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Research report

Ventrolateral periaqueductal gray lesion attenuates nociception but does not change anxiety-like indices or fear-induced antinociception in mice

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ABSTRACT

The exposure of rodents to an open elevated plus-maze (oEPM: four open arms raised from the floor) elicits naloxone-insensitive antinociception. Midazolam infusion into the dorsal portion of the periaqueductal gray (dPAG), a structure of the descending inhibitory system of pain, failed to alter oEPM-induced antinociception. Chemical lesion of dorsomedial and dorsolateral PAG attenuated defensive behavior in the standard EPM (sEPM), an animal model of anxiety, but failed to change oEPM-induced antinociception. The present study investigated the effects of bilateral lesion, with the injection of NMDA (*N*-methyl-D-aspartic acid), of the ventrolateral column of PAG (vlPAG) (i) on nociceptive response induced by 2.5% formalin injected into the right hind paw (nociception test) in mice exposed to the enclosed EPM (eEPM: four enclosed arms – a non-aversive situation) or to the oEPM and (ii) on anxiety indices in mice exposed to the sEPM without prior formalin injection. Results showed that oEPM-induced antinociception was not altered by lesion of vlPAG. Nevertheless, the lesion reduced the nociceptive response in mice exposed to the eEPM and increased general locomotor activity during the eEPM and oEPM exposure. Furthermore, vlPAG lesion did not alter anxiety-like indices in mice exposed to the sEPM. The results suggest that vlPAG does not play a role in oEPM-induced antinociception or in defensive reactions assessed in the sEPM. Moreover, vlPAG inactivation induces pain inhibition in mice not exposed to an aversive situation and seems to increase general activity.

1. Introduction

Reynolds [1] was the first to report that electrical stimulation of the midbrain periaqueductal gray (PAG) produces pain inhibition. Since then, many studies have characterized the PAG as an important structure of the descending pain inhibitory system [for a review, see Ref. [2]]. The effect elicited by PAG stimulation on nociception involves modulation of the nociceptive transmission in the spinal cord, a role that seems to depend on the rostral ventromedial medulla (RVM) function, since RVM lesion abolishes this type of pain inhibition [2–4].

The PAG is anatomically and functionally organized, being divided into four longitudinal parts that run parallel to the aqueduct, viz. the dorsomedial, dorsolateral, lateral and ventrolateral columns [5,6]. Chemical or electrical stimulation of dorsolateral and lateral PAG (dlPAG and lPAG) causes vigorous somatomotor activity, sympathoexcitation, cardio respiratory alterations and short-term non-opioid mediated analgesia [3,5,7–11]. On the other hand, the stimulation of the ventrolateral PAG (vlPAG) evokes "conservation-withdrawal" strategies or a passive coping reaction of quiescence/immobility, decreased vigilance and hyporeactivity, hypotension, bradycardia and an opioid analgesia which has a relatively long time course [5–7,9–13].

It has been shown that PAG also participates in some types of environmentally induced analgesia [9,14–17]. Concerning the fact that animals display pain inhibition when confronted with threatening situations, Bolles and Fanselow [18] emphasized that this reaction has a clear adaptive value, since it gives the animal an opportunity to exhibit defensive behavior, even though an injury has occurred, thereby increasing its chances of survival. Thus, considered to be an important structure of the brain defensive system (e.g., Ref. [19,20]), the PAG also participates in some types of aversive situation-induced antinociception.

It has been demonstrated that exposure of rodents to an elevated plus-maze (EPM: an apparatus in the shape of a plus sign, with two open and two enclosed arms, each with an open roof, elevated from the floor), a widely used animal model of anxiety [for a review, see Ref. [21]], besides inducing defensive behavioral responses, also elicits antinociception [22–25]. In experiments carried out in our laboratory, EPM-exposed mice do not display intense antinocicep-

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tion, assessed by the formalin nociception test. Nevertheless, when placed in a totally open elevated plus-maze (oEPM: with four open arms) mice [26,27] and rats [28] exhibit an intense antinociceptive response.

In order to investigate the role played by the dorsal portion of the PAG (dPAG: dm and dl columns) on oEPM-induced antinociception, we lesioned the dPAG by injecting a high dose of NMDA (*N*-methyl-D-aspartate) into this site. Bilateral dPAG lesion did not change oEPM-induced antinociception, but attenuated anxiety-like behavior in mice exposed to a standard EPM (sEPM: two open and two enclosed arms) [27]. Given that the vlPAG also modulates some forms of environmentally induced antinociception [9,14,16,17,29], the present study investigated the role of vlPAG on oEPM-induced antinociception and on sEPM-induced anxiety-like behavior in mice. To this end, a bilateral lesion was induced in the vlPAG by an injection of NMDA. The formalin test was used to assess nociception in the mice and was specifically chosen since it permits the assessment of pain sensitivity concurrently with exposure to an aversive situation, namely the oEPM.

2. Materials and methods

2.1. Subjects

The subjects were male Swiss mice (São Paulo State University/UNESP, SP, Brazil), weighing 25–35 g. They were housed in groups of 7 per cage (41 cm × 34 cm × 16 cm) and maintained under a 12:12 h light/dark cycle (lights on at 7:00 a.m.) in a temperature (23 ± 1 °C) and humidity (55 ± 5%) controlled environment. Food and drinking water were freely available. All mice were experimentally naive, and experimental sessions were carried out during the light phase of the cycle (9 a.m.–5 p.m.).

2.2. Drugs

NMDA (*N*-methyl-D-aspartic acid; RBI) dissolved in physiological saline (0.9% NaCl). This compound was injected bilaterally into vlPAG in a dose of 2 μg in 0.2 μl [30].

2.3. Surgery and excitotoxic lesion of vlPAG

Mice were bilaterally implanted with 7 mm stainless-steel guide cannulae (26 gauge; Cooper's Needleworks, Birmingham, UK) under chloral hydrate (500 mg/kg, i.p.) anesthesia. The guide cannulae were fixed to the skull with dental acrylic and jeweller's screws. Stereotaxic coordinates for the PAG were 4.1 mm posterior to the bregma, ±2.0 mm lateral to the midline and 2.1 mm ventral to skull surface, with the guide cannula angled 29° to the vertical axis. These coordinates were based on the Paxinos and Franklin mouse brain atlas [31].

NMDA solution was injected into the vlPAG immediately after the guide cannulae were implanted, while the mice were under anesthesia. Solutions were infused into the vlPAG through a microinjection unit (33 G cannula; Cooper's Needleworks), which extended 2.0 mm beyond the tip of each guide cannula. The microinjection unit was attached to a 5 μl Hamilton microsyringe via polyethylene tubing (PE-10) and administration was controlled by an infusion pump (BI 2000, Insight Equipamentos Científicos LTDA, Brazil) programmed to deliver a volume of 0.2 μl over a period of 60 s. The microinjection procedure consisted of gently restraining the animal, inserting the injection unit, infusing the solution, and keeping the injection unit in situ for a further 180 s. Confirmation of successful infusion was obtained by monitoring the movement of a small air bubble in the PE-10 tubing.

For each lesion group, a matching sham–lesion group (i.e. a group that was subjected to the same surgical procedure, but did not receive any intra-vlPAG injection) was added as a control group.

2.4. Formalin test

Nociception was assessed by the formalin test as previously described [32]. The formalin test causes a two-phase nociceptive response [33]. The first phase begins immediately after formalin injection and lasts approximately 5 min. It results from the direct stimulation of nociceptors [33,34]. The second phase begins 20 min after the injection and lasts approximately 40 min [35]. This phase is caused by C fibers activation [34,36] and also involves a period of sensitization during which inflammatory phenomena occur [36,37].

2.5. Apparatus and general procedure

The basic elevated plus-maze design was similar to that originally validated for mice [38]. The standard EPM (sEPM) consisted of two open arms (30 cm × 5 cm × 0.25 cm) and two enclosed arms (30 cm × 5 cm × 15 cm) connected to a common central platform (5 cm × 5 cm). The apparatus was constructed from wood (floor) and transparent glass (clear walls) and was raised to a height of 38.5 cm above floor level. The other two mazes were similarly constructed, but comprised either four enclosed arms (eEPM) or four open arms (oEPM).

Five to six days after surgery, mice were transported to the experimental room and left undisturbed for at least 30 min prior to testing. An aliquot of 50 μl of 2.5% formaldehyde solution was then injected into the dorsal surface of the right hind paw of each mouse, which was individually placed in a glass holding cage (30 cm × 20 cm × 25 cm). Twenty-five minutes after formalin injection, animals were exposed to the eEPM or oEPM, where the time spent on licking was noted for a period of 10 min (25–35 min after formalin injection). In order to investigate whether the NMDA lesion had effects on anxiety-like behavior, some mice were subjected to the same procedure as described above, except that they did not receive prior formalin injection and were only exposed to the sEPM.

On the sEPM, mice were individually placed on the central platform of the maze facing the left open arm. Both the eEPM and oEPM were similarly positioned in the experimental room and the experimenter placed the animal facing the arm that corresponded in direction to the sEPM left open arm, even though the eEPM had no open arms and the oEPM no enclosed arms. Between subjects, the mazes were thoroughly cleaned with 20% ethanol and a dry cloth. All sessions were video-recorded by a camera linked to a monitor and DVD in the adjacent laboratory.

2.6. Behavioral analysis

Videotapes were scored by a highly trained observer using an ethological analysis software package developed by Dr. Morato's group at the Faculdade de Filosofia, Ciências e Letras, University of São Paulo (USP) at Ribeirão Preto, Brazil. In addition to recording the time spent licking the formalin-injected paw (see above), the total number of arm entries (arm entry = all four paws on to an arm) was scored. For groups exposed to the sEPM (without prior formalin injection), the frequency of enclosed and open arm entries, % open arm entries [(open/total) × 100] and % open arm time [(open time in seconds/300) × 100] were also recorded. Although the duration of the test was 10 min, the data for exploration of the maze were calculated only during the first 5 min of the test (300 s).

2.7. Statistics

All results were initially subjected to Levene's test for homogeneity of variance and then analyzed by Student's *t*-test for unrelated samples (sham–lesion × lesion group), Mann–Whitney U test (sham–lesion × lesion group) or two-way analysis of variance (ANOVA) for independent factors (factor 1: maze type and factor 2: lesion type). Where indicated by significant *F* values, group differences were identified by Duncan's test. A *P*-value of 0.05 or less was required for significance.

2.8. Perfusion and verification of lesion sites

At the end of testing, animals received an overdose of the anesthetic thiopental (340 mg/kg, i.p.) and they were transcardially perfused with saline followed by 10% formaldehyde. The brains were removed and transferred to 10% formaldehyde. After 24 h and 48 h, they were placed in 20% and 30% sucrose solution, respectively, and then sectioned in a cryostat microtome (Leica CM 1850). The sections were mounted on gelatin-coated slides and stained with cresyl violet (Nissl staining). The anatomical location and size of NMDA lesions were estimated by assessing the typical neuronal cell loss and gliosis infiltration [39]. Nissl-staining methods are widely used to examine cytotoxic lesions [39,40] because these methods stain both glia and neuronal cell bodies, providing a very accurate delineation of the lesion site. Location of lesion sites was microscopically verified with reference to a mouse brain atlas [31].

2.9. Ethics

The experiments carried out in this study comply with the norms of the Brazilian Neuroscience and Behavior Society (SBNeC), based on the US National Institutes of Health Guide to the Care and Use of Laboratory Animals. Furthermore, all the procedures were analyzed and approved by the local Faculty Research Ethics Committee (Resolution CEP/FCF/Car. Number 20/2005; report number 10/2006).

3. Results

3.1. Histology

All NMDA and sham lesions were verified histologically with Nissl staining. Similarly to previously described electrolytic [41] and chemical [27,42] lesions of PAG, the lesion areas were extensive (see Figs. 1B and 2). In some cases, they included the lateral part of the PAG (lPAG) and also extended dorsally into the superior and/or inferior colliculi. The dorsal part of the PAG (dPAG, i.e.

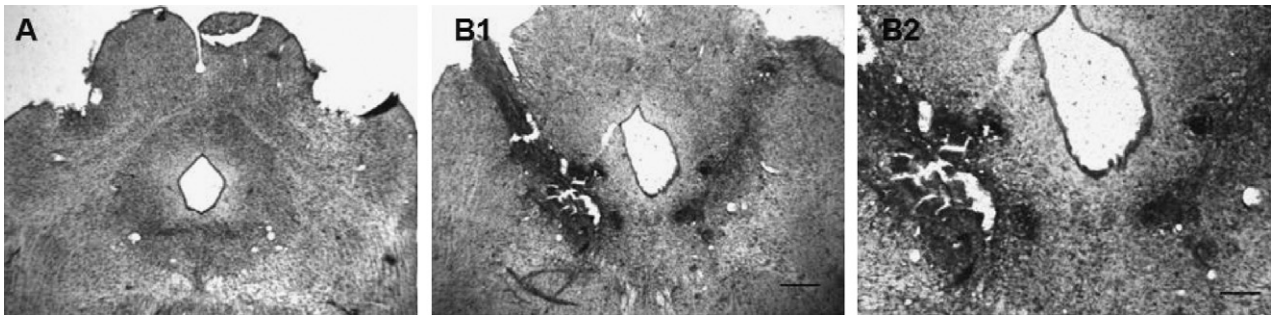

Fig. 1. NMDA lesion appearance. Photomicrographs of transverse cresyl violet-stained sections (Nissl staining), illustrating the extent and appearance of vlPAG bilateral (A) sham and (B) lesions, from representative cases. In the areas of lesion, note the extent of gliosis and loss of neurons. Scale bar = 400 μm in (A and B1); 200 μm in (B2).

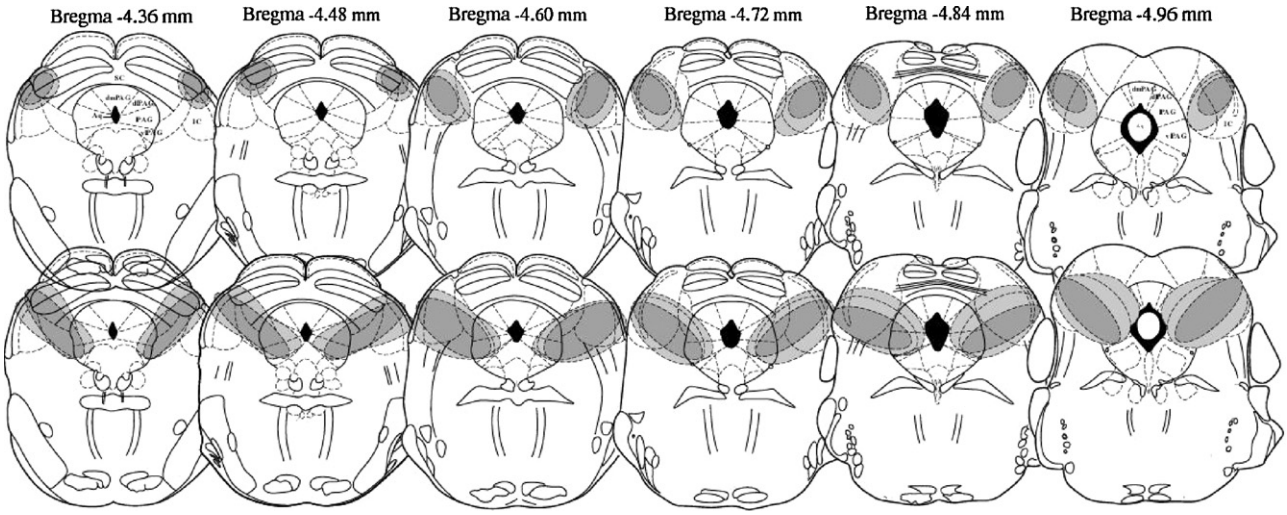

Fig. 2. Composite drawing showing the 'average' extent of NMDA lesion in the bilateral vlPAG. The average lesion is inferred from the layering of individual lesions on idealized cross-sections of vlPAG. The approximate distance from the interaural line is indicated at the top of the figures and is taken from the atlas of Paxinos and Franklin [31]. Abbreviations: Aq, aqueduct; dlPAG, dorsolateral periaqueductal gray; dmPAG, dorsomedial periaqueductal gray; lPAG, lateral periaqueductal gray; vlPAG, ventrolateral periaqueductal gray; SC, superior colliculus; IC, inferior colliculus.

dorsolateral and dorsomedial columns), however, was left intact. There was no sign of lesion in the sham–lesion rats, except where the guide cannulae were implanted (see Fig. 1A).

3.2. Behavioral results

Fig. 3 illustrates the effects of bilateral lesion of vlPAG on time spent licking the formalin injected paw in mice during phase 1 (0–5 min) and phase 2 (25–35 min) of the nociceptive test. In phase 1, the mice were in the glass holding cage (GC), while in phase 2, they were exposed to the eEPM or oEPM. Student's *t*-test for unrelated samples revealed that bilateral lesion of vlPAG did not change the nociceptive response during phase 1 of the formalin test ($t(50) = 0.43$; $P = 0.67$). However, in phase 2, the non-parametric Mann–Whitney U test indicated that both groups of oEPM exposed mice exhibited less time licking the injected paw than the corresponding eEPM group (Sham–lesion: $U = 1$; $Z = 4.27$; $P < 0.001$; Lesion: $U = 39$; $Z = 2.26$; $P < 0.05$). Furthermore, the same statistical test also revealed that the vlPAG lesion decreased the nociceptive response in eEPM exposed mice ($U = 55$; $Z = 2.18$; $P < 0.05$), but not in those exposed to the oEPM ($U = 58$; $Z = 0.49$; $P = 0.62$).

Fig. 4 shows the total arm entries (locomotor activity) and the time spent in the central square of the eEPM and oEPM, in vlPAG sham–lesion and lesion mice, injected with formalin. The results were recorded during the first 5 min of exposure to the EPM. Two-way ANOVA revealed significant effects for the maze-type factor [$F(1,48) = 82.59$, $P < 0.01$] and for the lesion factor [$F(1,48) = 7.32$, $P = 0.001$], but not for maze type × lesion interactions [$F(1,48) = 0.44$, $P = 0.51$]. The post-hoc test indicated that oEPM exposed mice exhibited fewer total entries than the eEPM groups. In addition, vlPAG lesion mice displayed a higher number of arm entries than sham–lesion animals. Regarding the time spent on the

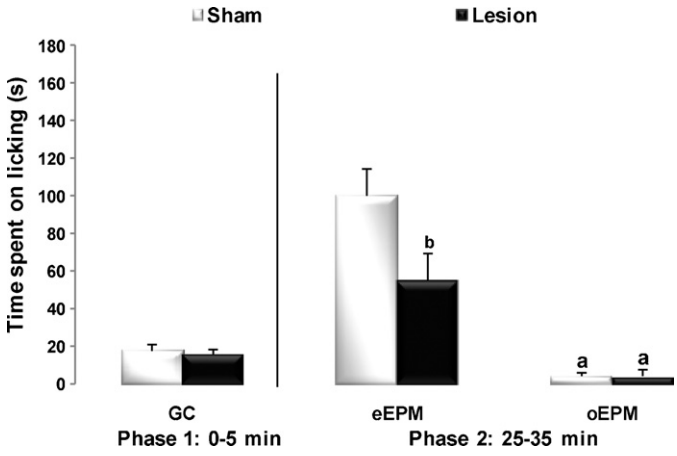

Fig. 3. Effects of bilateral lesion of vlPAG (with 0.2 μl NMDA) on time (s) spent on licking the paw injected with 2.5% formaldehyde, recorded during phase 1 (0–5 min) or phase 2 (25–35 min) of the nociceptive test. In phase 1 the mice were in the glass holding cage (GC) and in phase 2 they were exposed to the eEPM or oEPM; $n = 11$–15. [a,b] $P < 0.05$ versus eEPM and sham–lesion groups, respectively.

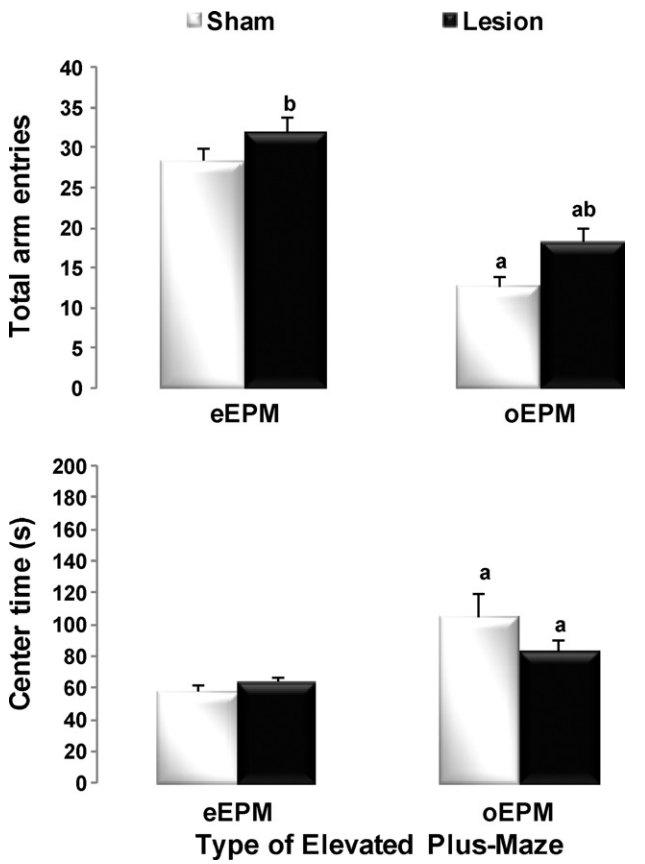

Fig. 4. Effects of vlPAG bilateral lesions on total arm entries and on the time spent in the central square of the eEPM and oEPM, in mice injected with 2.5% formaldehyde. Bilateral sham–lesion group (Sham), $n = 12–14$; bilateral lesion group (Lesion), $n = 11–15$. $^{a,b}P < 0.05$ compared with the eEPM and bilateral sham–lesion groups, respectively.

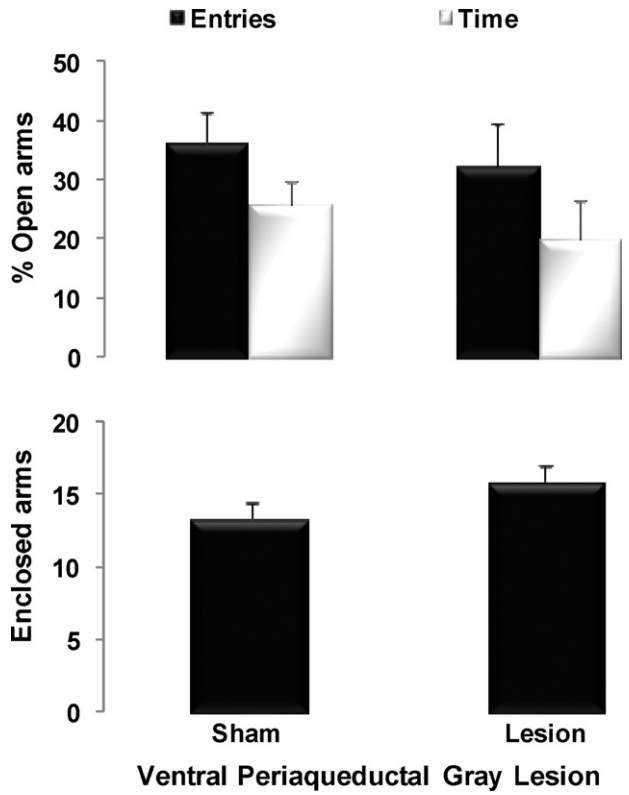

Fig. 5. Lack of effects of bilateral lesion of vlPAG on behavior of mice (not injected with 2.5% formalin in the right hind paw) exposed to the sEPM. Bilateral sham–lesion group (Sham), $n = 7$; Bilateral lesion group (Lesion), $n = 8$.

central square, the non-parametric Mann–Whitney U test revealed that oEPM exposed mice spent more time in the central part of the maze than eEPM-exposed mice, an effect that was not affected by vlPAG lesion (Sham–lesion: $U = 31$; $Z = -2.73$; $P < 0.01$; Lesion: $U = 36$; $Z = -2.41$; $P < 0.05$).

Fig. 5 shows the effects of vlPAG lesion on the behavior of mice exposed to the sEPM, without prior formalin treatment. The results were recorded during the first 5 min of exposure to the sEPM. Student's *t*-test for unrelated samples revealed that vlPAG lesion did not produce any significant change in conventional measures of anxiety [% Open arm entries: $t(13) = 0.46$, $P = 0.65$; % Open arm time: $t(13) = 0.78$, $P = 0.45$], or in general activity [Enclosed arm entries: $t(13) = -0.83$, $P = 0.42$], in mice exposed to the sEPM.

4. Discussion

The results showed that oEPM-induced antinociception was not altered by bilateral lesion of vlPAG in mice. Surprisingly, vlPAG lesion decreased the nociception response in eEPM-exposed mice and increased locomotor activity in both eEPM and oEPM exposed-groups. Furthermore, in contrast to previously observed results with dPAG lesion [27], bilateral vlPAG lesion did not produce anxiolytic-like effects in sEPM-exposed animals that had not received a formalin injection.

Although the PAG has been implicated in the modulation of defensive responses [20,43], including threat-induced antinociception [15–17], there are studies suggesting that these effects depend on factors such as the chance of escape from the aversive stimulus [9,44] and certain characteristics of the threatening stimulus: conditioned or unconditioned [45], or whether the stress is psychological or physical [9]. For instance, it has been demonstrated that either dorsal or ventrolateral PAG lesion reverts the antinociception induced by conditioned aversive stimuli in rats [46], whereas only vlPAG lesion disrupts unconditioned aversive stimuli-induced antinociception [14]. Thus, in light of such evidence and considering that the oEPM exposure represents an unconditioned aversive situation, it would be expected that vlPAG is involved in oEPM-induced antinociception. However, the present results demonstrated that vlPAG lesion did not change the time spent by mice exposed to the oEPM on licking their formalin-injected paw, suggesting that the vlPAG is not involved in the mediation of this environmentally induced defensive reaction.

Interestingly, present results showed that vlPAG lesion produced antinociception in mice exposed to the eEPM, a potentially non-aversive situation. The vlPAG column projects directly to the rostral ventromedial medulla (RVM), in which on and off cells exert important functions in the facilitatory and inhibitory modulation of pain, respectively [47]. Vanegas and Schaible [47] have emphasized that the nociceptive response to formalin injection is predominantly facilitated (rather than inhibited) in normal conditions (i.e. in the absence of psychological stress). In addition, it has been demonstrated that the formalin test activates the vlPAG [48–51]. Taken together, these facts seem to suggest that activation of vlPAG neurons might facilitate the nociceptive process by inhibiting the off cells and/or activating the on cells of the RVM in eEPM-exposed mice. Although attractive, such a hypothesis remains to be tested empirically.

In addition, we observed that bilateral lesion of the vlPAG increased locomotor activity in both eEPM- and oEPM-exposed mice. As shown in Fig. 4, vlPAG-lesion mice displayed higher numbers of arm entries than the sham–lesion groups. This locomotor disinhibitory effect produced by vlPAG lesion seems to corrobo-

rate previous studies showing that the vlPAG column modulated a state of quiescence/immobility in animals that had suffered an injury [52,53]. In this context, it has been demonstrated that the neurotransmission in the spinal-olivocerebellar tract, which is involved in motor coordination [54], is reduced by vlPAG activation [55]. Corroborating the evidence that the vlPAG column modulates locomotion, De Luca-Vinhas et al. [56] have demonstrated that inactivation of the vlPAG, by local midazolam injection, increases enclosed arm entries (without affecting the conventional anxiety-like indices) in rats exposed to the sEPM.

Although the present results showed that vlPAG lesion increased locomotion in both oEPM- and eEPM-exposed groups, it must be emphasized that oEPM-exposed mice displayed fewer arm entries and spent more time in the central square than eEPM-exposed mice, confirming the aversive characteristics of the oEPM. The reduction in locomotion and increase in time spent on the central platform suggest that mice are exploring the oEPM cautiously, probably exhibiting more risk assessment behaviors (e.g., stretch attend and flat-back postures) as previously reported in sEPM exposed animals (e.g., Ref. [23,57,58]).

We recently demonstrated that dorsomedial and dorsolateral lesion of the PAG selectively attenuated spatio-temporal measures of anxiety in sEPM-exposed mice [27]. This result was not replicated with the vlPAG lesion, since NMDA injection into this PAG column neither altered the anxiety-like indices (% open arm entries and % open arm time) nor changed general activity (enclosed arm entries) in mice exposed to the sEPM. Curiously, differently from the increased numbers of arm entries observed in oEPM- and eEPM-exposed groups, vlPAG-lesion mice did not display a higher number of enclosed arm entries than sham–lesion mice in the sEPM. These results contrast with previous findings that intra-vlPAG injection of midazolam, a benzodiazepine receptor agonist, increased enclosed arm entries (without affecting anxiety indices) in rats exposed to the sEPM [56]. The latter authors hypothesized that the vlPAG might mediate just some fear responses, such as freezing, and some types of antinociception controlled by the amygdala [29,59–63], but that aversive stimulus, inherent to the EPM, would not activate this portion of the PAG. Instead of that, the circuit responsible for the production of unconditioned responses, such as the avoidance of the open arms of the sEPM, actually may be the aversive brain system, comprising the dorsal PAG, medial hypothalamus and amygdala [64,65].

Regarding the involvement of other brain structures in the modulation of oEPM-induced antinociception, special attention has been given to the amygdala. We have previously observed that sEPM-open arm confinement-induced antinociception, which is in essence a form of oEPM exposure, is completely reverted by intra-amygdala injection of midazolam in mice concurrently exposed to the writhing (nociceptive) test [66,67]. Therefore, the role of the amygdala and other brain defensive system structures (e.g., hypothalamus and anterior cingulate cortex) [68] in the modulation of this type of environmentally induced antinociception in animals subjected to the formalin test needs to be investigated in further studies.

It is noteworthy that in the present study the lesions of the vlPAG were not limited to this midbrain structure. The NMDA injection also reached structures of the midbrain tectum, provoking neuronal lesions in the superior and inferior colliculi. As previously reported, electrolytic [41] and chemical [42] lesions of the PAG also provoke neuronal death in sites located in the dorsal midbrain, such as the superior and inferior colliculi. It has been reported that both of these structures belong to the brain aversive system [43,69], which also includes the medial hypothalamus, amygdala and dorsal periaquedutal gray [19]. However, we have recently demonstrated that superior and/or inferior colliculus lesions neither changed the nociceptive response in oEPM-exposed mice nor modified the locomotor activity and anxiety-like indices in sEPM-exposed mice [27].

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References

[1] Reynolds DV. Surgery in the rat during electrical analgesia induced by focal brain stimulation. Science 1969;164:444–5.
[2] Fields HL, Basbaum AI, Heinricher MM. Central nervous system mechanisms of pain modulation. In: McMahom SB, Koltzenburg M, editors. Wall and Melzack's textbook of pain. 5th ed. Churchill Livingstone: Elsevier; 2006. p. 125–42.
[3] Behbehani MM. Functional characteristics of the midbrain periaqueductal gray. Prog Neurobiol 1995;46:575–605.
[4] Heinricher MM, Tavares I, Leith JL, Lumb BM. Descending control of nociception: specificity, recruitment and plasticity. Brain Res Rev 2009;60:214–25.
[5] Bandler R, Shipley MT. Columnar organization in midbrain periaqueductal gray: modules for emotional expression? Trends Neurosci 1994;17:379–89.
[6] Carrive P. The periaqueductal gray and defensive behavior: functional representation and neuronal organization. Behav Brain Res 1993;58:27–47.
[7] Depaulis A, Keay KA, Bandler R. Quiescence and hyporeactivity evoked by activation of cell bodies in the ventrolateral midbrain periaqueductal gray of the rat. Exp Brain Res 1994;99:75–83.
[8] Hilton SM, Redfern WS. A search for brainstem cell groups integrating the defence reaction in the rat. J Physiol 1986;378:213–28.
[9] Keay KA, Bandler R. Parallel circuits mediating distinct emotional coping reactions to different types of stress. Neurosci Biobehav Rev 2001;25:669–78.
[10] Lovick TA. Interactions between descending pathways from the dorsal and ventrolateral periaqueductal gray matter in the rat. In: Depaulis A, Bandler R, editors. The midbrain periaqueductal gray: functional, anatomical and neurochemical organization. New York: Plenum Press; 1991. p. 101–20.
[11] Morgan MM. Differences in antinociception evoked from dorsal and ventral regions of the caudal and periaqueductal gray matter. In: Depaulis A, Bandler R, editors. The midbrain periaqueductal gray: functional, anatomical and neurochemical organization. New York: Plenum Press; 1991. p. 139–50.
[12] Depaulis A, Keay KA, Bandler R. Longitudinal neuronal organization of defensive reactions in the midbrain periaqueductal gray region of the rat. Exp Brain Res 1992;90:307–18.
[13] Lovick TA. Integrated activity of cardiovascular and pain regulatory systems: role in adaptative behavioral responses. Prog Neurobiol 1993;40:631–44.
[14] Bellgowan PS, Helmstetter FJ. Neural systems for the expression of hypoalgesia during nonassociative fear. Behav Neurosci 1996;110:727–36.
[15] Fanselow MS. The midbrain periaqueductal gray as a coordinator of action in response to fear and anxiety. In: Depaulis A, Bandler R, editors. The midbrain periaqueductal gray matter. New York: Plenum Press; 1991. p. 1–8.
[16] Harris JA. Descending antinociceptive mechanisms in the brainstem: their role in the animal's defensive system. J Physiol Paris 1996;90:15–25.
[17] Siegfried B, Frischknecht HR, Nunes-De-Souza RL. An ethological model for the study of activation and interaction of pain, memory and defensive systems in the attacked mouse. Role of endogenous opioids. Neurosci Biobehav Rev 1990;14:481–90.
[18] Bolles RC, Fanselow MS. A perceptual-defensive-recuperative model of fear and pain. Behav Brain Sci 1980;3:291–322.
[19] Graeff FG. Brain defense systems and anxiety. In: Roth M, Burrow GD, Noyes R, editors. Handbook of anxiety, vol.3. New York: Elsevier; 1990. p. 307–54.
[20] Graeff FG. Serotonin, the periaqueductal gray and panic. Neurosci Biobehav Rev 2004;28:239–59.
[21] Carobrez AP, Bertoglio LJ, Ethological. temporal analyses of anxiety-like behavior: the elevated plus-maze model 20 years on. Neurosci Biobehav Rev 2005;29:1193–205.
[22] Conceição IM, Maiolini Jr M, Mattia N, Vital MA, Santos BR, Smaili S, Frussa-Filho R. Anxiety-induced antinociception in the mouse. Braz J Med Biol Res 1992;25:831–4.
[23] Lee C, Rodgers RJ. Antinociceptive effects of plus-maze exposure: influence of opiate receptor manipulations. Psychopharmacology (Berlin) 1990;102:507–13.
[24] Stephens DN, Meldrum BS, Weidmann R, Schneider C, Grutzner M. Does the excitatory amino acid receptor antagonist 2-APH exhibit anxiolytic activity? Psychopharmacology (Berlin) 1986;90:166–9.
[25] Taukulis HK, Goggin CE. Diazepam-stress interactions in the rat: effects on autoanalgesia and a plus-maze model of anxiety. Behav Neural Biol 1990;53:205–16.

[26] Mendes-Gomes J, Nunes-De-Souza RL. Concurrent nociceptive stimulation impairs the anxiolytic effect of midazolam injected into the periaqueductal gray in mice. Brain Res 2005;1047:97–104.
[27] Mendes-Gomes J, Nunes-De-Souza RL. Anxiolytic-like effects produced by bilateral lesion of the periaqueductal gray in mice: influence of concurrent nociceptive stimulation. Behav Brain Res 2009;203:180–7.
[28] Cornélio AM, Nunes-De-Souza RL. Open elevated plus maze-induced antinociception in rats: a non-opioid type of pain inhibition? Physiol Behav 2009;96:440–7.
[29] Fanselow MS, Landeira-Fernandez J, Decola JP, Kim JJ. The immediate-shock deficit and postshock analgesia: implication for the relationship between the analgesic CR and UR. Anim Learn Behav 1994;22:72–6.
[30] Deacon RM, Croucher A, Rawlins JNP. Hippocampal cytotoxic lesion effects on species-typical behaviors in mice. Behav Brain Res 2002;132:203–13.
[31] Paxinos G, Franklin KBJ. The mouse brain in stereotaxic coordinates. 2nd ed. California: Academic Press; 2001.
[32] Abbott FV, Franklin KBJ, Westbrook RF. The formalin test: scoring properties of the first and second phases of the pain response in rats. Pain 1995;60:91–102.
[33] Dubuisson D, Dennis SG. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brainstem stimulation in rats and cats. Pain 1977;4:161–74.
[34] McCall WD, Tanner KD, Levine JD. Formalin induces biphasic activity in C-fibers in the rat. Neurosci Lett 1996;208:45–8.
[35] Bon K, Wilson SG, Mogil JS, Roberts WJ. Genetic evidence for the correlation of deep dorsal horn Fos protein immunoreactivity with tonic formalin pain behavior. J Pain 2002;3:181–9.
[36] Tjolsen A, Berge OG, Hunskaar S, Rosland JH, Hole K. The formalin test: an evaluation of the method. Pain 1992;51:5–17.
[37] Le Bars D, Gozariu M, Cadden SW. Animal models of nociception. Pharmacol Rev 2001;53:597–652.
[38] Lister RG. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology (Berlin) 1987;92:180–5.
[39] Sukikara MH, Mota-Ortiz SR, Baldo MV, Felicio LF, Canteras NS. A role for the periaqueductal gray in switching adaptive behavioral responses. J Neurosci 2006;26:2583–9.
[40] Heeb MM, Yahr P. Cell-body lesions of the posteriodorsal preoptic nucleus or posteriodorsal medial amygdala, but not the parvicellular subparafascicular thalamus, disrupt mating in male gerbils. Physiol Behav 2000;68:317–31.
[41] Blanchard DC, Williams G, Lee MC, Blanchard RJ. Taming of wild *Rattus novergicus* by lesions of the mesencephalic central gray. Physiol Psychol 1981;9:157–63.
[42] Dielenberg RA, Leman S, Carrive P. Effect of dorsal periaqueductal gray lesions on cardiovascular and behavioral responses to cat odor exposure in rats. Behav Brain Res 2004;153:487–96.
[43] Brandão ML, Troncoso AC, De Souza Silva MA, Huston JP. The relevance of neuronal substrates of defense in the midbrain tectum to anxiety and stress: empirical and conceptual considerations. Eur J Pharmacol 2003;463:225–33.
[44] Bandler R, Keay KA, Floyd N, Price J. Central circuits mediating patterned autonomic activity during active vs. passive emotional coping. Brain Res Bull 2000;53:95–104.
[45] Vianna DM, Brandão ML. Anatomical connections of the periaqueductal gray: specific neural substrates for different kinds of fear. Braz J Med Biol Res 2003;36:557–66.
[46] Helmstetter FJ, Tershner SA. Lesions of the periaqueductal gray and rostral ventromedial medulla disrupt antinociceptive but not cardiovascular aversive conditional responses. J Neurosci 1994;14:7099–108.
[47] Vanegas H, Schaible HG. Descending control of persistent pain: inhibitory or facilitatory? Brain Res Rev 2004;46:295–309.
[48] Keay KA, Bandler R. Distinct central representations of inescapable and escapable pain: observations and speculation. Exp Physiol 2002;87:275–9.
[49] Lumb BM, Parry DM, Semenenko FM, McMullan S, Simpson DA. C-nociceptor activation of hypothalamic neurones and the columnar organization of their projections to the periaqueductal grey in the rat. Exp Physiol 2002;87:123–8.
[50] Parry DM, Semenenko FM, Conley RK, Lumb BM. Noxious somatic inputs to hypothalamic-midbrain projection neurones: a comparison of the columnar organization of somatic and visceral inputs to the periaqueductal grey in the rat. Exp Physiol 2002;87:117–22.
[51] Rodella L, Rezzani R, Gioia M, Tredici G, Bianchi R. Expression of Fos immunoreactivity in the rat supraspinal regions following noxious visceral stimulation. Brain Res 1998;47:357–66.
[52] Morgan MM, Carrive P. Activation of the ventrolateral periaqueductal gray reduces locomotion but not mean arterial pressure in awake, freely moving rats. Neuroscience 2001;102:905–10.
[53] Morgan MM, Whitney PK, Gold MS. Immobility and flight associated with antinociception produced by activation of the ventral and lateral/dorsal regions of the rat periaqueductal gray. Brain Res 1998;804:159–66.
[54] Ito M. The cerebellum and neural control. New York: Raven Press; 1984.
[55] Cerminara NL, Koutsikou S, Lumb BM, Apps R. The periaqueductal grey modulates sensory input to the cerebellum: a role in coping behavior? Eur J Neurosci 2009;29:2197–206.
[56] De Luca-Vinhas MCZ, Macedo CE, Brandão ML. Pharmacological assessment of the freezing, antinociception, and exploratory behavior organized in the ventrolateral periaqueductal gray. Pain 2006;121:94–104.
[57] Fernandez Espejo E. Structure of the mouse behavior on the elevated plus-maze test of anxiety. Behav Brain Res 1997;86:105–12.
[58] Rodgers RJ, Lee C, Shepherd JK. Effects of diazepam on behavioral and antinociceptive responses to the elevated plus-maze in male mice depend upon treatment regimen and prior maze experience. Psychopharmacology (Berlin) 1992;106:102–10.
[59] Carrive P, Lee J, Su A. Lidocaine blockade of amygdala output in fear-conditioned rats reduces Fos expression in the ventrolateral periaqueductal gray. Neuroscience 2000;95:1071–80.
[60] Helmstetter FJ, Tershner SA, Poore LH, Belgowan PSF. Antinociception following opioid stimulation of the basolateral amygdala is expressed through the periaqueductal gray and rostral ventromedial medulla. Brain Res 1998;779:104–18.
[61] Ledoux JE, Farb C, Ruggiero DA. Topographic organization of neurons in the acoustic thalamus that project to the amygdala. J Neurosci 1990;10:1043–54.
[62] Oliveira LC, Nobre MJ, Brandao ML, Landeira-Fernandez J. Role of amygdala in conditioned and unconditioned fear generated in the periaqueductal gray. Neuroreport 2004;15:2281–5.
[63] Oliveira MA, Prado WA. Role of PAG in the antinociception evoked from the medial or central amygdala in rats. Brain Res Bull 2001;54:55–63.
[64] Brandão ML, Anseloni VZ, Pandossio JE, De Araujo JE, Castilho VM. Neurochemical mechanisms of the defensive behavior in the dorsal midbrain. Neurosci Biobehav Rev 1999;23:863–75.
[65] Brandão ML, Cardoso SH, Melo LL, Motta V, Coimbra NC. Neural substrate of defensive behavior in the midbrain tectum. Neurosci Biobehav Rev 1994;18:339–46.
[66] Baptista D, Bussadori K, Nunes-De-Souza RL, Canto-De-Souza A. Blockade of fear-induced antinociception with intra-amygdala infusion of midazolam: influence of prior test experience. Brain Res 2009;1294:29–37.
[67] Nunes-De-Souza RL, Canto-De-Souza A, Da-Costa M, Fornari RV, Graeff FG, Pela IR. Anxiety-induced antinociception in mice: effects of systemic and intra-amygdala administration of 8-OH-DPAT and midazolam. Psychopharmacology (Berlin) 2000;150:300–10.
[68] Brooks J, Tracey I. From nociception to pain perception: imaging the spinal and supraspinal pathways. J Anat 2005;207:19–33.
[69] Redgrave P, Dean P, Souki W, Lewis G. Gnawing and changes in reactivity produced by microinjections of picrotoxin into the superior colliculus of rats. Psychopharmacology 1981;75:198–203.