqueductal gray matter involved in organizing tonic immobility behavior in guinea pigs, Behav. Brain Res. 216 (2011) 94–99.
[27] L.H. Gargaglioni, N.C. Coimbra, L.G. Branco, Chemical lesions of the nucleus isthmi increase the hypoxic and hypercarbic drive to breathing of toads, Respir. Physiol. Neurobiol. 133 (2002) 289–299.
[28] W. Rössner, Stereotaktischer Hirnatlas Vom Meerschweinchen, 1st ed., Pallas Verlag, Munich, 1965.
[29] G. Paxinos, C. Watson, The rat brain in stereotaxic coordinate, The new coronal set, 5th ed., Elsevier Academic Press, New York, 2005.
[30] L.J. Mouton, G. Holstege, The periaqueductal gray in the cat projects to lamina VIII and the medial part of lamina VII throughout the length of the spinal cord, Exp. Brain Res. (1994) 253–264.
[31] C. Hamani, D. Mustansir, C.E. Macedo, M.L. Brandão, J. Shumake, F. Gonzalez-Lima, R. Raymond, A.M. Lozano, P.J. Fletcher, J.N. Nobrega, Antidepressant-like effects of medial prefrontal cortex deep brain stimulation in rats, Biol. Psychiatry 67 (2010) 117–124.
[32] S. Klein, M. Koch, K. Schwabe, Neuroanatomical changes in the adult rat brain after neonatal lesion of the medial prefrontal cortex, Exp. Neurol. 209 (2008) 199–212.
[33] J.B. Schulz, A. Wree, A. Schleicher, K. Zilles, Plasticity in the rat hippocampal formation following ibotenic acid lesion of the septal region: a quantitative [14C] deoxyglucose and acetylcholinesterase study, J. Cereb. Blood Flow Metab. 12 (1992) 1007–1021.
[34] F.C. Zhou, E.C. Azmitia, S. Bledsoe, Rapid serotonergic fiber sprouting in response to ibotenic acid lesion in the striatum and hippocampus, Dev. Brain Res. 84 (1995) 89–98.
[35] F.C. Zhou, E.C. Azmitia, Induced homotypic collateral sprouting of serotonergic fibers in hippocampus, Brain Res. 308 (1984) 53–62.
[36] C.R.A. Leite-Panissi, A.A. Ferrarese, A.L.B. Terzian, L. Menescal-De-Oliveira, Serotoninergic activation of the basolateral amygdala and modulation of tonic immobility in guinea pig, Brain Res. Bull. 69 (2006) 356–364.
[37] C.R. Monassi, L. Menescal-De-Oliveira, Serotonin 5-HT_2 and 5-HT_{1A} receptors in the periaqueductal gray matter differentially modulate tonic immobility in guinea pig, Brain Res. 1009 (2004) 169–180.
[38] A.B. Sargeant, L.E. Eberhardt, Death feigning by ducks in response to predation by red foxes (*Vulpes fulva*), Am. Midbrain Nat. 94 (1975) 108–119.
[39] R. Bandler, M.T. Shipley, Columnar organization in the midbrain periaqueductal gray: modules for emotional expression? Trends Neurosci. 17 (1994) 379–389.
[40] A.S. Bittencourt, A.P. Carobrez, L.P. Zamprogno, S. Tufik, L.C. Schenberg, Organization of single components of defensive behaviors within distinct columns of periaqueductal gray matter of the rat: role of n-methyl-d-aspartic acid glutamate receptors, Neuroscience 125 (2004) 71–89.
[41] A.S.P. Jansen, E. Farkas, M. Sams, A.D. Loewy, Local connections between the columns of the periaqueductal gray matter: a case of intrinsic neuromodulation, Brain Res. 784 (1998) 329–336.
[42] P.S. Katz, W.N. Fros, Intrinsic neuromodulation: altering neuronal circuits from within, Trends Neurosci. 19 (1996) 54–61.
[43] M.S. Fanselow, Shock-induced analgesia on the formalin test: effects of shock severity, naloxone, hypophysectomy, and associative variables, Behav. Neurosci. 1 (1984) 79–95.
[44] R.J. Rodgers, J.I. Randall, Social conflict analgesia: inhibition of early non-opioid component by diazepam or flumazepil fails to affect appearance of late opioid component, Brain Res. Bull. (1) (1987) 141–144.
[45] M.S. Fanselow, F.J. Helmstetter, Conditional analgesia, defensive freezing, and benzodiazepines, Behav. Neurosci. 102 (1988) 233–243.
[46] G. Carli, F. Farabollini, G. Fontani, F. Grazzi, Physiological characteristics of pressure immobility. Effect of morphine, naloxone and pain, Behav. Brain Res. 12 (1984) 55–63.
[47] M.S. Fanselow, The midbrain periaqueductal gray as coordinator of action in response to fear and anxiety, in: A. Depaulis, R. Bandler (Eds.), The midbrain Periaqueductal Gray Matter: Functional, New York, Plenum Publishing Corporation, Anatomical and Immunohistochemical Organization, 1991, pp. 151–173.
[48] I.B. Kinscheck, L.R. Watkins, D.J. Mayer, Fear is not critical to classically conditioned analgesia: the effects of periaqueductal gray lesions and administration of chlordiazepoxide, Brain Res. 298 (1984) 33–44.
[49] T.A. Lovick, Ventrolateral medullary lesions block the antinociceptive and cardiovascular responses elicited by stimulating the dorsal periaqueductal grey matter in rats, Pain 21 (1985) 241–252.