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Nucleus accumbens dopaminergic neurotransmission switches its modulatory action in chronification of inflammatory hyperalgesia

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Abstract

Dopaminergic neurotransmission in the nucleus accumbens, a central component of the mesolimbic system, has been associated with acute pain modulation. As there is a transition from acute to chronic pain ('chronification'), modulatory structures may be involved in chronic pain development. Thus, this study aimed to elucidate the role of nucleus accumbens dopaminergic neurotransmission in chronification of pain. We used a rat model in which daily subcutaneous injection of prostaglandin E_2 in the hindpaw for 14 days induces a long-lasting state of nociceptor sensitization that lasts for at least 30 days following the end of the treatment. Our findings demonstrated that the increase of dopamine in the nucleus accumbens by local administration of GBR12909 (0.5 nmol/0.25 μL), a dopamine reuptake inhibitor, blocked prostaglandin E_2-induced acute hyperalgesia. This blockade was prevented by a dopamine D2 receptor antagonist (raclopride, 10 nmol/0.25 μL) but not changed by a D1 receptor antagonist (SCH23390, 0.5, 3 or 10 nmol/0.25 μL), both co-administered with GBR12909 in the nucleus accumbens. In contrast, the induction of persistent hyperalgesia was facilitated by continuous infusion of GBR12909 in the nucleus accumbens (0.021 nmol/0.5 μL/h) over 7 days of prostaglandin E_2 treatment. The development of persistent hyperalgesia was impaired by SCH23390 (0.125 nmol/0.5 μL/h) and raclopride (0.416 nmol/0.5 μL/h), both administered continuously in the nucleus accumbens over 7 days. Taken together, our data suggest that the chronification of pain involves the plasticity of dopaminergic neurotransmission in the nucleus accumbens, which switches its modulatory role from antinociceptive to pronociceptive.

Introduction

Nucleus accumbens (NAc) is a central component of mesolimbic system classically involved in reward and motivation (Goeders & Smith, 1983; Ambroggi *et al.*, 2008; Carlezon & Thomas, 2009; Floresco, 2015). Recently, dopaminergic mesolimbic circuitry has been associated with the activity of pain pathway. Various studies have shown that experimental increases of mesolimbic dopaminergic activity attenuates acute nociceptive behavior (Chudler & Dong, 1995; Altier & Stewart, 1998, 1999; Magnusson & Fisher, 2000; Haghparast *et al.*, 2012) whereas its decrease promotes hyperalgesia (Saade *et al.*, 1997; Magnusson & Martin, 2002). Regarding the role of mesolimbic dopamine receptors in acute pain modulation, it has been shown that dopamine D2 receptor activation produces an antinociceptive effect (Morgan & Franklin, 1991; Altier & Stewart, 1998, 1999; Taylor *et al.*, 2003; Haghparast *et al.*, 2012) while the role of dopamine D1 receptor activation in the modulation of nociception has not been completely defined (Altier & Stewart, 1998; Magnusson & Fisher, 2000; Schmidt *et al.*, 2002; Taylor *et al.*, 2003; Haghparast *et al.*, 2012).

In addition to acute pain modulation, it is noteworthy that NAc dopamine is associated with emotional learning mechanisms which may play an essential role in the transition from acute to chronic pain ('chronification') (Apkarian, 2008). Chang *et al.* (2014) have demonstrated a correlation between activity of NAc and persistent neuropathic pain, showing that disruption of NAc neuronal activity leads to reduction in neuropathic pain-related behavior. Moreover, studies have suggested an enhanced dopaminergic activity in the NAc during the beginning of neuropathic pain development (Austin *et al.*, 2010; Chang *et al.*, 2014). These studies demonstrated decreased expression of dopamine D1 and D2 receptors in the NAc of rats with neuropathic pain (Austin *et al.*, 2010; Chang *et al.*, 2014).

Studies on pain chronification have generally focused on neuropathic pain. However, among many causes, chronic hyperalgesia commonly results from an inflammatory episode (Woolf, 2011). Ferreira *et al.* (1990) thus developed an animal model that simulates the chronic pain arising from an inflammatory event. This animal model induces sensitization that lasts for at least 30 days after the cessation of 14 successive daily intraplantar injections of prostaglandin E_2 (PGE_2). This model is similar to a clinical condition of

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chronic hyperalgesia generated by frequent sensitization of the nociceptor that lasts for a long time in the absence of any inflammatory stimulus (Woolf, 1983, 2011). The development of chronic hyperalgesia is probably related to neuroplastic changes in pain pathway and its neural structures of modulation. Given that NAc dopaminergic neurotransmission is involved in long-term maladaptive neuroplasticity, such as associated with drug addiction (Smith *et al.*, 2013; Song *et al.*, 2014) and depressive disorders (Nestler & Carlezon, 2006; Francis *et al.*, 2015), it is plausible that NAc dopamine plays an important role in pain chronification. Therefore, the aim of this study was to investigate the role of dopaminergic neurotransmission of NAc in the development of chronic hyperalgesia induced by PGE_2 in rats.

Materials and methods

Animals

Experiments were performed with male Wistar rats (250–280 g), obtained from the Multidisciplinary Center for Biological Investigation (CEMIB) at the State University of Campinas (UNICAMP) and kept under a 12/12-h light–dark cycle, with controlled humidity (60–80%) and temperature (22–25 °C). Food and water were available *ad libitum*. Rats were taken to the testing area at least 1 h before testing. Each experiment used from five to 12 rats per group. All behavioral testing was performed between 09:00 and 16:00 h. Animal care and handling procedures were in accordance with International Association for the Study of Pain (IASP) guidelines for the use of animals in pain research and approved by the institutional Committee for Ethics in Animal Experimentation at UNICAMP (CEUA/IB-UNICAMP), São Paulo, Brazil, protocol number 1767-1. All effort was made to limit the number of animals used.

Drugs and doses

All agents used in this study were obtained from Sigma Aldrich (St Louis, MO, USA): PGE_2; GBR12909, 1-(2-[bis(4-fluorophenyl) methoxy]ethyl)-4-(3-phenylpropyl)piperazine dihydrochloride, a dopamine reuptake inhibitor; SCH23390, 7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride, a selective dopamine D1/D5 receptor antagonist; and raclopride, 3,5-dichloro-*N*-(1-ethylpyrrolidin-2-ylmethyl)-2-hydroxy-6-methoxybenzamide (+)-tartrate salt, a selective dopamine D2/D3 receptor antagonist. PGE_2 was diluted in saline and 1% ethanol (Merck, Darmstadt, Germany). GBR12909 dihydrochloride (0.1, 0.2 and 0.5 nmol/0.25 μL, and 0.021 nmol/0.5 μL) was diluted in 5% dimethyl sulfoxide (DMSO; Sigma) and 0.9% NaCl solution (saline). Raclopride tartrate (0.5, 3 and 10 nmol/0.25 μL, and 0.125 nmol/0.5 μL) and SCH23390 hydrochloride (0.5, 3 and 10 nmol/0.25 μL, and 0.416 nmol/0.5 μL) were diluted in 0.9% NaCl solution.

Doses were determined for acute administration in the NAc, based on doses used in the literature: GBR12909 (Weikop *et al.*, 2007), SCH23310 (Schmidt *et al.*, 2002) and raclopride (Taylor *et al.*, 2003). First, the dose of GBR12909, a dopamine reuptake inhibitor, was determined using three different doses to find the lowest effective dose. From this, the doses of both dopamine D1 and D2 receptor antagonists were established using three different doses of each one to find the lowest dose able to prevent the effect of co-administered GBR12909. For chronic treatments, the effective doses determined in acute experiments were used and daily released through osmotic mini-pumps over 7 days.

Evaluation of mechanical hyperalgesia

The mechanical nociceptive threshold was measured by the electronic von Frey method (Vivancos *et al.*, 2004). In a quiet room, rats were placed in acrylic cages (12 × 20 × 17 cm) with wire grid floors, 15–30 min before the start of testing. During this adaptation period, the paws were tested three times. The test consisted of evoking a hindpaw flexion with a hand-held force transducer adapted with a 0.7-mm^2 polypropylene tip (electronic von Frey hair; IITC Life Science, Woodland Hills, CA, USA). A tilted mirror placed under the grid provided a clear view of the rat hindpaw. The investigator was trained to apply the tip between the five distal footpads with a gradual increase in pressure. The stimulus was automatically discontinued, and its intensity was recorded when the paw was withdrawn. The maximal force applied was 80 ***g***. The endpoint was characterized by the removal of the paw in a clear flinch response after paw withdrawal. Animals were tested before and after drug treatments. The investigator was blind to all experimental treatments. The results are expressed as the mean mechanical threshold evaluated 3 h after PGE_2 or saline injection in the hindpaw (acute hyperalgesia) and as the mean mechanical threshold evaluated during continuous treatment with PGE_2 or saline in the hindpaw (persistent hyperalgesia).

PGE_2 administration in the rat hindpaw

For injection in the hindpaw, animals were briefly retained and a hypodermic 26-gauge needle was inserted in the plantar surface of the hindpaw. PGE_2 or vehicle was subcutaneously administered in a volume of 50 μL (Vivancos *et al.*, 2003) once to induce acute hyperalgesia or once a day over 14 consecutive days to induce persistent hyperalgesia.

Acute hyperalgesia

Intracranial NAc cannulation

Stereotaxic surgeries were conducted in rats under deep anesthesia induced by an intraperitoneal (i.p.) injection of ketamine (80 mg/kg, Dopalen, Vetbrands, Miramar, FL, USA) and xylazine (10 mg/kg, Anasedan, Vetbrands). The anterior cranial region was shaved and cleaned with a cotton tip applicator soaked in a solution of povidone-iodine (PVP-I 10%; Rioquímica, São José do Rio Preto, SP, Brazil). According to defined coordinates from the bregma – anteroposterior (AP) +1.3 mm, mediolateral (ML) ±1.8 mm and dorsoventral (DV) +7.0 mm – guide cannulas (23 gauge, 15 mm) were bilaterally implanted. A stainless steel screw was fixed in the skull and the cannulas were fixed both to one another and to the stainless steel screw through dental cement (Vipi Flash acrylic resin, Pirassununga, SP, Brazil). The retracted skin and soft tissues were repositioned and the skin was sutured over dental cement using 5-0 suture (Procare, Medico Huaian Co., Huaian, Jiangsu, China). Thus, the wound was kept closed and just the guide cannulas were exposed crossing the skin. Stainless steel dummy cannulas were inserted to keep the guide cannulas free of debris. After surgery, dipyrone (300 mg/kg) was administered (i.p.). Rats were housed individually and allowed to recover for 7–10 days. The injection sites were verified by histological examination (50-μm sections stained with Cresyl Violet acetate) and plotted on coronal maps adapted from the atlas of Paxinos & Watson (1986).

Drug administration in the NAc

Cannulated rats were briefly retained and the stainless steel dummy cannula was removed from guide cannulas and replaced with a

microinjector (30 gauge, 16 mm) connected via a 30-cm length of polyethylene tube to a 2- μL Hamilton syringe. Ten minutes before return to the testing box, drugs or vehicle (0.25 μL) was bilaterally infused over 45 s. Injections were confirmed by following the movement of a small air bubble within the polyethylene tubing. Microinjectors were left in place for a further 45 s to minimize backflow along the cannula track. In two different groups, the dopamine receptor antagonists at different doses were injected immediately before the dopamine reuptake inhibitor. Each animal experienced only one microinjection session (Fig. 1).

Persistent hyperalgesia

Mini-pump implantation

For animals submitted to continuous treatment in the NAc, osmotic mini-pumps (Alzet Osmotic Pumps, Model 1007D, 90- μL reservoir volume, 7 days of delivery, 0.5 μL/h release rate) were implanted subcutaneously between the scapular blades. Each pump, filled with drug or vehicle, was connected to an injector (26 gauge) by a polyethylene tube. The injector cannulas were placed in the NAc bilaterally as already described for intracranial NAc cannulation. After surgery, dipyrone (300 mg/kg) was administered (i.p.) in the rats (Fig. 2).

Rotarod test

Motor activity was measured as previously described (Tsuda *et al.*, 1996) using the rotarod (Ugo Basile, Varese, Italy) after bilateral injection of drug or its vehicle in the NAc. Animals were assessed for their ability to maintain balance on a rotating bar at a constant speed of 18 r.p.m. The time from when the animal mounted the rod to when it fell from the rod was recorded. The animals were trained for 2 days before testing, which includes three training sessions each day. The cut-off time was 180 s.

Statistical analysis

Statistical analysis of the results obtained was performed in GRAPHPAD PRISM version 4.00 for Windows (GraphPad Software, San Diego, CA, USA). The results are presented as the mean ± SEM of 5–12 rats per group. To analyse the PGE_2-induced acute hyperalgesia and the effect of dopaminergic neurotransmission-related drugs or their vehicles administered in the NAc on hyperalgesia, one-way analysis of variance (ANOVA) with one between-subject factor (i.e. treatment) was used. One-way ANOVA allowed us to obtain the degree of significance in nociceptive response among groups and was followed by a Bonferroni multiple comparison test to compare the groups. Two-way repeated-measures ANOVA was used to analyse persistent hyperalgesia development with PGE_2 or saline treatment in the hindpaw and the effect of dopaminergic neurotransmission-related drugs or their vehicles administered in the NAc on hyperalgesia. Two-way ANOVA with one between-subject factor (i.e. treatment) and one within-subject factor (i.e. time) was used to determine if there were significant differences in nociceptive response among and between groups and if there was an interaction between factors, time and treatment. This was followed by the Bonferroni multiple comparison test to compare the main effect (i.e. treatment) between groups. An unpaired *t*-test was used to evaluate the effect of treatment comparing two groups (i.e. treated and control groups) at a specific time point.

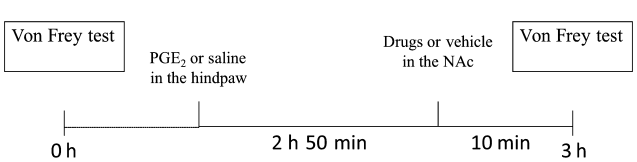


FIG. 1. Schematic experimental design to induce acute hyperalgesia. Rats were submitted to surgery 7–10 days before the basal von Frey test at 0 h. Immediately after the basal von Frey test, PGE_2 or saline was subcutaneously injected in the rat hindpaw. The peak of PGE_2-induced hyperalgesic response occurs 3 h after its injection, when the von Frey test was developed again. Ten minutes before the latter von Frey test, drugs or their vehicle were administered in the NAc. PGE_2, prostaglandin E_2; NAc, nucleus accumbens.

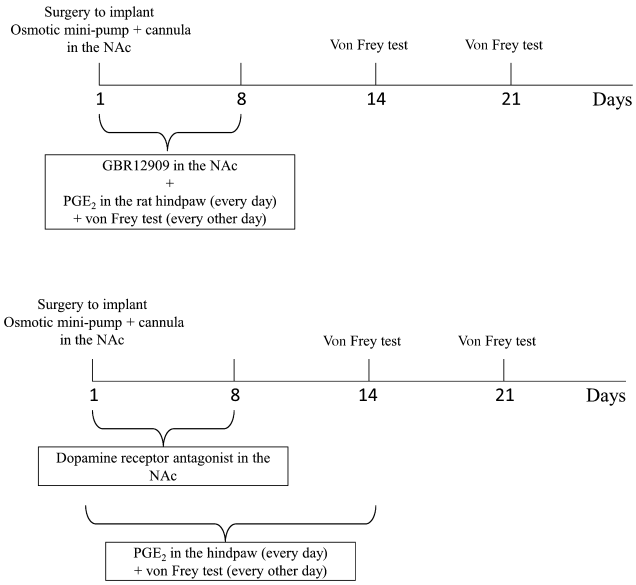


FIG. 2. Schematic experimental design to induce persistent hyperalgesia. Surgery to implant cannulas connected to osmotic mini-pumps was developed at day 1. Top: GBR12909 or its vehicle was released in the NAc for 7 days. PGE_2 or saline was injected in the rat hindpaw once a day over 7 days to induce persistent hyperalgesia and the von Frey test was developed every other day over 7 days, and then at days 14 and 21. Bottom: SCH23390 or raclopride and their vehicle were released in the NAc for 7 days. PGE_2 or saline was injected in the rat hindpaw once a day over 14 days to induce persistent hyperalgesia and the von Frey test was developed every other day over 14 days and then at day 21. PGE_2, prostaglandin E_2; NAc, nucleus accumbens.

Results

Dopaminergic neurotransmission in the NAc blocks acute hyperalgesia induced by PGE_2

Hyperalgesia induced by PGE_2 (100 ng/50 μL) administered in the rat hindpaw was evaluated by the von Frey test performed 3 h after its intraplantar administration. The dopamine reuptake inhibitor, GBR12909 (0.5 nmol/0.25 μL per side), bilaterally administered in the NAc 10 min before the von Frey test, blocked the hyperalgesia induced by PGE_2 (Fig. 3; $F_{7,66} = 17.14$; $P < 0.01$). The administration of GBR12909 (0.5 nmol) by itself did not change the mechanical nociceptive threshold in rats treated with saline (50 μL) in the hindpaw (Fig. 3; $F_{7,66} = 17.14$, $P > 0.05$). Rats treated with GBR12909 intra-NAc did not present impaired motor activity as assessed by the rotarod test (Vehicle – time on rotarod:

179.0 ± 0.5164 s; GBR12909 – time on rotarod: 179.2 ± 0.4014 s; $t = 0.2548$, $P = 0.59$).

Dopaminergic neurotransmission in the NAc via D2 but not D1 receptor activation impaired acute inflammatory hyperalgesia

To verify whether D1 or D2 receptor activation is involved in the anti-hyperalgesic effect of dopamine in the NAc, antagonists of the dopamine D1 or D2 receptor were administered immediately before GBR12909 (0.5 nmol per side). Bilateral co-administration of the dopamine D2 receptor antagonist raclopride (0.5, 3 or 10 nmol) and GBR12909 prevented the anti-hyperalgesic effect of enhanced dopaminergic neurotransmission in a dose-dependent manner (Fig. 4B; $F_{5,33} = 32.09$, $P < 0.01$). However, the bilateral co-administration of D1 receptor antagonist, SCH23390 (0.5, 3 or 10 nmol) and GBR12909 did not alter the anti-hyperalgesic effect of enhanced dopaminergic neurotransmission in the NAc (Fig. 4A; $F_{5,35} = 14.36$, $P > 0.05$). The bilateral administration of SCH23390 or raclopride alone in the NAc did not alter the acute hyperalgesia induced by PGE_2 (100 ng/50 μL) in the hindpaw (Fig. 4C; $F_{5,29} = 11.92$, $P > 0.05$ and Fig. 4D, $F_{5,37} = 15.62$; $P > 0.05$, respectively). However, dopamine D2 receptor antagonist administration in the NAc facilitated the acute hyperalgesia induced by a lower dose of PGE_2 (30 ng/50 μL) in the hindpaw (Fig. 4D; $F_{5,37} = 15.62$, $P < 0.05$). Dopamine D1 or D2 receptor antagonists did not alter mechanical threshold by themselves (Fig. 4C; $F_{5,29} = 11.92$, $P > 0.05$ and Fig. 4D; $F_{5,37} = 15.62$, $P > 0.05$; respectively).

Dopaminergic neurotransmission in the NAc facilitates persistent hyperalgesia induced by PGE_2

Administration of PGE_2 (100 ng/50 μL) in the hindpaw over 14 days persistently reduces the mechanical nociceptive threshold for 30 days after PGE_2 treatment cessation, as previously described by Ferreira *et al.* (1990). Mechanical threshold decreased gradually over time with PGE_2 treatment (Fig. 5A; time: $F_{7,10} = 11.59$, $P < 0.0001$ and interaction: $F_{7,10} = 5.684$, $P < 0.0001$). As shown in Fig. 5A, the mechanical nociceptive threshold evaluated just before PGE_2 administration started to decrease significantly at day 7 ($F_{1,10} = 86.01$, $P < 0.05$), and remained reduced up to day 14 ($F_{1,10} = 86.01$, $P < 0.05$). The decrease in mechanical nociceptive threshold persisted for at least 7 days after the end of the PGE_2 treatment, as evaluated at day 21 (Fig. 5B; $t = 7.721$, $P < 0.0001$). Saline administration for 14 days did not change the mechanical nociceptive threshold.

As shown in Fig. 5C, the local administration of PGE_2 (100 ng/50 μL) in the rat hindpaw once a day associated with sustained infusion of vehicle in the NAc during 7 consecutive days induced hyperalgesia over time of treatment (time: $F_{4,12} = 20.71$, $P < 0.0001$ and interaction: $F_{8,12} = 4.633$, $P = 0.0003$) that did not persist after treatment cessation (at day 7, $F_{2,12} = 20.66$, $P < 0.05$; at day 14, $F_{2.12} = 20.66$, $P > 0.05$ and Fig. 5D at day 21, $F_{2,12} = 52.51$, $P > 0.05$). However, administration of PGE_2 (100 ng/50 μL) in the hindpaw during seven consecutive days simultaneously with sustained infusion of GBR12909 (0.021 nmol/0.5 μL/h) in the NAc over 7 days induced persistent hyperalgesia that lasted for at least 14 days after the end of the treatment (Fig. 5C: at day 7, $F_{2.12} = 20.66$, $P < 0.01$; at day 14, $F_{2.12} = 20.66$, $P < 0.05$; and Fig. 5D: at day 21, $F_{2,12} = 52.51$, $P < 0.001$). There was no difference between persistent hyperalgesia induced by 7 days of PGE_2 treatment in the hindpaw plus GBR12909 in the NAc and the persistent hyperalgesia induced by 14 days of PGE_2 treatment in the hindpaw plus vehicle in the NAc (Fig. 5E: at day 14, $F_{2,15} = 52.91$, $P > 0.05$; and Fig. 5F: at the day 21, $F_{2,15} = 52.56$, $P > 0.05$). To rule out the effect of pump implantation on induction of hyperalgesia, vehicle was infused bilaterally by mini-pumps in the NAc over 7 days concomitant with PGE_2 administration in the hindpaw. There was no difference between PGE_2 alone in the hindpaw and PGE_2 in the hindpaw plus vehicle mini-pumps in the NAc treatments (Fig. 5G: at day 7, $F_{2,12} = 15.78$, $P > 0.05$; at the day 14, $F_{2,12} = 15.78$, $P > 0.05$; and Fig. 5H: at the day 21, $F_{2,12} = 0.56$, $P > 0.05$).

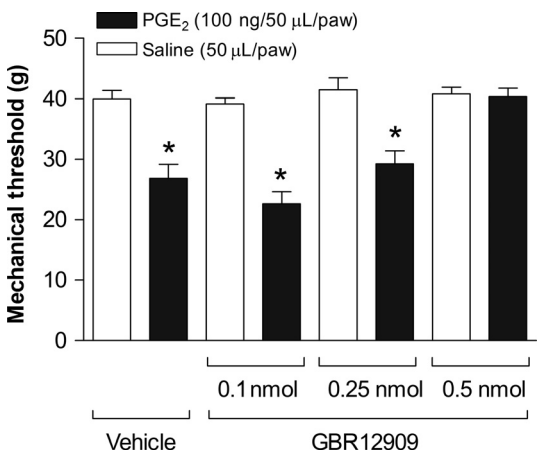


FIG. 3. Effect of enhanced dopaminergic neurotransmission in the NAc on acute mechanical hyperalgesia induced by PGE_2. Administration of the dopamine reuptake inhibitor GBR12909 at 0.5 nmol, but not at 0.1 or 0.25 nmol, bilaterally in the NAc prevented mechanical acute hyperalgesia evaluated 3 h after PGE_2 injection ($n = 7$–12; $P < 0.01$; one-way ANOVA followed by Bonferroni test). GBR12909 (0.5 nmol) by itself did not change the mechanical nociceptive threshold in rats treated with saline in the hindpaw ($P > 0.05$). *Statistical difference when compared with the other groups. PGE_2, prostaglandin E_2.

Dopaminergic neurotransmission in the NAc mediates persistent hyperalgesia by D1 and D2 receptor activation

As dopaminergic neurotransmission facilitated persistent PGE_2-induced hyperalgesia development, to verify which dopamine receptor participates in this process, the antagonists of dopamine D1 or D2 receptors were bilaterally administered in the NAc over seven consecutive days associated with PGE_2 (100 ng/50 μL) in the hindpaw once a day for 14 days. Neither dopamine D1 and D2 receptor antagonists, SCH23390 (0.125 nmol/0.5 μL/h) and raclopride (0.416 nmol/0.5 μL/h), respectively, administered in the NAc changed the hyperalgesic response during the 14 days of persistent hyperalgesia induction by PGE_2 treatment. As shown in Fig. 6A, PGE_2-treated groups, associated with vehicle, SCH23390 or raclopride in the NAc, had reduced mechanical threshold over time as compared with the control group ($F_{3,20} = 75.68$, all $P > 0.01$; for time: $F_{7,20} = 35.69$, $P < 0.0001$). However, the hyperalgesia observed at day 14 was not maintained after cessation of PGE_2 treatment as evaluated at day 21. Bilateral administration of the D1 receptor antagonist SCH23390 in the NAc over 7 days impaired establishment of persistent hyperalgesia (Fig. 6B; $F_{3,20} = 29.87$, $P < 0.001$). Yet bilateral administration of the D2 receptor antagonist raclopride in the NAc over 7 days also impaired establishment of persistent hyperalgesia (Fig. 6B; $F_{3,20} = 29.87$; $P < 0.05$). There

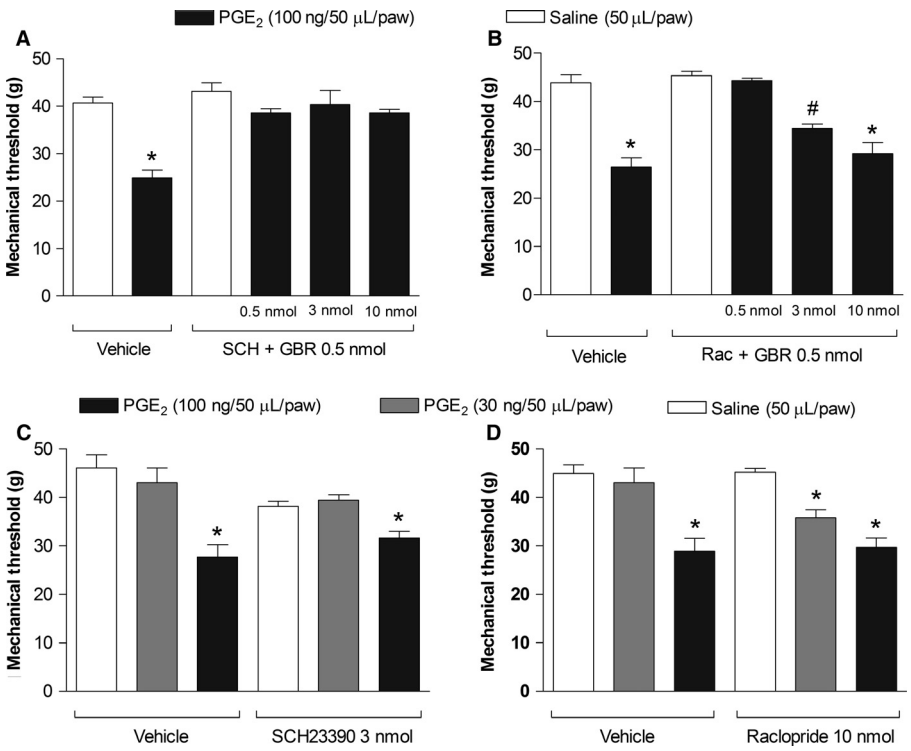


FIG. 4. Effect of dopamine D1 and D2 receptor antagonists on the anti-hyperalgesic action of dopaminergic neurotransmission in the NAc. (A) The dopamine D1 receptor antagonist SCH23390 (0.5, 3 and 10 nmol) administered in NAc did not reverse the anti-hyperalgesic effect of GBR12909 (0.5 nmol) on PGE_2-induced acute hyperalgesia (n = 6–7; $P > 0.05$). (B) The dopamine D2 receptor antagonist raclopride (0.5, 3 and 10 nmol) administered in NAc reversed, in a dose-dependent manner, the anti-hyperalgesic effect of GBR12909 (0.5 nmol) on PGE_2-induced acute hyperalgesia (n = 6–7; $P < 0.01$). (C) The dopamine D1 receptor antagonist SCH23390 (3 nmol) did not alter the acute hyperalgesia induced by PGE_2 (n = 5–7; $P > 0.05$). (D) The dopamine D2 receptor antagonist raclopride (10 nmol) facilitated the hyperalgesia induced by 30 ng PGE_2 in the hindpaw (n = 5–8; $P < 0.05$). $^{*,\#}$Significantly different from the other groups. The mechanical threshold was measured 3 h after saline or PGE_2 administration. PGE_2, prostaglandin E_2; GBR, GBR12909; SCH, SCH23390; Rac, raclopride.

was no difference in mechanical threshold of the group receiving vehicle in the NAc over 7 days and saline in the hindpaw over 14 days (Fig. 6A and B).

All injections of drugs or of their vehicles were within the NAc (Fig. 7).

Discussion

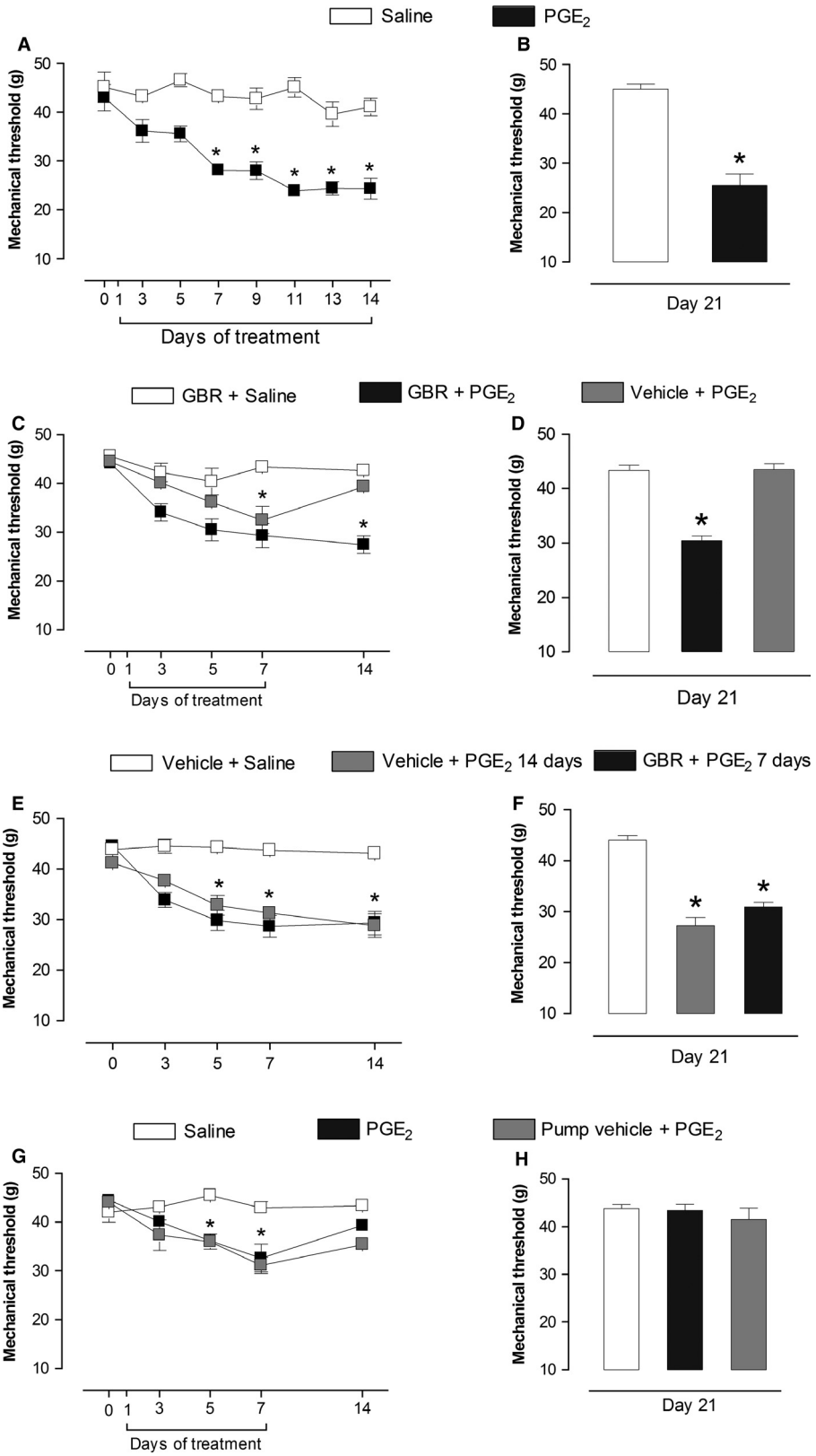
Our findings demonstrate for the first time the differential involvement of NAc dopaminergic neurotransmission in the modulation of inflammatory hyperalgesia in peripheral tissue. In acute hyperalgesia, this modulation is negative, i.e. dopamine released in the NAc prevents the acute hyperalgesic response. However, dopamine plays a facilitatory role in persistent hyperalgesia development, demonstrating that there is a different neurochemical processing in the NAc during the chronification of hyperalgesia.

NAc dopaminergic neurotransmission and acute hyperalgesia

Many studies have focused on NAc dopaminergic activity and its role in acute pain modulation, demonstrating that an experimental increase of mesolimbic dopaminergic activity attenuates the acute nociceptive behavior whereas its decrease leads to hyperalgesia (Altier & Stewart, 1998; Gear *et al.*, 1999; Taylor *et al.*, 2003; Wood, 2004; Haghparast *et al.*, 2012). Our findings corroborate these previous reports (Franklin, 1989; Morgan & Franklin, 1991; Altier & Stewart, 1999; Gao *et al.*, 2001; Taylor *et al.*, 2003) and demonstrate that increased endogenous dopamine levels in the NAc, due to administration of the dopamine reuptake inhibitor GBR12909, prevents the acute PGE_2-induced hyperalgesic response. But enhanced dopaminergic neurotransmission in the NAc did not change mechanical threshold. This suggests that NAc dopaminergic activity may have an adaptive role in nociception, as the presence of abnormal tissue status, such as inflammation, might engage supraspinal mechanisms of pain modulation.

Classically, dopamine exerts its actions through at least five different receptor subtypes of two subfamilies: D1-like receptors (D1 and D5) and D2-like receptors (D2, D3 and D4). The receptors belonging to each subfamily, D1- or D2-like, show similar sensitivities to agonists and antagonists, and thus it is not possible pharmacologically to differentiate dopamine D1 from D5 or D2 from D3 receptors (Missale *et al.*, 1998). It is noteworthy that dopamine D1 and D2 receptors are expressed in around 95% of medium spiny neurons of NAc, wherein part of them expresses either dopamine D1 or D2 receptors and a smaller part expresses both receptors (Hasbi *et al.*, 2009; Perreault *et al.*, 2011). As D1 and D2 receptors are the major dopamine receptors found in the NAc, they are considered to be involved in the anti-hyperalgesic effect induced by dopamine.

Indeed, our study showed that, in acute conditions, the anti-hyperalgesic effect of enhanced dopaminergic neurotransmission in the NAc is mediated by the dopamine D2 but not D1 receptor. A dopamine D2 receptor antagonist, raclopride, was co-administered with GBR12909 in the NAc and blocked the anti-hyperalgesic effect of increased dopaminergic activity, allowing the recovery of PGE_2-induced hyperalgesia. In contrast, a dopamine D1 receptor antagonist, SCH23390, did not change the anti-hyperalgesic action of enhanced dopaminergic activity in the NAc. Participation of the



dopamine D2 receptor in analgesia has been described by other authors (Morgan & Franklin, 1991; Altier & Stewart, 1998, 1999; Taylor *et al.*, 2003; Haghparast *et al.*, 2012). In addition, we demonstrated that the dopamine D2 receptor in the NAc can be related to endogenous control of the nociceptive pathway once its antagonist facilitated the acute hyperalgesic response when a subliminal dose of PGE_2 was administered in the hindpaw. As the NAc dopamine D2 receptor has an anti-hyperalgesic action and

FIG. 5. Effect of sustained enhanced dopaminergic neurotransmission in the NAc on the induction of persistent hyperalgesia. (A) Persistent hyperalgesia was induced by administration of PGE_2 (100 ng per paw) over 14 consecutive days. Mechanical threshold was evaluated just before PGE_2 administration. Mechanical threshold decreased after 7 days of PGE_2 treatment, and remained decreased up to day 14, as compared with saline treatment ($n = 6$; $P < 0.05$). (B) Decreased mechanical nociceptive threshold persisted for at least 7 days after the end of PGE_2 treatment ($P < 0.0001$). (C) Bilateral continuous infusion of GBR12909 (0.021/0.5 μL/h) in the NAc over seven consecutive days facilitated the development of persistent hyperalgesia induced by PGE_2 (100 ng per paw) once a day over 7 days, when compared with PGE_2 in the hindpaw over 7 days and saline in the NAc ($n = 5$; $P < 0.05$). GBR12909 administered in the NAc did not induce hyperalgesia by itself ($n = 5$; $P < 0.05$). (D) Bilateral continuous infusion of GBR12909 (0.021 nmol/0.5 μL/h) in the NAc over seven consecutive days facilitated the establishment of persistent hyperalgesia induced by PGE_2 (100 ng per paw) injected once a day over 7 days, when evaluated at day 21 and compared with administration of PGE_2 in the hindpaw over 7 days and infusion of vehicle in the NAc ($n = 6$ rats; $P < 0.001$). GBR12909 administered in the NAc did not induced hyperalgesia by itself ($n = 6$; $P > 0.05$). (E) Bilateral infusion of GBR12909 (0.021/0.5 μL/h) in the NAc over 7 consecutive days concomitant with 7 days of PGE_2 treatment in the hindpaw induced persistent hyperalgesia similar to that induced by PGE_2 treatment in the hindpaw over 14 days when compared with the control group ($n = 6$; $P < 0.05$). (F) Decreased mechanical nociceptive threshold persisted up to day 21 when treated groups were compared with the vehicle control group ($P < 0.05$). (G) Administration of PGE_2 (100 ng per paw) over 7 days associated or not with osmotic mini-pumps did not induce persistent hyperalgesia after cessation of PGE_2 treatment ($n = 5$; $P > 0.05$). (H) Osmotic mini-pumps alone did not contribute to persistent hyperalgesia establishment evaluated at day 21 ($n = 5$, $P > 0.05$). Mechanical threshold was evaluated just before PGE_2 administration. *Statistically different from other groups. PGE_2, prostaglandin E_2; GBR, GBR12909.

binds to dopamine released when a stimulus is presented, it is possible that the antagonist, raclopride, prevents the action of dopamine, favoring hyperalgesia. Note that raclopride by itself did not alter mechanical threshold when saline was administered in the hindpaw, suggesting that NAc dopamine D2 receptor activation is not related to mechanical nociception in normal tissue.

NAc dopaminergic neurotransmission and chronification of hyperalgesia

Using an animal model developed by Ferreira *et al.* (1990), persistent PGE_2-induced hyperalgesia was established to evaluate the role of NAc dopaminergic neurotransmission in the chronification of hyperalgesia. Persistent hyperalgesia is induced in rodents chronically treated with PGE_2 in the hindpaw, probably due to plastic changes of neural pathways involved in pain processing. Inflammatory mediators continuously sensitizing nociceptors and their central synaptic targets promote a maladaptive plasticity that results in persistent hyperalgesia (Ellis & Bennett, 2013). From this, our findings surprisingly demonstrate that there is a different neurochemical processing in the NAc during the chronification of hyperalgesia. Unlike the acute hyperalgesia the persistent hyperalgesia development is facilitated when the dopamine reuptake inhibitor, GBR12909, is chronically administered in the NAc concomitantly to PGE_2 in the hindpaw during 7 days, persistent hyperalgesia is facilitated. Thus, a lower mechanical threshold is observed at days 7 and 14 after the end of PGE_2 treatment, despite PGE_2 being injected in the hindpaw only during 7 days and not 14 days as usual to induce persistent hyperalgesia (Ferreira *et al.*, 1990). Whether PGE_2 is administered in the hindpaw over 7 days alone or with vehicle in the NAc, persistent hyperalgesia is not maintained after the end of PGE_2 treatment. Note that persistent dopaminergic neurotransmission did not alter basal mechanical threshold, again suggesting that dopamine modulates the nociceptive processing from sensitized peripheral fibers. Increased dopamine activity in the NAc has been described in an animal model of neuropathic pain (Austin *et al.*, 2010) and in patients with fibromyalgia (Wood *et al.*, 2007). This increased dopaminergic activity suggests an enhanced mesolimbic learning state that may play an essential role in chronification of pain. As enhanced dopaminergic activity in the NAc facilitates persistent hyperalgesia development, it is important to consider possible plastic changes in

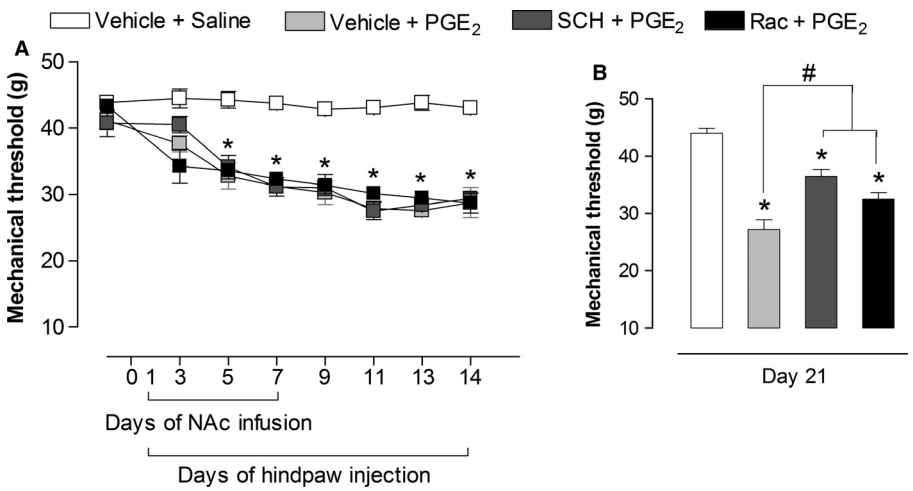


FIG. 6. Effect of sustained blockade of dopamine D1 and D2 receptors in the NAc on the induction of persistent hyperalgesia. (A) Bilateral continuous infusion of SCH23390 (0.125 nmol/0.5 μL/h) or raclopride (0.416 nmol/0.5 μL/h) in the NAc over 7 consecutive days did not alter the hyperalgesia induced by PGE_2 (100 ng per paw) injected once a day over 14 days when compared with infusion of vehicle in the NAc ($n = 6$ rats; $P > 0.05$). All groups differed from the group receiving vehicle bilaterally in the NAc over 7 days and saline in the hindpaw over 14 days ($P < 0.01$). (B) Bilateral continuous infusion of SCH23390 or raclopride in the NAc over seven consecutive days impaired the establishment of persistent hyperalgesia evaluated at day 21 when compared with infusion of vehicle in the NAc and PGE_2 in the hindpaw ($n = 6$ rats; $P < 0.05$). All these groups differed from the group receiving vehicle in the NAc and saline in the hindpaw ($P < 0.01$). *Significantly different from vehicle/saline group; #Significantly different from vehicle/PGE_2 group. PGE_2, prostaglandin E_2; SCH, SCH23390; Rac, raclopride; NAc, nucleus accumbens.

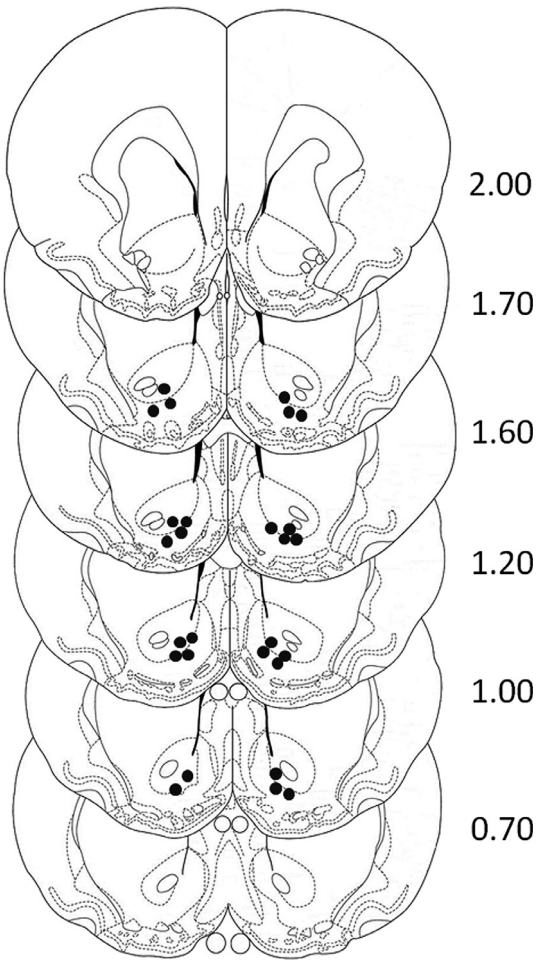


FIG. 7. Location of the injection sites in the NAc. All injection sites were within the NAc. Coronal sections were taken from a brain atlas (Paxinos & Watson, 1986) to demonstrate the areas of the injection sites (filled circles). Because some injections were mapped to identical locations, there are fewer circles shown than the total number of injections performed. The numbers on the right refer to the rostral distance (mm) to bregma.

the dopaminergic pathway in response to sustained increased tone of dopamine in the NAc. The dopaminergic system has a strong capability to restore its balance, in the face of fluctuations in tone and receptor binding, through the cooperative effect of different homeostatic mechanisms involving the dopamine transporter (DAT), dopamine receptors, autoreceptors and tyrosine hydroxylase. Indeed, changes in dopamine receptor expression in the NAc have been shown in neuropathic pain conditions (Austin *et al.*, 2010; Chang *et al.*, 2014).

As dopamine participates in modulation of acute hyperalgesia and given a possible dysregulation of dopaminergic neurotransmission in the development of persistent hyperalgesia, dopamine receptors may play a key role in this process. Participation of the dopamine D1 receptor in the development of persistent inflammatory hyperalgesia was confirmed by blockade of its activity. Our behavioral data suggest that continuous activation of the dopamine D1 receptor is necessary to induce persistent hyperalgesia, as its antagonist, SCH23990, administered in the NAc over the first 7 days of the 14 days of PGE_2 treatment in the hindpaw, impaired persistent hyperalgesia development. Note that the dose of SCH23390 used per day (3 nmol) was the same as used in the acute hyperalgesia protocol. Nevertheless, this dose had no effect on the acute condition, indicating an altered action of dopamine via the D1 receptor during the chronification of hyperalgesia. Interestingly, in spite of dopamine D2 receptor being associated with an antinociceptive effect of dopamine in acute hyperalgesia, our study suggests that the dopamine D2 receptor also participates in the development of persistent hyperalgesia. The dopamine D2 receptor antagonist, raclopride, administered in the NAc during the first 7 days of the 14 days of PGE_2 treatment in the hindpaw, impaired persistent hyperalgesia development. Regarding the pro-hyperalgesic role of dopamine in persistent hyperalgesia development, its effect might be inhibited by either dopamine D1 or D2 receptor antagonists. Thus, activation of both dopamine D1 and D2 receptors during the period of persistent hyperalgesia induction seems to be necessary for establishment of persistent hyperalgesia. This suggests a cooperative activation of dopamine D1 and D2 receptors in persistent hyperalgesia development as occurs in the expression of several reward and motivation-related behaviors mediated by NAc (Hopf *et al.*, 2003; Hasbi *et al.*, 2009). Given that dopamine D1 and D2 receptors in NAc possibly comprise two different populations of medium spiny neurons that are involved in two parallel NAc pathways (Chang *et al.*, 2014), it may seem surprising to observe a similar behavioral change when a dopamine D1 or D2 receptor antagonist was administered in the NAc. An important point to be considered is the small proportion of NAc neurons co-expressing D1 and D2 receptors constituting a dopamine D1/D2 receptor heteromer. Activation of this heteromer by dopamine triggers calcium intracellular signaling usually involved in synaptic plasticity (Hasbi *et al.*, 2011; Perreault *et al.*, 2011) and it may be underlying development of persistent hyperalgesia.

As the dopamine system has strong capability to adapt to changes in tone and receptor binding, it is important to consider whether the chronic treatment with dopamine reuptake inhibitor or receptor antagonists would cause adaptive changes in the NAc. It has been described that chronic administration (28 days) of GBR12909 has no effect on dopamine D1 and D2 receptor or DAT expression in the NAc (Tella *et al.*, 1996). However, it was also reported that GBR12909 administered for 7 days induces a decrease of DAT density in the NAc (Kunko *et al.*, 1997). The difference in the experimental protocol regarding GBR12909 dose and route of administration may explain these contradictory results. Given a possible change in DAT expression, the consequent increase of endogenous dopamine levels in the synaptic cleft would not alter our result, and one of the goals of the present study was to evaluate the effect of increased dopaminergic neurotransmission on persistent hyperalgesia development. In relation to dopamine receptor antagonists, some studies administering SCH23390 or raclopride over a long period such as 21 days have demonstrated adaptive changes related to expression of these receptors (Hall & Sallemark, 1987; Hess *et al.*, 1988; Lappalainen *et al.*, 1990; Yu *et al.*, 1998). However, it is noteworthy that 7 days of raclopride administration did not induce changes in D2 receptor mRNA in the NAc (Kopp *et al.*, 1992). Although there is no report in the literature on adaptive changes in the NAc induced by 7 days of SCH23390 administration, it is plausible that this period is not enough to induce plastic changes on dopamine receptors in the NAc.

NAc is a structure of the mesolimbic system classically involved in emotional and motivational processes. In spite of a number of studies considering the role of NAc dopamine in modulating nociceptive responses (Altier & Stewart, 1998; Gear *et al.*, 1999; Taylor *et al.*, 2003; Wood, 2004; Haghparast *et al.*, 2012), the mechanism through which it acts remains unclear. Regarding NAc connections,

there are two possible pathways to NAc participation in pain modulation. First, NAc may modulate the affective/motivational component of pain through its reciprocal connections with the anterior cingulate cortex, amygdala and prefrontal cortex, all limbic structures. Furthermore, there are efferent projections from NAc to mediodorsal thalamic nucleus, also involved with the limbic system and emotional responses (Salgado & Kaplitt, 2015). Second, NAc may also modulate the sensory/discriminative component of pain as there are connections of NAc with periaqueductal gray, a structure that is part of the descending circuit of pain modulation. In addition, there is a direct projection from spinal cord to NAc, probably containing nociceptive information (Cliffer *et al.*, 1991). Considering NAc and pain, there seems to be neurochemical differences in the modulatory mechanisms acting in acute and chronic conditions, probably related to plastic changes in the NAc as well as in other neural structures involved in pain modulation. Our data suggest that NAc dopaminergic neurotransmission is involved in the chronification of hyperalgesia and its modulatory role switches on the transition from acute to chronic conditions, changing from anti- to pro-hyperalgesic.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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Abbreviations

ANOVA, analysis of variance; DAT, dopamine transporter; DMSO, dimethyl sulfoxide; NAc, nucleus accumbens; PGE2, prostaglandin E2.

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