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Research report

Rat exposure in mice with neuropathic pain induces fear and antinociception that is not reversed by 5-HT$_{2C}$ receptor activation in the dorsal periaqueductal gray

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HIGHLIGHTS

- Rat exposure induces antinociception in mice with chronic constriction injury.
- Intra-dPAG infusion of a 5-HT$_{2C}$ agonist does not reduce defensive behaviors in mice confronted by a predator.
- Activation of 5-HT$_{2C}$ receptors in the dPAG does not alter nociception in mice with chronic constriction injury.

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ABSTRACT

Previous studies have demonstrated that serotonin 5-HT$_{2C}$ receptors in the dorsal periaqueductal gray (dPAG) mediate both anxiety and antinociception in mice submitted to the elevated plus maze. The present study examined the effects of intra-dPAG infusion of the serotonin 5-HT$_{2C}$ receptor agonist (MK-212) in the defensive reactions and antinociception in mice with neurophatic pain confronted by a predator. Neuropathic pain was induced by chronic constriction injury (CCI) of the sciatic nerve, and predator confrontation was performed using the rat exposure test (RET). Our results demonstrated that both sham-operated and CCI mice exhibited intense defensive reactions when confronted by rats. However, rat-exposed CCI mice showed reduced pain reactivity in comparison to CCI mice exposed to a toy rat. Intra-dPAG infusion of MK-212 prior to predator exposure did not significantly alter defensive or antinociceptive responses. To our knowledge, our results represent the first evidence of RET-induced antinociception in mice. Moreover, the results of the present study suggest that 5-HT$_{2C}$ receptor activation in the dPAG is not critically involved in the control of predator-evoked fearful or antinociceptive responses.

1. Introduction

Field and laboratory studies have demonstrated that many different predatory stimuli (e.g., live cats, foxes, and weasels, as well as related stimuli such as fur, odor, urine, feces, and anal gland secretions) are capable of evoking defensive responses in rats and mice [for a review,see Ref. 1]. Considering that rats are natural predators of mice [2], a growing body of evidence has suggested that exposing mice to live rats could be an ethologically supported paradigm to unravel the neurobiology of aversive states [3–6]. Previous studies have demonstrated that mice confronted by a rat show intense defensive reactions, including risk assessment, avoidance, freezing, and flight [3,6,7], as well as inhibition of ongoing non-defensive behaviors [8,9], pregnancy disruption [10] and activation of neuroendocrine stress systems [4].

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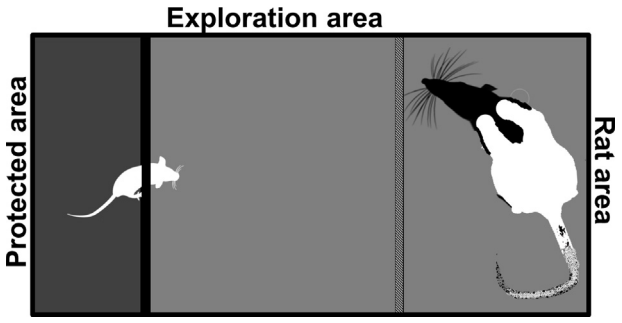

Fig. 1. Schematic diagram of the rat exposure test (RET) apparatus.

Several lines of evidence suggest that defensive reactions elicited by predatory threats are mediated by cortical and limbic areas, such as the medial prefrontal cortex, amygdala, hippocampus, and hypothalamic subnuclei [11–13]. The orchestration of defensive responses also relies largely on top-down and bottom-up signals from the dorsal periaqueductal gray (dPAG) [14–16]. The dPAG is also known to modulate nociception [17–19], suggesting that this region plays a pivotal role in the interaction between anxiety and pain.

Nociception is a protective response that helps to limit tissue damage. However, antinociception also plays an important role by increasing the chances of individuals' escape and survival during challenging situations. The dPAG shows a high expression of serotonin (5-HT) receptors [20], which are activated by serotonergic projections from the dorsal raphe nucleus [21,22] and mediate fear- and anxiety-like behaviors [22,23]. In line with this, we have demonstrated that anxiety-induced antinociception in mice exposed to the elevated plus maze (EPM) is modulated by 5-HT signaling in the dPAG [17,18,23–26]. However, although antinociception has observed in mice confronted by weasels, no study has evaluated antinociception in mice confronted by a rat. Furthermore although considerable evidence supports the role of 5-HT in mediating EPM-induced anxiety and antinociception in mice [22,27–29], it remains unknown whether 5-HT_{2C} receptors in the dPAG mediate predator-evoked defensive reactions and antinociception in mice.

Inhibitory and facilitatory nociceptive pathways are mediated by 5-HT receptors in the dPAG [30]. We have recently shown that neuropathic pain induced by the antineoplastic agent oxaliplatin, which possesses toxic properties that lead to neuropathic pain syndrome with paresthesia and dysesthesia, increases 5-HT_{2C} receptor expression in the dPAG [31]. Although several studies support the role of dPAG 5-HT_{2C} receptors in acute and chronic pain [17,31], it remains unclear whether neuropathic pain-related changes in 5-HT_{2C} receptor function are responsible for hyperalgesia that develops following chronic constriction injury (CCI) of the sciatic nerve [32–35]. Therefore, the present study examined changes in defensive behaviors and nociception induced by predator exposure in mice with CCI, as well as the possible involvement of dPAG 5-HT_{2C} receptors. Our results demonstrated that CCI-induced chronic pain was blocked in mice that were confronted by rats. However, administration of the 5-HT_{2C} agonist MK-212 into the dPAG was not able to reduce defensive behaviors in sham-operated or CCI mice and induced only minor allodynia-like effects in CCI mice.

2. Materials and methods

2.1. Animals

Subjects were male Swiss mice from the university in-house colony, weighing 25–35 g and housed in groups of 10 per cage (41 cm × 34 cm × 28 cm). Predators were adult male Long-Evans hooded rats, weighing 450–600 g and housed in groups of 2 per cage (41 cm × 34 cm × 28 cm). All rodents were maintained in a temperature-controlled environment (24 ± 1 °C) under a normal 12:12 h light-dark cycle (lights on: 7:00 a.m.). Food and drinking water were freely available except during the brief test periods. The experiments described in this study were performed in compliance with the recommendations of the National Council for the Control of Animal Experimentation (CONCEA). This study was approved by the Ethics Committee on Animal Experiments of the Federal University of São Carlos, Brazil (Res. 019/2008).

2.2. Drugs

The preferential 5-HT_{2C} receptor agonist MK-212 (6-chloro-2-[1-piperazinyl]pyrazine hydrochloride; Tocris Cookson Inc., USA), was dissolved in sterile saline solution (0.9% NaCl with 2% Tween 80) at 0.21 or 0.63 nmol/0.1 μL. The sterile saline solution alone was used as the vehicle control. Drug dosages were chosen based on pilot experiments and previous studies by our research group [36]. Intra-dPAG infusions of vehicle or MK-212 were performed 5 min before each exposure test (toy or live rat).

Apomorphine (Siegfried Zofinger, Switzerland) was dissolved in saline solution (0.9% NaCl) at 3.0 mg/kg and administered subcutaneously to Long-Evans hooded rats in a single dose 30 min before each experiment. The stimulus rat was exchanged for a recently injected rat after 50 min in the RET chamber in order to maintain a uniform activity level in the stimulus rats during the test sessions [3,4].

2.3. Surgical procedures and microinjection

2.3.1. Chronic constriction injury of the sciatic nerve

The CCI procedure was based on previous studies [34,37]. Mice were anesthetized by intraperitoneal injection of a ketamine/xylazine solution (100 mg/kg and 10 mg/kg, respectively), and the sciatic nerve was exposed unilaterally at the mid-thigh level. Three ligatures consisting of sterile non-inflammatory 6.0 mononylon were placed 1 mm apart around the nerve and tightened until they elicited a brief twitch in the hindlimb. The incision was then closed in layers. Sham-operated animals underwent the same procedure with the exception of the nerve ligatures.

2.3.2. Stereotaxic surgery

On the 7th day after the CCI procedure, a stainless-steel guide cannula (25-gauge, 7 mm; Insight Instruments, Brazil) was implanted in the mice under surgical anesthesia using a stereotaxic frame (Insight Instruments). The stereotaxic coordinates for the target site within the dPAG were 4.1 mm posterior to bregma, 1.3 mm lateral to the midline, and 1.2 mm ventral to the 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. The guide cannula was fixed to the skull with dental cement and jeweler's screws. A dummy cannula (33-gauge stainless steel wire; Fishtex®, Brazil) was inserted into the guide cannula at the time of surgery in order to prevent occlusion. Following surgery, the mice received ceftriaxone (4 mg/kg) to facilitate post-operative recovery and prevent infection [38].

Vehicle or MK-212 solutions were injected through the guide cannula into the dPAG using a microinjection needle (33-gauge stainless steel cannula; Insight Instruments) that was extended 2 mm beyond the tip of the guide cannula. The microinjection needle was connected to a 10 μL microsyringe via polyethylene tubing (PE-10); the flow rate was controlled by an infusion pump (BI 2000; Insight Instruments) programmed to deliver 0.1 μL of solution over a period of 60 s. The microinjection procedure consisted of gently

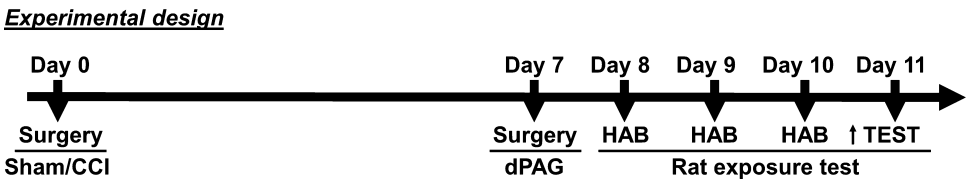

Fig. 2. Schematic representation of the experimental protocol. CCI = chronic constriction injury; dPAG = dorsal periaqueductal gray; HAB = habituation. The upward arrow represents the day of drug infusion into dPAG.

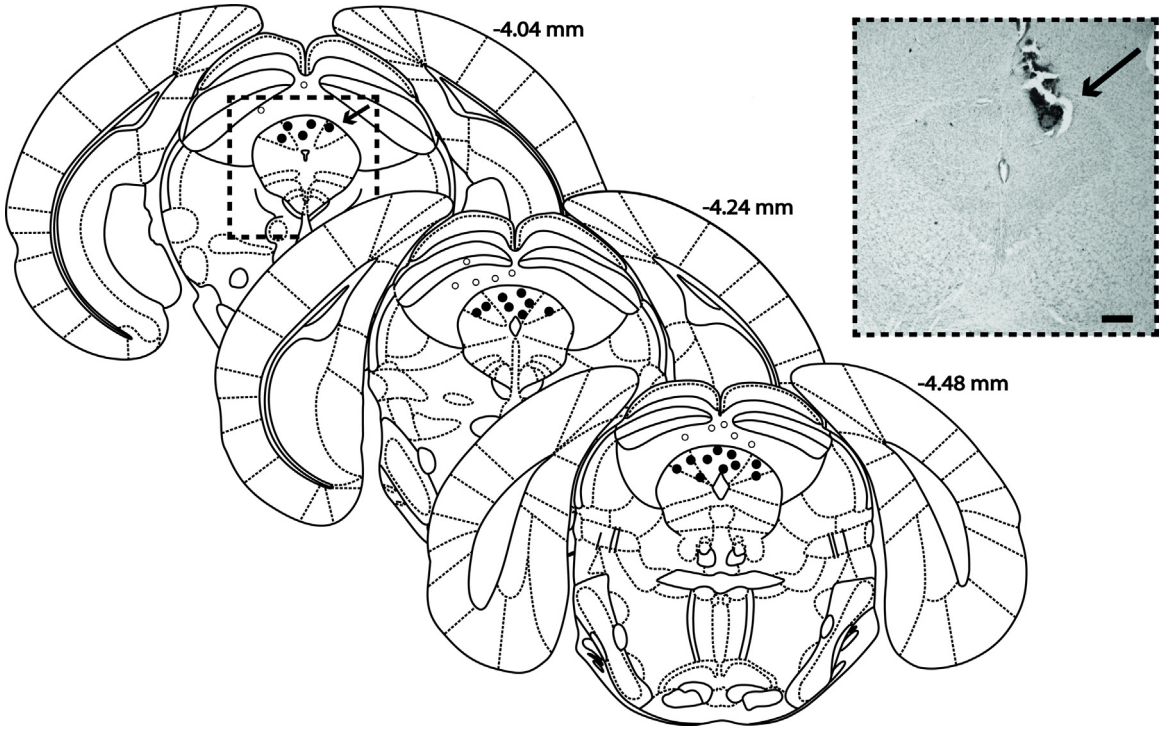

Fig. 3. Schematic representation of microinfusion sites within (filled circles) or outside (empty circles) the dPAG. Sections are between 4.04 and 4.48 mm from the bregma according to the mouse brain atlas by Paxinos and Franklin [40]. Because of overlap, the number of points represented is smaller than the actual number of implanted mice. Scale line = 50 μm.

restraining the animal, removing the dummy cannula, and introducing the injection needle. After the injection, the needle was kept in place for a further 60 s to prevent solution backflow. The infusion was confirmed by monitoring the movement of a small air bubble in the PE-10 tubing [25].

2.4. Rat exposure test

The RET apparatus was similar to that originally described by Yang et al. [6] and was also based on that used by Beijamini and Guimarães [39]. The apparatus comprised a wooden box (59 × 29 × 18 cm) divided into a rat area (20 × 29 × 18 cm) separated from the mouse exploration area (29 × 29 × 18 cm) by a metal grid wall and a protected area (10 × 29 × 18 cm) separated from the exploration area by a black partition with a small door (4 × 4 cm) (Fig. 1). All tests were conducted during the light phase of the light/dark cycle under moderate illumination (20 lux measured at the center of the exploration surface). Experiments were recorded using a vertically mounted video camera connected to a desktop computer. The apparatus was cleaned between experiments with a 20% alcohol solution and dried with paper towels.

The RET procedure consisted of habituation sessions on days 8, 9, and 10 after CCI and a testing session on day 11 (Fig. 2). In each habituation session, mice were placed in the center of the exploration area and allowed to freely explore the apparatus for 10 min in the presence of a toy rat with dimensions and appearance similar to those of a live rat. On the test day, mice received intra-dPAG injections of vehicle or MK-212, as described above (0.21 or 0.63 nmol/0.1 μL; n = 7–9/group), and were individually placed in the center of the exploration area in the presence of a toy rat or an apomorphine-treated male Long-Evans rat.

The behavioral analysis comprised spatiotemporal, ethological, exploratory and nociceptive measures. The spatiotemporal measures were the time spent in the protected area and time spent in contact with (including climbing) the grid wall. The ethological measures were the frequency of SAP (stretched-attend posture, a risk-assessment posture in which the body is stretched forward but the hindpaws remain in position). Total frequency of entries in the protected area and rearings were taken as the exploratory measures. Chronic pain was assessed by measuring the frequency of shaking the injured hindpaw [6].

2.5. Histology

At the end of the test session, each mouse was deeply anesthetized with a ketamine/xylazine solution and received a 0.1 μL infusion of 1% Evans blue, according to the microinjection procedure described above. The animals were then transcardially perfused with sterile saline followed by 10% formaldehyde solution. Brains were removed and stored in 10% formaldehyde before

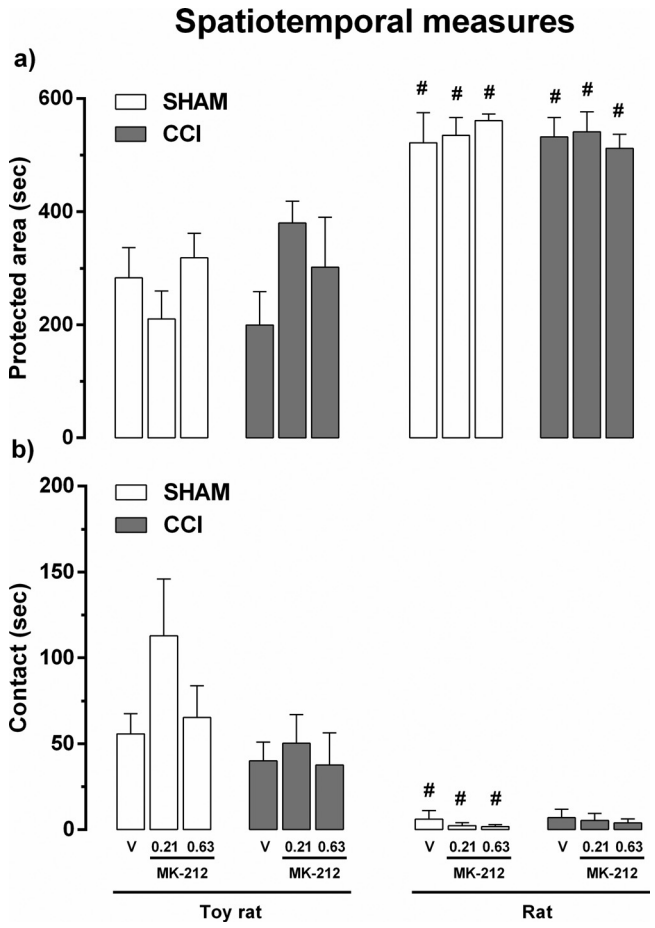

Fig. 4. Effects of MK-212 infusion (0.21 or 0.63 nmol/0.1 μL) within the dPAG on spatiotemporal measures. (a) Time in protected area; (b) Time in contact with grid wall. Data are presented as mean ± standard error of the mean (SEM). # P < 0.05 compared to respective group exposed to toy rat (stimulus effect). n = 7–9 mice per group. SHAM = constriction surgery control; V = vehicle.

sectioning using a cryostat (CM1850; Leica Biosystems, Germany). The coronal sections were mounted by thawing on glass slides, Nissl stained, and coverslipped with Permount. Sections were analyzed using a microscope (BX-71; Olympus, Japan) with a mounted camera linked to a desktop computer. Images were processed using acquisition and analysis software (CellSens 1.9; Olympus, Japan). The injection sites were verified histologically according to a mouse brain atlas [40]. Data from animals with injection sites outside the dPAG were excluded from the study.

2.6. Statistical analysis

All data were analyzed by 3-way analysis of variance (ANOVA) (factor 1: condition—sham-operated or CCI; factor 2: stimulus—toy rat or rat exposure; and factor 3: treatment—vehicle or MK-212). Significant F values were subjected to Duncan's multiple range tests. A P value of 0.05 or less was required for significance.

3. Results

Histological analyses confirmed that 93 mice (n = 7–9/group) had accurate microinjection cannula placements within the dPAG (Fig. 3), representing an accuracy rate of 70% of all mice used.

Figs. 4 and 5 present the behavioral effects (spatiotemporal and ethological measures, respectively) of intra-dPAG MK-212 injections in sham-operated and CCI mice exposed to toy or live rats. Three-way ANOVA revealed significant effects of predator exposure on time in the protected area ($F_{1,76} = 83.86$, $p < 0.05$), contact with the grid wall ($F_{1,76} = 45.53$, $p < 0.05$), SAP frequency ($F_{1,76} = 52.98$, $p < 0.05$), and protected rearing ($F_{1,75} = 94.45$, $p < 0.05$). However, ANOVA did not reveal significant effects of MK-212 treatment on these parameters. The post hoc Duncan test indicated that both sham-operated and CCI mice exposed to rats spent significantly more time in the protected area and less time in contact with the grid wall. Moreover, predator exposure induced significant increases in SAP and protected rearing compared to mice exposed to the toy rat ($p < 0.05$). These data support the induction of an aversive state by predator exposure and suggest that dPAG 5-HT_{2C} receptors are not involved in these fearful reactions.

Fig. 6 presents the effects of predator exposure on exploratory behavior in sham-operated and CCI mice treated with MK-212. Three-way ANOVA revealed statistically significant effects of rat exposure ($F_{1,76} = 24.61$, $p < 0.05$) and MK-212 treatment ($F_{2,76} = 3.43$, $p < 0.05$), as well as CCI vs. rat exposure interaction ($F_{1,76} = 10.11$, $p < 0.05$), on the number of entries into the protected compartment. The post hoc Duncan test showed decreased total entries in sham-operated mice exposed to live rats compared to those exposed to the toy rat ($p < 0.05$). In addition, CCI mice with intra-dPAG MK-212 infusion (0.21 nmol) that were exposed to the toy rat showed an increase in the total entries compared to the respective sham-operated controls ($p < 0.05$). Three-factor ANOVA also revealed statistically significant effects of CCI ($F_{1,76} = 5.38$, $p < 0.05$) and rat exposure ($F_{1,76} = 6.77$, $p < 0.05$) on protected compartment entries. However, post hoc analyses demonstrated no differences in exploratory measures among sham-operated or CCI mice exposed to rats or to the toy rat, regardless of MK-212 treatment.

Fig. 7 illustrates the effects of intra-dPAG MK-212 injection on the shaking frequency of the injured hindpaw in sham-operated and CCI mice exposed to toy or live rats. Three-way ANOVA revealed a significant effect of rat exposure ($F_{1,76} = 35.01$, $p < 0.05$), treatment vs. rat exposure interaction ($F_{2,76} = 4.09$, $p < 0.05$), and MK-212 treatment vs. constriction surgery vs. rat exposure interaction ($F_{2,76} = 3.57$, $p < 0.05$) on the shaking frequency of the injured hindpaw. The post hoc Duncan test indicated that CCI significantly increased the shaking response in comparison to the sham procedure ($p < 0.05$) in mice exposed to the toy rat, confirming the usefulness of the CCI protocol for the study of chronic pain. Furthermore, shaking behavior was reduced in CCI mice exposed to rats compared to CCI mice exposed to the toy rat ($p < 0.05$), indicating fear-induced antinociception. In contrast, intra-dPAG infusion of MK-212 (0.21 nmol) did not alter predator exposure-induced antinociception in CCI mice, but rather increased the frequency of shaking behavior in CCI mice exposed to the toy rat compared to the vehicle control group ($p < 0.05$), suggesting the induction of allodynia.

4. Discussion

In the present study, we observed strong defensive behaviors in mice that were confronted by rats. Defensive behaviors were characterized by increases in the time spent in the protected area and frequency of SAPs, as well as nearly complete avoidance of contact with the grid wall of the predator area. Defensive behavioral patterns were followed by the attenuation of injured paw-shaking seen in CCI mice, consistent with antinociception induced by an aversive state [25,41–43]. Furthermore, we found that activation of 5-HT_{2C} receptors in the dPAG of CCI mice did not affect fear-like responses or changes in nociception induced by predator confrontation. To the best of our knowledge, ours are the first study to demonstrate antinociception induced by the RET, as well as the lack of effects

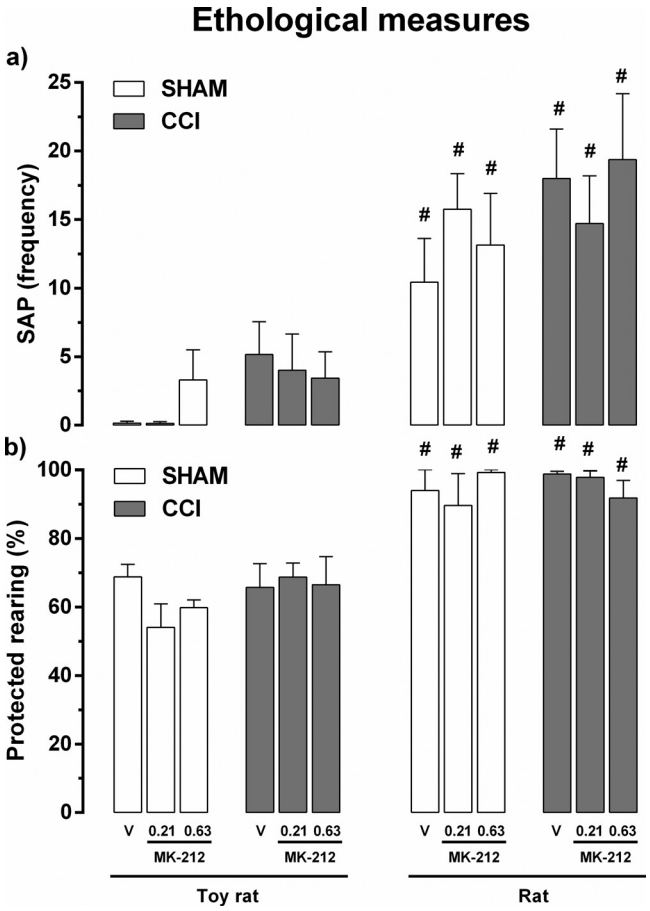

Fig. 5. Effect of MK-212 infusion (0.21 or 0.63 nmol/0.1 μL) within the dPAG on ethological measures. (a) Stretched-attend postures (SAP) frequency; (b) Protected rearings (% in relation to the total rearings). Data are presented as mean ± SEM. #P < 0.05 compared to respective group exposed to toy rat (stimulus effect). n = 7–9 mice per group.

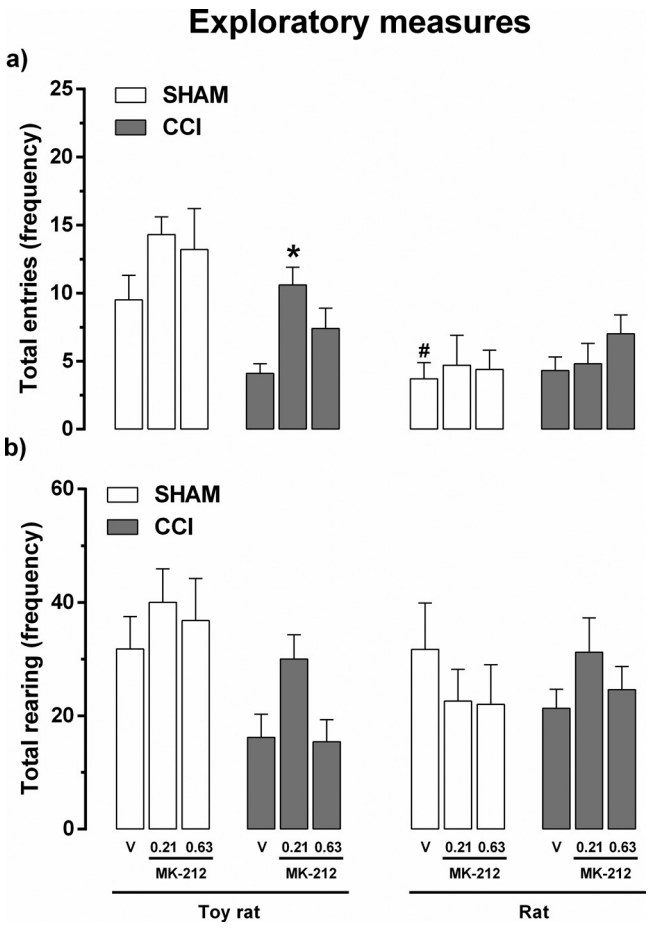

Fig. 6. Effect of MK-212 infusion (0.21 or 0.63 nmol/0.1 μL) within the dPAG on exploratory measures in mice subjected to the RET. (a) frequency of entries in the protected area; (b) total frequency of rearing. Data are presented as mean ± SEM. *P < 0.05 compared to respective vehicle group (treatment effect). #P < 0.05 compared to respective group exposed to toy rat (stimulus effect). n = 7–9 mice per group.

of dPAG 5-HT_{2C} receptor activation on predator-induced fear or neuropathic pain.

Predator exposure is an experimental approach that has been consistently shown to induce fear and anxiety in laboratory rodents [3,4,6]. The present study confirms earlier findings demonstrating that mice confronted by a rat exhibit intense defensive reactions including increased avoidance, risk assessment behaviors, and defensive burying [3], as well as stress hormone release [4]. We also extend these findings by demonstrating that the RET approach is a suitable model for the study of fear-induced antinociception. The present data showing that CCI mice confronted by a rat display almost a complete attenuation of hyperalgesia induced by sciatic nerve constriction are in line with results of previous studies demonstrating that social conflict [43–45] and predator cues [42,46,47] can trigger analgesia and antinociception. These findings also corroborate previous studies demonstrating that mice exposed to the elevated plus maze show antinociception [25,48,49]. Interestingly, in an elegant study, Mendes-Gomes and Nunes-de-Souza [24] demonstrated that mice subjected to the EPM with all arms open (oEPM) showed significantly more analgesia-like effects than mice subjected to the standard EPM. This result suggests that neural systems mediating nociception could be differentially activated according to the level of aversion elicited by the task. Thus, it is likely that nociceptive regulation by 5-HT receptors in the dPAG may also differ depending on the aversive stimulus.

To determine whether 5-HT_{2C} receptors are involved in specific components of fear-like responses induced by predator confronta-

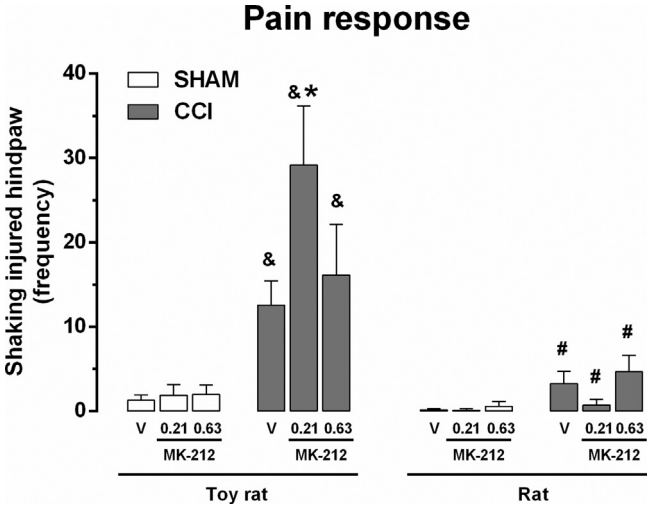

Fig. 7. Effect of MK-212 infusion (0.21 or 0.63 nmol/0.1 μL) within the dPAG on pain responses. Data are presented as mean ± SEM. *P < 0.05 compared to respective vehicle group (treatment effect). #P < 0.05 compared to respective group exposed to toy rat (stimulus effect). & P < 0.05 compared to respective SHAM group (condition effect). n = 7–9 mice per group.

tion, we used an apparatus that allowed the animals to perform both avoidance (protected area) and approach (open area) behaviors. This approach typically creates an exploratory conflict in which mice tend to present increased risk assessment behaviors, such as SAP, along with the nociceptive response (shaking the injured hindpaw). Mice subjected to the present experimental design showed both avoidance of the predator area and risk-assessment behaviors (e.g., SAP). Our decision to use these measures was based on evidence from the literature suggesting that this set of behaviors represents different modules of the defensive reaction [29] that can be differentially modulated by drug treatments [50,51]. For instance, while anxiolytics tend to reduce freezing and increase risk assessment in highly aversive situations where no escape route is available, these drugs typically reduce risk-assessment behaviors and avoidance in highly aversive situations if an escape route is available [29]. In the present study, however, avoidance and risk-assessment behaviors were not altered by intra-dPAG infusion of a 5-HT_{2C} agonist. This result is in direct opposition to previous findings demonstrating that intra-dPAG injection of *meta*-chlorophenylpiperazine (mCPP), a 5-$HT_{2B/2C}$ agonist, decreases anxiety-like behaviors in mice exposed to the EPM [26]. Gomes et al. demonstrated similar anxiolytic effects after intra-dPAG infusion of MK-212 at the doses used in the present study in mice exposed to the EPM [36]. Furthermore, we have previously demonstrated that the blockade of 5-HT_{2C} receptors in the dPAG abolishes the anxiolytic effects induced by increased serotonergic input from the dorsal raphe nucleus [23]. In contrast, data from the present study demonstrating that activation of dPAG 5-HT_{2C} receptors did not reduce predator-induced fear-like reactions corroborates the results of a study by Yamashita et al., which demonstrated that intra-dPAG infusion of MK-212 has no effect on flight behavior assessed in rats subjected to the elevated T-maze, a behavior associated with panic disorder [52]. Another study found that intra-dPAG administration of MK-212 increases inhibitory avoidance in the T-maze, a behavior associated with generalized anxiety [53]; however, we cannot confirm these findings, since any anxiogenic effects of MK-212 may not have been apparent using the behavioral measures chosen for our study. Thus, we suggest that 5-HT_{2C} receptors in the dPAG are involved in the modulation of mild aversive states evoked by paradigms such as the EPM rather than responses to more intensely fearful situations.

Several studies have demonstrated that dPAG 5-HT_{2C} receptors are also involved in the modulation of anxiety-induced antinociception [17,24]. For example, a study from our group demonstrated that activation of these receptors increases antinociceptive effects induced by confinement to the open arm of the EPM [17]. In another study, we showed that mice confined to the open arm following intra-dPAG injection of the 5-$HT_{2B/2C}$ agonist *meta*-chlorophenylpiperazine showed reduced writhing behavior induced by intraperitoneal injection of acetic acid, suggesting that the activation of dPAG 5-HT_{2C} receptors increases anxiety-induced antinociception [17]. By contrast, 5-HT_{2C} agonists injected in the dPAG of mice subjected to the standard EPM induced anxiolytic effects [36]. Furthermore, Mendes-Gomes and Nunes-de-Souza suggested that fear, but not anxiety, induces antinociception, since mice exposed to the oEPM show increased antinociception following formalin injection into the hindpaw compared to mice exposed to the standard EPM or to the EPM with all four arms enclosed [24]. On the other hand, Pobbe et al. [54] observed anxiolytic-like effects after intra-dPAG infusion of the 5-$HT_{2A/2C}$ agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) in mice exposed to the mouse defense test battery (MDTB) but found no effects in mice exposed to the RET. This lack of effect of intra-dPAG DOI administration could result from drug nonspecificity or the strong aversiveness of the paradigm, since DOI is a rather preferential 5-HT_{2A} agonist, suggesting that the lack of anxiolytic-like effects of this drug in mice exposed to the RET could be explained by the high level of aversion elicited by the task. Although the authors assert that the MDTB and oEPM open arm are more aversive than the RET because the MDTB and open-arm confinement do not include escape routes, we believe that the RET represents a particularly aversive ethological situation. The predator stimulus in the MDTB consists of an anesthetized rat and not a freely moving predator, which may explain the contrasting effects of DOI observed in the MDTB and the RET.

The present study demonstrated a significant increase in shaking of the injured hindpaw in mice with CCI confronted by a toy rat compared to sham-operated mice under the same conditions. This shaking behavior typically manifests as a vibration-like shaking of the hindpaw in the air and is an indicator of spontaneous pain similar to symptoms commonly described in patients with neuropathic pain [55,56]. The data from the present study corroborate previous findings demonstrating that CCI is a useful tool for investigating the neurobiology of neuropathic pain [34,57,58]. As described above, predator exposure is an innately aversive stimulus that evokes defensive responses [1,6,7] and, like other aversion-based tests, it can induce antinociception [17,18,25,41,42,47]. In the present study, CCI mice exposed to rats showed a consistent reduction in shaking behavior, indicating that predator confrontation induces antinociception for this type of chronic pain. Thus, our results confirm previous findings showing that the RET is a suitable model for the study of fear reactions [3,4,6] and support the utility of this approach for investigating the interaction between pain and emotion [17,18,41].

We also demonstrated that intra-dPAG infusion of MK-212 slightly increased hindpaw shaking, suggesting an increase in nociception. We believe that this effect could be explained by an increase in 5-HT_{2C}-mediated transmission following CCI [17] or by an anxiolytic-like effect of MK-212 [36] that could not be observed using the behavioral measures chosen. This is supported by studies showing that neuropathic pain can induce an increase in 5-HT_{2C} receptor expression in the dPAG [31,57,59], suggesting that the increased nociceptive response following dPAG 5-HT_{2C} receptor activation in toy-exposed CCI mice could overcome an increase in facilitatory descending pathway activity after central sensitization produced by neuropathic pain, resulting in pro-nociceptive effects [17]. On the other hand, studies showing that activation of dPAG 5-HT_{2C} receptors promotes anxiolytic effects [23,26], suggesting that the 5-HT2C activation could result in the increased nociceptive response observed in the present study. However, since we found no anxiolytic-like effects following the administration of MK-212, this is merely speculative, and further studies are required to investigate these hypotheses.

In conclusion, the present study demonstrates that the RET is a useful tool with which to investigate the neurobiology of aversive states and fear-induced antinociception, as well as to study the mechanisms underlying neuropathic pain. We also conclude that dPAG 5-HT_{2C} receptors may not play a critical role in intense fearful reactions, in contrast to their role in anxiety-like responses that has been demonstrated using the EPM and MDTB. However, further studies are needed to elucidate the precise involvement of 5-HT_{2C} receptors in the neurobiology of anxiety and neuropathic pain.

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