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Interplay between 5-HT_{2C} and 5-HT_{1A} receptors in the dorsal periaqueductal gray in the modulation of fear-induced antinociception in mice

Daniela Baptista-de-Souza [a, c, e], Vinícius Pelarin [a, c], Lucas Canto-de-Souza [b, e], Ricardo Luiz Nunes-de-Souza [b, c, e], Azair Canto-de-Souza [a, c, d, e, *]

[a] Dept. Psychology, Federal University of São Carlos-UFSCar, São Carlos, SP, 13565-905, Brazil
[b] Lab. Pharmacology, School of Pharmaceutical Sciences, Univ. Estadual Paulista – UNESP, Araraquara, SP, 14800-903, Brazil
[c] Joint Graduate Program in Physiological Sciences UFSCar/UNESP, São Carlos, SP, 13565-905, Brazil
[d] Graduate Program in Psychology UFSCar, Rod. Washington Luís, Km 235, São Carlos, SP, 13565-905, Brazil
[e] Institute of Neuroscience and Behavior, Av. Do Café, 2.450, 14050-220, Ribeirão Preto, SP, Brazil

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ABSTRACT

The confinement of rodents to the open arm of the elevated-plus maze provokes antinociception (OAA). As a type of defensive reaction, the OAA has been investigated through systemic and intramesencephalic (e.g., dorsal portion of the periaqueductal gray – dPAG) injections of anxiolytic-like drugs [e.g., serotonergic (5-HT) receptor agonists or antagonists]. Here we investigated the effects of (i) intra-dPAG injections of a 5HT_{2C} receptor agonist (MK-212; 0.21 or 0.63 nmol) and antagonist (SB 242084; 0.01, 0.1 or 1.0 nmol); (ii) combined injections of SB 242084 and MK-212 into the dPAG; (iii) combined injections of SB 242084 with 8-OHDPAT (10 nmol) into the dPAG on the OAA in male Swiss mice. Nociception was assessed with the writhing test induced by acetic acid injection. Results showed that (i) intra-dPAG injection of MK-212 (0.63 nmol) increased the OAA; (ii) intra-dPAG SB 242084 (1.0 nmol) prevented the OAA; (iii) SB 242084 (0.1 nmol, a dose devoid of intrinsic effect on nociception) blocked the OAA enhancement provoked by MK-212 and enabled 8-OH-DPAT to prevent the OAA. These results suggest that OAA is mediated by 5-HT_{2C} receptors within the dPAG. Intra-dPAG SB242084 administration provoked similar results on the effects produced by MK-212 and 8-OH-DPAT on OAA. In addition, the dPAG 5-HT_{1A} and 5-HT_{2C} receptors interact each other in the modulation of OAA.

1. Introduction

It has long been reported that the nociceptive system can be inhibited by various environmental threatening stimuli (e.g., Helmstetter and Fanselow, 1987; Bolles and Fanselow, 1980; Fardin et al., 1984; Miczek et al., 1982; Rodgers and Johnson, 1995; Siegfried et al., 1990; Terman et al., 1984; Watkins and Mayer, 1982). The relationship between fear/anxiety state and analgesia has been demonstrated in a range of tests including the elevated plus-maze (EPM) (Cornélio and Nunes-de-Souza, 2007; Lee and Rodgers, 1990; Mendes-Gomes and Nunes-de-Souza, 2009), a widely used animal model of anxiety (Carobrez and Bertoglio, 2005). Interestingly, EPM open-arm confined rodents display high magnitude antinociceptive response (e.g., Cornélio and Nunes-De-Souza, 2009; Mendes-Gomes and Nunes-de-Souza, 2009; Rodgers et al., 1992), a typical environmentally induced analgesic response. The underlying mechanisms involved in the EPM open-arm induced antinociception (OAA) has been investigated through systemic and intracerebral (e.g. amygdala and periaqueductal gray matter) injections of antianxiety drugs (Jimenez-Velazquez et al., 2006; Mendes-Gomes and Nunes-de-Souza, 2009; Nunes-de-Souza et al., 2000; Paul et al., 2002; Tavares et al., 2018), and previous studies have also shown that serotonin (5-HT) neurotransmission modulates areas where nociception and aversion converge (Butler and Finn, 2009).

Regarding the role of 5-HT in the defensive behaviors, Deakin and Graeff (1991) have postulated that 5-HT plays a dual role in

* Corresponding author. Federal University of São Carlos, Department of Psychology, Rod. Washington Luis, km 235, Monjolinho, Sao Carlos CEP: 13565-905, SP, Brazil.
E-mail address: souzaalm@ufscar.br (A. Canto-de-Souza).

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the modulation of emotional states induced by aversive situations. According to them, while activation of the 5-HT pathway to forebrain areas [e.g., from dorsal raphe nucleus (DRN) to amygdala) would *facilitate* mainly subtle emotional responses (e.g., those related to generalized anxiety), activation of the 5-HT periventricular pathway (e.g., from the lateral winds of the DRN to dPAG and hypothalamus) would *attenuate* extreme defensive reactions (e.g., fight/flight responses, which have been related to panic attack). Moreover, previous studies have emphasized that 5-HT neurotransmission in the dPAG also modulates anxiety-like behaviors (Zanoveli et al., 2003). In this context, it has been suggested that while the activation of 5-HT_{1A} and 5-HT_{2A} receptors attenuates panic-like responses (De Paula Soares and Zangrossi, 2004; Zanoveli et al., 2003), serotonin would play an anxiogenic-like effect in the 5-HT_{2C} receptor subtype (De Paula Soares and Zangrossi, 2004). Concerning the role of 5-HT in the modulation of nociception, previous studies have demonstrated that antinociception induced by electrical stimulation of the midbrain tectum (including the dPAG) is blocked by local injection of 5-HT_2 antagonist (Brandao et al., 1999; Coimbra and Brandão, 1997; Lohof et al., 1987). In addition, most serotonin receptors located in the dPAG are 5-HT_{1A} and 5-HT_2 types (Brandao et al., 1991; Lovick, 1993), and previous studies have emphasized the involvement of these 5-HT receptor subtypes in the modulation of some forms of antinociception induced by aversive stimuli (De Luca-Vinhas et al., 2003, 2006; Nunes-de-Souza et al., 2000).

5-HT_{1A}, an inhibitory G-coupled protein receptor (GCPR), and 5HT_2, a stimulatory GCPR (Azmitia, 2007; Pytliak et al., 2011; Shih et al., 1991), are widely distributed in brain defense areas, including the midbrain periaqueductal gray matter (PAG) (Barnes and Sharp, 1999; Hannon and Hoyer, 2008), where 5-HT plays a role in the modulation of defensive behavior (Deakin and Graeff, 2013; Graeff, 2004; Graeff et al., 1996) as well as in antinociception induced by aversive situations (Baptista et al., 2012; Castilho and Brandão, 2001; Coimbra and Brandão, 1997; Coimbra et al., 2006; De Luca-Vinhas et al., 2003, 2006). Regarding the involvement of the PAG 5-HT_1 and 5-$HT_{2A/2C}$ receptors in the modulation of nociceptive response, previous studies have demonstrated that intra-PAG injections of BAYR1531 (a 5-HT_{1A} receptor agonist) reduced the antinociception induced by social defeat in mice (Canto-de-Souza et al., 1998), whereas local injection of ketanserin, a preferential 5-$HT_{2A/2C}$ receptor antagonist, inhibited the antinociception induced by electrical stimulation of this limbic midbrain structure in rats (De Luca-Vinhas et al., 2003, 2006). Furthermore, we have demonstrated that the activation of 5-HT_{2C} receptors located within the dorsal portion (dorsolateral and dorsomedial) of the PAG (dPAG) was capable to enhance the OAA in mice (Baptista et al., 2012).

Interestingly, 5-HT_{1A} and 5-$HT_{2A/2C}$ receptors seem to interact each other in the modulation of behavioral and endocrine responses (Hensler and Truett, 1998; Zhang et al., 2001). In brief, Valdez et al. (2002) demonstrated that 5-$HT_{2A/2C}$ receptor activation induces a significant attenuation of 5-HT_{1A} receptor activity in the cingulate cortex of rats. However, it remains to be determined whether the 5-HT_{1A} and 5-HT_{2C} receptors located in the PAG also play an interactive role in the modulation of nociception. Thus, our hypothesis is that 5-HT_{1A} and 5-HT_{2C} receptors located in the dPAG somehow interplay each other in the modulation of OAA.

Here, we performed three experiments to test our hypothesis. First, we investigated the effects of (i) intra-dPAG injections of the 5-HT_{2C} receptor agonist (MK-212) and antagonist (SB 242084) (Experiment 1). Then, we investigated the effects of intra-dPAG SB 242084 combined with local injection of MK-212 on OAA (Experiment 2). Finally, we examined the effects of intra-dPAG SB 242084 combined with intra-dPAG injection of the 5-HT_{1A} receptor agonist, 8-OH-DPAT, on the EPM-OAA in mice (Experiment 3).

2. Experimental procedures

2.1. Subjects

Subjects were adult male Swiss mice (Federal University of São Carlos, SP, Brazil), weighing 25–30 g, housed in groups of 10 per cage (cage size: 41 cm × 34 cm × 16 cm). They were maintained under a normal 12:12 h light-dark (LD) cycle (lights on: 7:00 a.m.) in a temperature (24 ± 1 °C) controlled environment. Food and drinking water were freely available except during the brief test periods. The experiments were carried out during the light phase of the LD cycle (9:00–16:00). Different batches of experimentally naive mice were used for each experiment.

2.2. Ethics

The experiments reported in this study were performed in compliance with the recommendations of the Brazilian Guidelines for Care and Use of Animals for Scientific and Educational Purposes, elaborated by The National Council of Control of Animal Testing (CONCEA). This study was also approved by the Ethics Committee on Use of Animals of the Federal University of São Carlos (Res. 046/2009).

2.3. Drugs

8-OH-DPAT [(±)-8-hydroxy-2-(di-*n*-propylamino) tetralin hydrobromide; Sigma, (10 nmol)] (5-HT_{1A} partial receptor agonist); MK-212 [6-chloro-2-(1-piperazinyl)] pyrazine hydrochloride, Tocris Cookson Inc., (0.21 or 0.63 nmol) (5-HT_{2C} preferential receptor agonist); SB 242084 [6-chloro-2,3-dihydro-5-methyl-*N*-[6-[(2-methyl-3-pyridinyl)oxy]-3-pyridinyl]-1H-indole-1-carboxyamide dihydrochloride] (0.01, 0.1 or 1.0 nmol) (selective 5-HT_{2C} receptor antagonist), were used for the intra-dPAG treatment.

The doses used in intra-dPAG treatment were based on previous studies (Baptista et al., 2012; Gomes and Nunes-de-Souza, 2009; Nunes-de-Souza et al., 2000; Vicente and Zangrossi, 2012). The drugs were prepared in a vehicle of physiological saline with 2% of Tween 80 and a final volume of 0.1 μl was injected. The same vehicle/volume was used to be injected into the dPAG of animals of the control group (vehicle group).

2.4. Surgery and microinjection

A stainless-steel guide cannula (25-gauge × 7 mm; Insight Instruments, Brazil) was implanted in mice under ketamine + xylazine anesthesia (100 mg/kg and 10 mg/kg, i.p.) in a stereotaxic frame (Insight Instruments, Brazil). The guide cannula was fixed to the skull with dental acrylic and one jeweler's screw. Stereotaxic coordinates for the target site in the dPAG were 4.1 mm posterior to bregma, 1.3 mm lateral to the midline and 1.2 mm ventral to skull surface. The guide cannula was implanted at an angle of 26° to the vertical and was aimed to terminate 2 mm from the target site. To reduce the incidence of occlusion, a dummy cannula (33-gauge stainless steel wire; Fishtex®, Brazil) was inserted into the guide cannula at the time of surgery. During the surgery animals received ketoprofen (benzeneacetic acid, 5 mg/kg, i.p.) and ceftriaxone (ceftriaxone sodium hemieptahydrate, 4 mg/kg, i.p.) (Garber et al., 2011). Before behavioral tests, mice were allowed 4–5 days to recover from surgery. Solutions were injected into the dPAG by a microinjection unit (33-gauge stainless steel cannula, Insight Instruments, Brazil), that extended 2 mm beyond

the tip of the guide cannula. The microinjection unit was connected to a 10 μl Hamilton microsyringe via polyethylene tubing (PE-10) and the rate of flow was controlled by an infusion pump (BI, 2000–Insight Instruments, Brazil) programmed to deliver 0.1 μl of each solution over a period of 60 s. The microinjection procedure consisted of gently restraining the mouse, inserting the injection unit, infusing the solution for 60 s and keeping the injection unit in place for 90 s. The movement of a small air bubble in the PE-10 tubing, during and after the microinjection, confirmed the delivery of the solution.

2.5. Apparatus and general procedure

The basic EPM design was very similar to that originally described by Lister (1987). It comprised two open arms (OA: 30 cm × 5 cm × 0.25 cm) and two enclosed arms (EA: 30 cm × 5 cm × 15 cm) that extended in a cross from a common central platform (5 cm × 5 cm), the entire maze being raised to a height 38.5 cm above floor level. Confinement to an OA or EA was achieved by placing an easily removable gate at the proximal end of each arm of the EPM. All testing was conducted under moderate illumination (77 lux; measured on the central platform of the EPM) during the light phase of the LD cycle.

Nociception was assessed by the writhing test as previously described by Vander Wende and Margolin (1956). In the present study, writhes were induced by injecting 0.1 ml/10 g body weight (b.w.) of 0.6% acetic acid i.p., 5 min after the intra-dPAG drug injection. They were then individually confined to either an OA or an EA of the EPM for 5 min, during which the number of writhes was recorded. Between subjects, the maze was thoroughly cleaned with 20% ethanol and dried with a cloth. All sessions were video-recorded with a camera linked to a monitor in an adjacent laboratory. This experimental protocol was repeated in all experiments described below.

2.5.1. Experiment 1: effects of intra-dPAG injections of 5-HT_{2C} receptor agonist and antagonist on OAA in mice

Seventy-nine mice received intra-dPAG injection of vehicle, MK-212 [0.21 (n = 18) or 0.63 (n = 16) nmol] or saline and SB 242084 [0.01 (n = 16), 0.1 (n = 14) or 1.0 (n = 15) nmol] and 5 min later, an i.p. injection of 0.6% acetic acid (0.1 ml/10 g b.w.). Immediately after acetic acid injection, each mouse was confined either in the enclosed arm or open arm of the EPM to record the number of writhes.

2.5.2. Experiment 2: effects of combined treatment with intra-dPAG injection of SB 242084 and MK-212 on OAA in mice

This experiment aimed to investigate whether the enhancement of OAA provoked by intra-dPAG injection of MK-212 would be changed by prior local injection of SB 242084, a selective 5-HT_{2C} receptor antagonist.

Sixty-seven mice received intra-dPAG injection of SB 242084 (0.1 nmol) and 5 min later intra-dPAG injection of MK-212 (0.63 nmol), [saline + vehicle (n = 14), saline + MK-212 (n = 18), SB + vehicle (n = 17), SB + MK-212 (n = 18)] and 5 min later, all animals received i.p. injection of 0.6% acetic acid (0.1 ml/10 g b.w.). Immediately after acetic acid injection, each mouse was confined either in the enclosed arm or open arm of the EPM to record the number of writhes.

2.5.3. Experiment 3: effects of combined treatment with SB 242084 and 8-OH-DPAT both intra-dPAG on OAA in mice

This experiment was delineated to investigate whether the lack of effects of intra-dPAG injection of 8-OH-DPAT (Baptista et al., 2012), a 5-HT_{1A} receptor agonist, is changed by prior local injection of SB 242084, a selective 5-HT_{2C} receptor antagonist.

A group of sixty-two mice received intra-dPAG injection of SB 242084 (0.1 nmol) and 5 min later a second intra-dPAG injection of 8-OHDPAT (10 nmol), [saline + vehicle (n = 14), saline+8-OH-DPAT (n = 15), SB + vehicle (n = 17), SB +8-OHDPAT (n = 16)] and 5 min later, all animals received i.p. injection of 0.6% acetic acid (0.1 ml/10 g b.w.). Immediately after acetic acid injection, each mouse was confined either in the enclosed arm or open arm of the EPM to record the number of writhes.

2.6. Histology

At the end of testing, all animals received a 0.1 μl infusion of 1% methylene blue as described in Section 2.4 (Surgery and microinjection). The animals were then killed by anesthetic overdose (300 mg/kg ketamine + 30 mg/kg xylazine, i.p.), their brain were removed, and injection sites were verified histologically against the atlas of Paxinos and Franklin (2001). Data from animals with injection sites outside the periaqueductal gray were excluded from the study.

2.7. Statistical analysis

In Experiment 1, the data were analyzed by two-way analysis of variance (ANOVA) (treatment × type of confinement). In Experiments 2 and 3, the data were analyzed by three-way ANOVA (treatment 1 × treatment 2 × type of confinement). Significant *F* values were followed up by Duncan's multiple range test. A p value ≤ 0.05 was required for significance.

3. Results

The histological analysis confirmed that 250 mice received cannula implantation in the PAG, being 92% of them within the dPAG and 8% within the lateral PAG (a single representative image is shown in Fig. 1). Sixty-one subjects were used to investigate the effects of intra-dPAG MK-212 (vehicle, 0.21 or 0.63 nmol) on OAA. Sixty subjects were used to investigate the effects of intra-PAG SB 242084 (saline, 0.01, 0.1 or 1.0 nmol) on OAA. Sixty-eight animals were used to investigate the effects of combined treatment intra-dPAG with SB 242084 (0.1 nmol) and MK-212 (0.63 nmol) on OAA. Sixty-one animals were used to investigate the effects of combined treatment intra-dPAG with SB 242084 (0.1 nmol) and 8-OHDPAT (10 nmol) on OAA.

3.1. Experiment 1: Intra-dPAG MK-212 or SB 242084 enhanced and attenuated, respectively, the OAA in mice

Fig. 2A shows the effects of intra-dPAG MK-212 on OAA. Two-way ANOVA revealed statistically significant effects for the type of confinement factor [$F_{(1,73)} = 70.14$, $p < 0.05$], for the treatment factor [$F_{(2,53)} = 4.23$, $p < 0.05$], and for treatment versus type of confinement interaction [$F_{(2,53)} = 4.55$, $p < 0.05$]. Duncan's test indicated that the number of writhes was significantly higher in EA-confined animals than in the OA group, regardless of the dose of MK-212 the mice had received. However, the OA-confined animals that received 0.63 nmol of MK-212 displayed lower number of writhes than their respective control groups (Fig. 2A).

Fig. 2B shows the effects of intra-dPAG SB 242084 on OAA. Two-way ANOVA also revealed statistically significant effects for the type of confinement factor [$F_{(1,52)} = 55.96$, $p < 0.05$], and for treatment versus type of confinement interaction [$F_{(3,52)} = 3.97$, $p < 0.05$], but did not reveal significant effects for the treatment factor [$F_{(3,52)} = 2.18$, $p > 0.05$]. Duncan's post hoc test confirmed that the number of writhes was significantly lower in OA-confined

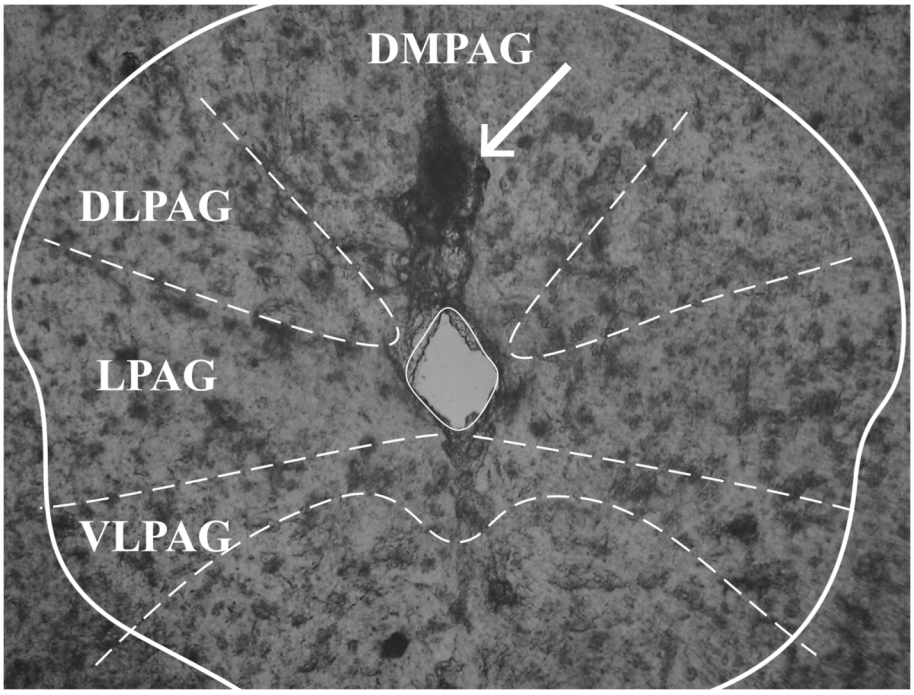

Fig. 1. Photomicrography of a coronal section (Bregma −4.24 mm) of a representative subject showing an injection site (white arrow) within the mouse dPAG.

than in EA-confined animals, except for the group treated with SB 242084 (1.0 nmol), which, in turn, also exhibited significantly higher number of writhes than OA-confined group treated with saline (Fig. 2B).

3.2. Experiment 2: blockade of 5-HT$_{2C}$ in the dPAG attenuated the enhancement of OAA induced by MK-212 in mice

Three-way ANOVA for the SB 242084 + MK 212 treatment revealed statistically significant effects for type of confinement factor [($F_{(1,60)} = 99.92$, $p < 0.05$)], for treatment with MK-212 [$F_{(1,60)} = 14.89$, $p < 0.05$)], SB 242084 treatment × MK-212 treatment interaction ($F_{(1,60)} = 10.68$, $p < 0.05$] and for SB 242084 treatment × MK-212 treatment × type of confinement interaction [$F_{(1,60)} = 8.15$, $p < 0.05$]. No effects were observed for treatment with SB 242084 factor [$F_{(1,60)} = 0.33$, $p > 0.05$]. Post hoc analyses confirmed that the number of writhes was significantly higher in EA-confined animals than in the OA group, and that OA-confined animals treated with 0.63 nmol of MK-212 (saline + MK-212) exhibited lower number of abdominal contortions than their respective control group (saline + vehicle). Intra-dPAG injection of SB 242084 (SB 242084 + vehicle) *per se* did not significantly alter the number of writhes in both EA- and OA-confined groups. Interestingly, animals treated with SB 242084 + MK-212 and confined to the OA showed no statistical differences in the number of writhes compared to their respective control group (saline + vehicle). Overall, these results indicate that the enhancement of the OA-induced antinociception observed in mice treated with MK-212 was prevented by SB 242084 (Fig. 3).

3.3. Experiment 3: Intra-dPAG SB 242084 injected before local injection of 8-OH-DPAT reversed the OAA in mice

For SB 242084 + 8-OH-DPAT treatment, three-way ANOVA revealed statistically significant effects for type of confinement factor [$F_{(1,54)} = 56.64$, $p < 0.05$], 8-OHDPAT treatment [($F_{(1,54)} = 9.50$, $p < 0.05$], SB 242084 treatment × 8-OHDPAT treatment interaction ($F_{(1,54)} = 6.45$, $p < 0.05$), type of confinement factor × 8-OH-DPAT treatment ($F_{(1,54)} = 5.16$, $p < 0.05$). Again, no effects were observed for SB 242084 treatment [$F_{(1,54)} = 2.52$, $p > 0.05$]. Post hoc analyses confirmed that the number of writhes was significantly higher in EA-confined animals than in the OA group, except for animals treated with SB 242084 + 8-OH-DPAT and confined to the OA. These animals exhibited higher number of abdominal contortions compared to all OA-confined groups. Overall, these results indicate that the combination of SB 242084+ 8-OH-DPAT treatment reversed the OAA (Fig. 4).

4. Discussion

The present study attempted to clarify the role of the 5-HT$_{1A}$ and 5-HT$_{2C}$ receptors located in the dPAG in the modulation of the OAA. Here we demonstrated that intra-dPAG injections of 5-HT$_{2C}$ agonist (MK-212) and antagonist (SB 242084), respectively, intensified and blocked OAA. At intrinsically inactive dose, intra-dPAG SB 242084 prevented the enhancement of OAA induced by 5-HT$_{2C}$ receptor activation in the dPAG. Despite unchanged OAA alone, the blockade of 5-HT$_{2C}$ receptors in the dPAG enabled the activation of local 5-HT$_{1A}$ receptors to prevent OAA.

As shown in Experiment 1, while activation of the 5-HT$_{2C}$ receptor in the dPAG enhanced OAA, the blockade alone of this 5-HT receptor subtype impaired OAA. Indeed, intra-dPAG injection of SB 242084 produced an opposite effect, i.e. it completely blocked this type of environmentally induced antinociception. Together, these results are suggestive that 5-HT modulates OAA at 5-HT$_{2C}$ receptors in the dPAG.

The involvement of the PAG 5-HT$_{2C}$ receptors in the modulation of pain response has been previously emphasized (Baptista-de-Souza et al., 2014; de Freitas et al., 2014; Furuya-da-Cunha et al., 2016; Obata et al., 2007). Thus, aiming to better characterize the role of the dPAG 5-HT$_{2C}$ receptor in the modulation of OAA, we injected 0.1 nmol of SB 242084 (a dose without intrinsic effects on nociceptive response; see results of Exp. 1) into the dPAG before local injection of 0.63 nmol (a dose that intensified OAA) of MK-212.

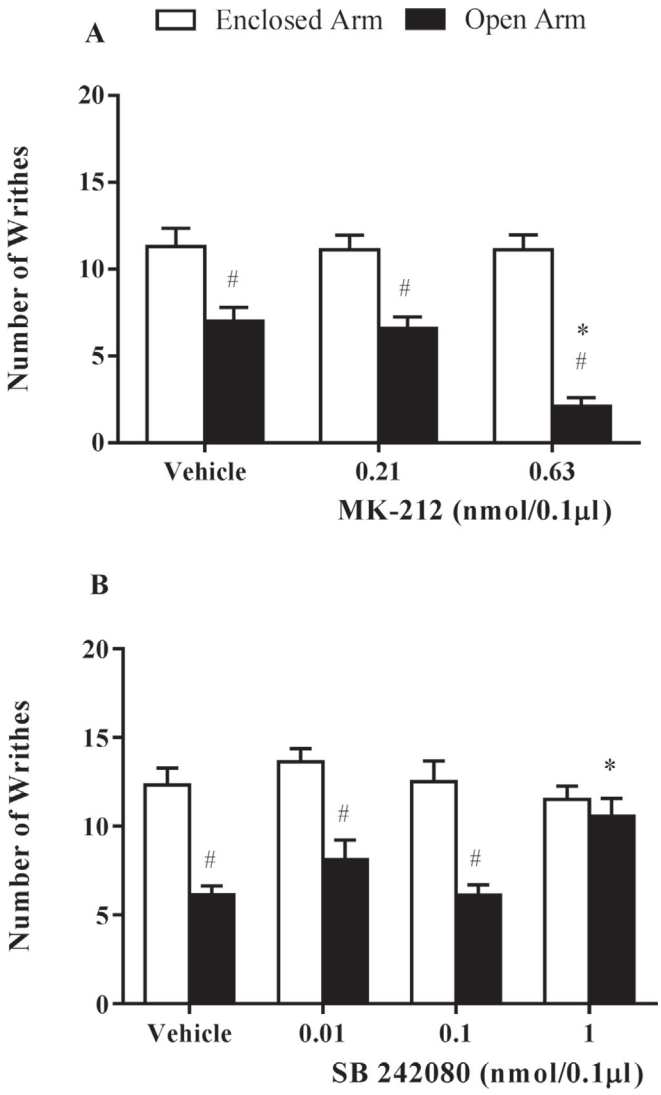

Fig. 2. Effects of intra-dPAG MK-212 (0.21 and 0.63 nmol/0.1 µl) (A) or intra-dPAG SB 242084 (0.01, 0.1 and 0.1 nmol/0.1 µl) (B) on OAA in mice (n = 8–12). Data are presented as mean + SEM. $^{\#}p < 0.05$ versus EA-confined group. $^{*}p < 0.05$ versus respective control group.

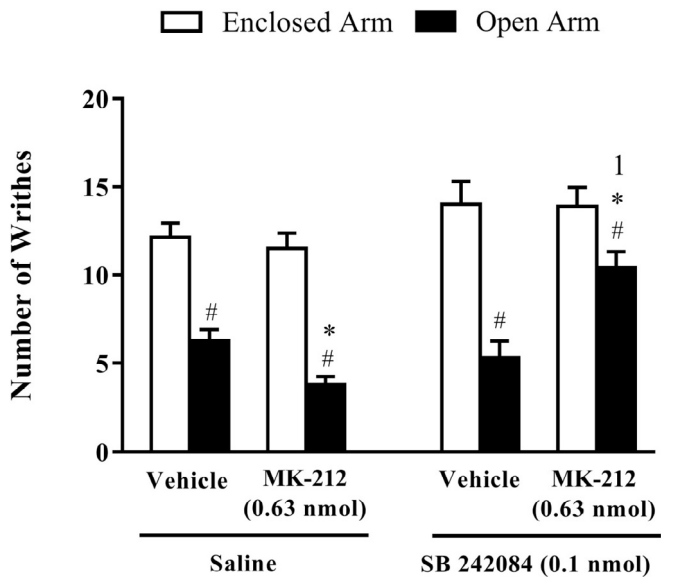

Fig. 3. Effects of intra-dPAG SB 242084 (0.1 nmol/0.1 µl) + MK-212 on OAA in mice (n = 8–11). Data are presented as mean + SEM. $^{\#}p < 0.05$ versus EA-confined group. $^{*}p < 0.05$ versus respective control group. $^{1}p < 0.05$ versus saline + MK-212 group or 8-OH-DPAT group.

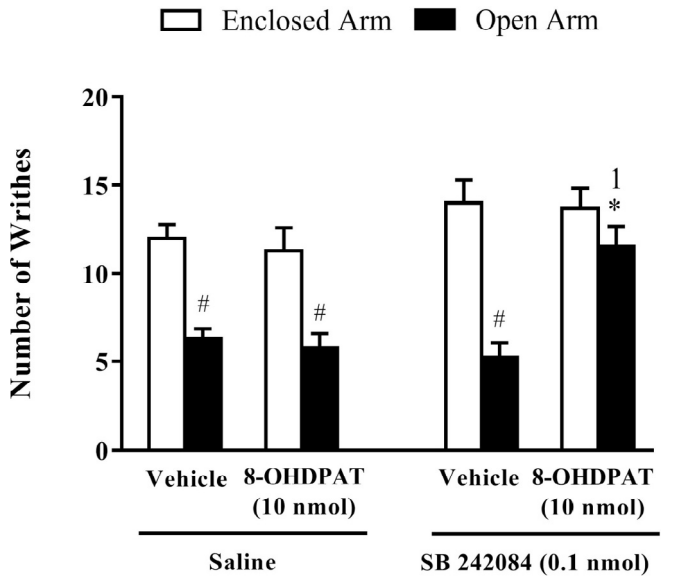

Fig. 4. Effects of intra-dPAG SB 242084 (0.1 nmol/0.1 µl) + 8-OH-DPAT (10 nmol/0.1 µl) on OAA in mice (n = 7–10). Data are presented as mean + SEM. $^{\#}p < 0.05$ versus EA confined group. $^{*}p < 0.05$ versus respective control group. $^{1}p < 0.05$ versus saline + 8-OH-DPAT group.

As shown in Fig. 3B, although SB 242084 *per se* did not prevent OAA, this 5-HT_{2C} receptor antagonist completely blocked the OAA enhancement induced by MK-212. Indeed, the combined injections "SB 242084 + MK-212" not only blocked the facilitatory effect of MK-212 on OAA; they also prevented OAA. These results are similar to those recently reported by Tavares et al. (2018), who demonstrated that intra-amygdala injection of SB 242084 prevented the increase of OAA induced by local microinjections of MK-212. Thus, it seems that the 5-HT intensifies OAA through activating 5-HT_{2C} receptors located in both amygdala (Tavares et al., 2018) and dPAG (present results).

The midbrain PAG is also highly dense in 5-HT_{1A} receptors (Brandao et al., 1991; Hannon and Hoyer, 2008) and previous studies have demonstrated that intra-PAG injection of 5-HT_{1A} receptor agonist reduces social defeat-induced analgesia (Canto-de-Souza et al., 1998). The hypothesis that the activation of this 5-HT receptor subtype in the dPAG could attenuate the OAA has been previously investigated, and the results have shown that intra-dPAG injection of 8-OH-DPAT, a 5-HT_{1A} receptor full agonist, was incapable to change the OAA (Baptista et al., 2012).

Moreover, Valdez et al. (2002) showed that activation of 5-$HT_{2A/2C}$ receptor selectively attenuates 5-HT_{1A} receptor activity in neurons of the anterior cingulate cortex of rats. To test the hypothesis that 5-HT_{1A} and 5-HT_{2C} receptors located in the dPAG somehow interplay each other in the modulation of OAA, we injected SB 242084 into the dPAG to block 5-HT_{2C} receptors, and then injected 8-OH-DPAT into the same brain site to activate 5-HT_{1A} receptors. Interestingly, such a combination of drugs completely blocked OAA. In other words, activation of 5-HT_{1A} receptors impairs OAA only when 5-HT_{2C} receptors are blocked within the dPAG. These results strongly suggest an opposing role of 5-HT_{1A} and 5-HT_{2C} receptors in the modulation of OAA. It remains to be determined whether the

role of 5-HT in the 5-HT_{1A} and 5-HT_{2C} receptors in modulation of OAA is also related to its ability to change anxiety/fear-related responses at the same receptors located in this midbrain area (e.g., Deakin and Graeff, 1991; De Paula Soares and Zangrossi, 2004; Yamashita et al., 2011; Zanoveli et al., 2003).

Furthermore, it has to be highlighted that PAG controls the nociceptive transmission through connections with rostral ventromedial medulla (RVM) wherein modulates *ON*- and *OFF*-cells (responsible, respectively, for facilitating and inhibiting pain at spinal cord level) (Fields, 2004; Palazzo et al., 2008). If so, it would not be unreasonable to suggest that the RVM *OFF*-cells might receive projections from dPAG fibers containing 5-HT_{2C} and 5-HT_{1A} receptors, accentuating the OAA as a consequence of the 5-HT_{2C} activation and reversing this phenomenon in the meantime of this serotonergic receptor is blocked and the 5-HT_{1A} is activated in the midbrain PAG.

Finally, it is important to take into account that other neurotransmitter systems may be involved in the modulation of OAA. For instance, several studies have shown the involvement of noradrenergic neurotramission on antinociception (Freitas et al., 2005; Jones, 1991; Mokha et al., 1985; Munro, 2007; Romero et al., 2012), including specific connections between locus coeruleus and dorsal raphe nucleus (Kaehler et al., 1999; Lee et al., 2005; Pudovkina et al., 2002; Tjolsen et al., 1991). Furthermore, it has been demonstrated the relationship of α2-adrenoceptors and 5-HT_{1A} somatodendritic autoreceptors (Felippotti et al., 2011; Mongeau et al., 1998), wherein there are α2-adrenoceptors located in serotoninergic terminals (Sastre-Coll et al., 2002). Thus, further studies are needed to investigate whether noradrenaline also plays a role in the modulation of OAA.

5. Conclusions

Taken together, the present study demonstrated that (i) 5-HT_{2C} receptors of the dPAG play an important role in the modulation of OAA, (ii) local activation of the 5-HT_{1A} receptors alone does not change OAA, however (iii) 5-HT_{1A} and 5-HT_{2C} receptors located within this midbrain area interacts each other in the modulation of OAA.

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The Author(s) declare(s) that there is no conflict of interest.

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