D1- and D2-like dopamine receptors within the nucleus accumbens contribute to stress-induced analgesia in formalin-related pain behaviours in rats

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Abstract

Background: Stressful experiences can produce analgesia, termed stress-induced analgesia (SIA). Meanwhile, it has been widely established that the mesolimbic dopamine pathway and nucleus accumbens (NAc) have a profound role in pain modulation. In this study, we examined the role of accumbal dopamine receptors in antinociception caused by forced swim stress (FSS) in order to understand more about the function of these receptors within the NAc in FSS-induced analgesia.

Method: Stereotaxic surgery was unilaterally performed on adult male Wistar rats weighing 230–250 g (some on the left and some on the right side of the midline). Two supergroups were microinjected into the NAc with a D1-like dopamine receptor antagonist, SCH-23390, at doses of 0.25, 1 and 4 μg/0.5 μl saline per rat or Sulpiride as a D2-like dopamine receptor antagonist at the same doses [0.25, 1 and 4 μg/0.5 μl dimethyl sulfoxide (DMSO) per rat]; while their controls just received intra-accumbal saline or DMSO at 0.5 μl, respectively. The formalin test was performed after rats were subjected to FSS (6 min, 25 ± 1 °C) to assess pain-related behaviours.

Results: The results demonstrated that intra-accumbal infusions of SCH-23390 and Sulpiride dose-dependently reduced FSS-induced antinociception in both phases of the formalin test. However, the percentage decrease in area under the curve (AUC) values calculated for treatment groups compared to formalin-control group was more significant in the late phase than the early phase.

Conclusion: Our findings suggest that D1- and D2-like dopamine receptors in the NAc are involved in stress-induced antinociceptive behaviours in the formalin test as an animal model of persistent inflammatory pain.

What does this study add?: Forced swim stress (FSS) induces the antinociception in both phases of formalin test. Blockade of accumbal dopamine receptors attenuate the antinociception induced by FSS. Stress-induced analgesia is dose-dependently reduced by dopamine receptor antagonists in both phases, although it is more prominent during the late phase.
1. Introduction

Pain is a multidimensional occurrence which is categorized into sensory, affective and cognitive components interposed by various neurobiological mechanisms (Garland, 2012). A variety of experimental approaches indicated that endogenous pain modulatory systems can be activated by some painful or stressful stimuli (Watkins and Mayer, 1986; Butler and Finn, 2009). This phenomenon referred to as stress-induced analgesia/antinociception (SIA) contributes to the maintenance of homeostasis and the survival of animals. However, depending on its severity, the opioid and non-opioid mechanisms are involved in SIA (Chapman et al., 2008; Xie et al., 2008). The specific neural regions involved in SIA comprise cortex, hippocampus, amygdala, hypothalamus,periaqueductal grey matter (PAG), rostral ventromedial medulla (RVM) and spinal cord (Butler and Finn, 2009). For many decades, investigations were focused more on endogenous opioids; however, it has been indicated that other neurotransmitters including serotonin, acetylcholine, nitric oxide, endocannabinoids and GABA have been implicated in facilitation or inhibition of SIA (Butler and Finn, 2009). The mesolimbic dopamine system is one of the most sensitive stress-responsive pathways which have been implicated in pain management (Tidey and Miczek, 1996; Altier and Stewart, 1999).

Dopamine is the principal neurotransmitter that controls motivated behaviour (Berridge and Kringelbach, 2008). Moreover, dopamine receptors are classified into two types: D1-like (which includes D1 and closely related D5 receptors) and D2-like (incorporating D2, closely related D3 and D4 receptors) receptors (Missale et al., 1998). Several lines of evidence suggest that in tonic pain, D2 receptors mediate the inhibitory role of dopamine in pain modulation (Morgan and Franklin, 1991; Magnusson and Fisher, 2000; Taylor et al., 2003) while D1 receptors are not involved (Magnusson and Fisher, 2000; Hagelberg et al., 2004). Previous studies have identified the nucleus accumbens (NAc) as one of the most critical part of the mesolimbic dopaminergic pathway which is involved in pain modulation as well as other functions ranging from motivation and reward to feeding and drug addiction (Gear et al., 1999). Furthermore, functional magnetic resonance imaging (fMRI) indicated that subcortical structures, such as the NAc, amygdala and PAG are activated in response to pain (Becerra et al., 2001).

On the other hand, it has been shown that acute exposure to stress augments extracellular levels of dopamine in the NAc and related regions (Bannon et al., 1983; Sorg and Kalivas, 1993). However, there are conflicting reports regarding dopamine involvement in SIA (Kulkarni, 1980; Tricklebank et al., 1984). Animal models of SIA typically combine aversive and noxious stimuli. Meanwhile, forced swimming stress (FSS) as an aversive stimulus was used frequently to study the impact of stress on pain in rats and mice. In addition, being a noxious stimulus, tail flick, hot plate and formalin test were routinely used following exposure to stress paradigms (Butler and Finn, 2009). It has also been demonstrated that the duration of stress and water temperature markedly change the neurochemical basis of SIA (O’Connor and Chipkin, 1984). Accordingly, we examined the role of accumbal dopamine receptors in antinociception caused by forced swim stress to understand more about the function of nucleus accumbens in FSS-induced analgesia.

2. Materials and methods

2.1 Animals

Experiments were performed on 61 adult male albino Wistar rats weighing 230–250 g (Pasteur Institute, Tehran, Iran). Animals were housed in a room at 22 ± 2 °C, under an inverted 12-h light-dark cycle (lights on at 7:00 a.m.) with free access to food and water. The animals were randomly allocated to different experimental groups and each animal was used only once. Additionally, subjects were habituated to the handling and testing procedures prior to testing. All procedures were performed according to the Guide for the care and use of laboratory animals (National Institutes of Health Publication No. 80–23, revised 1996) and were approved by the Research and Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran.

2.2 Drugs

Drugs used in this study include SCH-23390, (R)-(+)7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride as a D1-like dopamine receptor antagonist (Tocris Bioscience, Bristol, UK) and Sulpiride, (S)-(+)5-aminsulfonyl-N-[([1-ethyl-2-pyrrolidinyl]methyl]-2-methoxybenzamide as a D2-like dopamine receptor antagonist (Tocris Bioscience, Bristol, UK) which were dissolved in sterile normal saline and dimethyl sulfoxide (DMSO; Sigma-Aldrich, Germany), respectively. Formalin 2.5% was prepared by diluting 37% formaldehyde (Merck,
Germany) with physiological saline solution (0.9%). All drugs were freshly prepared on the day of experiment.

### 2.3 Surgical preparation

Rats were intraperitoneally anesthetized with a mixture of xylazine 2% (10 mg/kg) and ketamine 10% (100 mg/kg) and placed in a stereotaxic instrument (Stoelting, Wood Dale, Illinois, USA). Then, a small craniotomy was performed and the surface of the skull was exposed. A stainless steel guide cannula (23 gauge, 11 mm length) was unilaterally inserted 1 mm above the NAc at the following coordinates: 1.5 ± 0.2 mm anterior to bregma, 1.4 ± 0.2 mm lateral to midline and 7.3 mm ventral from the skull surface (Paxinos and Watson, 2007). Guide cannula was then anchored to the skull using two stainless steel screws and dental acrylic cement. A stainless steel stylet was used to occlude the guide cannula after the cement was completely hardened. Stereotaxic surgery was performed on both sides of the midline in which some rats were randomly underwent surgery on the left and some on the right side (Supporting Information Fig. S1). Animals were separately kept for 5–7 days before performing the experiments.

### 2.4 Drug administration

Each microinjection was performed by lowering a stainless steel injector cannula (a 30-gauge needle) with a length of 1 mm longer than the guide cannula into the NAc. The injector cannula was connected to a 1-μl Hamilton syringe by polyethylene tubing (PE-20), and then 0.5 μl of drug solution or vehicle (saline or DMSO) was infused over 60 s. During the time of microinjections, animals were free to move around the cage.

### 2.5 Experimental procedure

#### 2.5.1 Forced swim stress

On the test day, animals were initially transferred to the test room and allowed to acclimatize for half an hour. FSS was performed 5 min after the microinjection of either drug or vehicle. Each animal was placed in a cylindrical plastic tank (50 cm high, 30 cm in diameter) which was filled with water up to a level of 30 cm and maintained at 25 ± 1 °C for 6 min (Attarzadeh-Yazdi et al., 2013). After completion of the stress protocol, each rat was carefully dried with a towel and after almost 10 min, formalin was injected either into right or left hind paw while the microinjections had been performed contralaterally. This was done because each side of brain mostly receives nociceptive information from the contralateral side of the body. All experiments were carried out daily between 8:00 a.m. and 4:00 p.m.

#### 2.5.2 Formalin test

In all experiments, after the microinjection of either vehicle or the drugs and following exposure to forced swim stress, 50 μl of formalin 2.5% was injected subcutaneously into the plantar surface of either right or left hind paw. Rats were immediately placed in a transparent Plexiglas chamber with an open roof (35 × 35 × 35 cm³) and a mirror angled at 45° to observe spontaneous activity of the affected paw. The four behavioural categories are as follows: 0, the position and posture of the injected hind paw was indistinguishable from the another hind paw; 1, the injected paw had little or no weight placed on it; 2, the injected paw was elevated and was not in contact with any surface; 3, the injected paw was licked, bitten or shaken. Time spent in each type of behaviour was recorded in 5-min blocks for 60 min test period. A weighted average nociceptive score, ranging from 0 to 3, was calculated by multiplying the time spent in each category by the category weight, summing these products and dividing by the total time (300 s) for each 5-min time block:

\[
\text{Nociceptive score} = \\
\frac{(t_0 \times 0) + (t_1 \times 1) + (t_2 \times 2) + (t_3 \times 3)}{t_0 + t_1 + t_2 + t_3}
\]

By utilizing this method, an ordinal scale of nociceptive scores was generated with a range of 0–3 (Ronaghi et al., 2011). The procedure was accomplished by histological verification of samples and data analysis.

### 2.6 Experimental protocols

In the first part of the study, rats were assigned to control groups including formalin-, saline- and DMSO-control groups (n = 5–6 in each group); in which the effects of baseline nociceptive behaviour, surgical manipulation and microinjection volume were determined. Among these, saline-control and DMSO-control groups received 0.5 μl saline or DMSO (as drug vehicle) into the NAc, respectively; followed by formalin injection after exposing the animals to stress protocol.
2.6.1 The effect of intra-accumbal administration of SCH-23390 on antinociceptive behaviours caused by forced swim stress in formalin test

To evaluate the effect of D1-like dopamine receptor antagonist on antinociceptive responses induced by forced swim stress, animals received unilateral injections of SCH-23390 (at 0.25, 1 and 4 μg/0.5 μl saline; n = 5–6 in each group) in the NAc, 5 min prior to exposure to FSS (for 6 min, 25 ± 1 °C water). Rats were then dried with a towel and submitted to hind paw formalin injection to assess the animal’s pain-related behaviours at 5 min intervals for a 60-min period. Additionally, to investigate the effect of D1-like dopamine receptor antagonist alone on the nociceptive behaviours, animals received the maximum dose of SCH-23390 (4 μg/0.5 μl saline) into the NAc (n = 5).

2.6.2 The effect of intra-accumbal administration of Sulpiride on antinociceptive behaviours caused by forced swim stress in formalin test

In order to examine the possible role of D2-like dopamine receptors located in the NAc in antinociception induced by forced swim stress, Sulpiride was unilaterally microinjected into the NAc at various doses (0.25, 1 and 4 μg/0.5 μl DMSO; n = 5–6 in each group) just 5 min before exposure to FSS (for 6 min, 25 ± 1 °C water). In another set of experiment, rats only received the highest dose of Sulpiride (4 μg/0.5 μl DMSO; n = 5) into the NAc just 5 min before formalin injection.

2.7 Histological verification

After implementation of the experiments, animals were deeply anesthetized with ketamine and xylazine and were transcardially perfused with 0.9% saline and 10% formaldehyde solution. Rats were then sacrificed, and their brains were removed. 50-μm transverse brain sections were cut and the location of cannula tract was confirmed using the rat brain atlas (Supporting Information Fig. S1) (Paxinos and Watson, 2007).

2.8 Statistics

Pain score is expressed as mean ± SEM (standard error of mean) and p < 0.05 is the defined level for statistical significance. Data were processed by commercially available software Graph Pad Prism® 6.0. The mean nociceptive scores of different groups were compared by two-way ANOVA followed by Bonferroni’s multiple comparisons test, to specify the treatment and time effects on pain behaviours. In order to evaluate the nociceptive responses during the early or late phases, area under the curve (AUC) was calculated as raw pain scores x time by the linear trapezoidal method and a single value was subjected to one-way ANOVA followed by protected Newman–Keuls test. Besides, one-way ANOVA followed by Dunnett’s test was used to calculate the percentage decrease of the AUC values calculated for pain scores in the saline or DMSO-control and the experimental groups compared to AUC values of formalin-control group during the early and late phases.

3. Results

In this study, two-way ANOVA with Bonferroni’s post hoc [treatment effect: F (2, 143) = 28.24, p < 0.0001; time effect: F (11, 143) = 16.99, p < 0.0001; treatment and time interaction effect: F (22, 143) = 7.863, p < 0.0001; Fig. 1A] revealed that the application of FSS could significantly attenuate the nociceptive responses during the formalin test following administration of saline or DMSO in control groups which resulted as baseline in all time set intervals, compared to formalin-control group (No FSS). Similarly, one-way ANOVA followed by the Dunnett’s multiple comparison test indicated that the calculated AUC values of saline and DMSO- control groups are remarkably identical [F (2, 15) = 32.02, p < 0.0001; Fig. 1B]. Fig. 1C showed that the percentage decrease in AUC values of saline- and DMSO-control groups is partly similar and had no significant difference in both phases of the formalin test.

3.1 The effect of intra-accumbal administration of SCH-23390 on antinociceptive behaviours caused by forced swim stress in formalin test

To determine whether D1-like dopamine receptors in the NAc are involved in formalin-induced pain behaviours following a forced swimming session (6 min), SCH-23390 was administered into the NAc. Fig. 2 depicts that the application of the forced swim stress could significantly attenuate the nociceptive responses in the formalin test. Also, intra-accumbal administration of different doses of the antagonist (0.25, 1 and 4 μg/0.5 μl saline per rat) was found to have the potential to significantly prevent the antinociceptive responses of swim stress in both phases [Treatment effect: F (5,264) = 60.35, p < 0.0001; time effect: F (11, 264) = 3.348, p = 0.0002; treatment and time interaction effect: F
The acquired AUC values of different doses of the antagonist were compared with those calculated for control groups (saline and formalin). This was done separately for the early and late phases (Fig. 3A and B). One-way ANOVA followed by Newman–Keuls multiple comparison test showed that the antinociceptive response of swim stress was dose-dependently suppressed by injection of different doses of SCH-23390 in the early \([F (5, 28) = 14.2, p < 0.0001; \text{Fig. 3A}]\) and late \([F (5, 28) = 24.25, p < 0.0001; \text{Fig. 3B}]\) phases. In comparison with the formalin-control group, AUC values of saline-control group and all treatment groups were reduced in both phases. However, this reduction was not significant in SCH-23390 at the dose of 4 \(\mu g\). In addition, the results revealed that compared to saline-control group, SCH-23390 at highest dose (4 \(\mu g/0.5 \mu l\)) decreased the analgesic effect of stress during both phases \((p < 0.001)\). This was while in comparison with the saline-control group, SCH-23390 at the dose of 1 \(\mu g\) markedly antagonized analgesic effect of stress during the late phase \((p < 0.01)\), but not in the early phase. On the other hand, administration of the maximum dose of SCH-23390 (4 \(\mu g/0.5 \mu l\)) alone into the NAc could not affect the AUC calculated value, compared to the formalin-control group.

As it can be seen in Fig. 4, the results are shown as percentage decrease in the AUC values of saline-control and experimental groups compared to the AUC values of formalin-control group which are scored as zero. As a consequence, one-way ANOVA followed by Dunnett’s test showed significant differences in percentage decrease of the AUC values calculated for pain scores in the saline-control and experimental groups for the early \([F (4, 26) = 20.47, p < 0.0001]\) and late \([F (4, 26) = 36.57, p < 0.0001]\) phases compared to the AUC values of formalin-control group. Saline \((0.5 \mu l/rat) + \text{FSS}\) induced the most antinociceptive effect which is shown as the highest percentage decrease in the AUC values. In comparison with the formalin-control group, percentage decrease of the AUC values of the groups which had received SCH-23390 at the doses of 1 and 4 \(\mu g\) were less in the late phase than the early phase; demonstrating that the involvement of D1-like dopamine receptors in the NAc on antinociceptive behaviours caused by forced swim stress is more noticeable during the late phase than the early phase.

### 3.2 The effect of intra-accumbal administration of Sulpiride on antinociceptive behaviours caused by forced swim stress in formalin test

To investigate the role of D2-like dopamine receptors in the NAc on formalin-induced pain behaviours following exposure to the stress paradigm, different doses of Sulpiride \((0.25, 1 \text{ and } 4 \mu g/0.5 \mu l \text{ DMSO})\) were injected unilaterally into the NAc.
per rat), as a selective D2-like receptor antagonist, were microinjected into the NAc. As observed in Fig. 5, administration of different doses of Sulpiride failed to produce any effect on antinociceptive responses of swim stress in both phases, compared to DMSO-control group [treatment effect: F (5, 341) = 44.29, \( p < 0.0001 \); time effect: F (11, 341) = 10.86, \( p < 0.0001 \); treatment and time interaction effect: F (55, 341) = 3.888, \( p < 0.0001 \)]. Also, one-way ANOVA and Newman–Keuls multiple comparison test revealed that Sulpiride affected the antinociceptive response caused by swim stress during the early [F (5, 36) = 11.11, \( p < 0.0001 \); Fig. 6A] and late [F (5, 36) = 25.35, \( p < 0.0001 \); Fig. 6B] phases. The results showed that compared to formalin-control group, DMSO and all doses of Sulpiride following FSS reduced AUC values in both phases. Fig. 6A and B reveal that compared to DMSO-control group, the antinociceptive response of swim stress was dose-dependently suppressed by injection of different doses of Sulpiride during both phases. Indeed, this effect was more remarkable at doses of 1 \( \mu g/0.5 \mu l \) and 4 \( \mu g/0.5 \mu l \) (\( p < 0.05, p < 0.01 \)). Additionally, the maximum dose of Sulpiride (4 \( \mu g/0.5 \mu l \)) alone could not affect the pain scores in comparison with the formalin-control group.

On the other hand, in Fig. 7, one-way ANOVA following Dunnett’s test showed significant differences in percentage decrease of the AUC values calculated for pain scores in the DMSO-control and the experimental groups during the early [F (4, 30) = 13.74, \( p < 0.0001 \)] and late [F (4, 30) = 30.06, \( p < 0.0001 \)].
phases compared to those calculated for the formalin-control group. DMSO (0.5 μl/rat) + FSS induced the highest percentage decrease in responses compared to the formalin-control group during both phases. In comparison with the formalin-control group, percentage decrease of the AUC values of all treatment groups were less in the late phase than the early phase. Altogether, as shown in Fig. 7, it appears that the effect of Sulpiride as a selective D2-like dopamine receptor antagonist on antinociceptive behaviours caused by FSS was more observable during the late phase compared to the early phase.

4. Discussion

The findings of the present experiment indicated that microinjection of D1/D2-like dopamine receptor antagonists into the NAc significantly decreased antinociceptive effects of stress during the early and late phases of formalin-induced nociception. Multiple studies buttress the view that stress may commence several changes in neuronal and hormonal status and may induce analgesia in both animals and humans. This phenomenon known as SIA is activated by endogenous pain inhibitory systems (Watkins and Mayer, 1986; Chapman et al., 2008; Butler and Finn, 2009). This is in consonance with our results which showed that FSS (for 6 min, 25 ± 1 °C) could significantly attenuate the nociceptive responses during the two phases of formalin test.

It has been shown that the dopaminergic system and dopamine play a critical role in endogenous analgesia, and are involved in pain modulation in several regions of the central nervous system such as the basal ganglia, anterior cingulate cortex (ACC), insula, thalamus, PAG and the spinal cord (Wood, 2008). However, there are conflicting reports regarding the involvement of dopamine in SIA; for example, some findings showed that intraperitoneal administration of haloperidol and pimozide, both of which are dopamine D2 antagonists, increased antinociception following footshock stress in both paw lick and tail flick tests (Tricklebank et al., 1984). In contrast to these findings, antinociception induced by prolonged immobilization was blocked by haloperidol (Kulkarni, 1980).

There are numerous studies indicating that the mesolimbic dopamine system is one of the most sensitive stress-responsive pathways which have been
implicated in pain management (Tidey and Miczek, 1996; Altier and Stewart, 1999). Exposure to acute stress can activate VTA-dopamine neurons via the release of substance P (SP) and endogenous opioids. (Bannon et al., 1983; Sorg and Kalivas, 1993). In addition, previous preclinical and clinical studies support a role for the NAc as one of the most essential parts of the mesolimbic dopamine pathway in the processing of pain (Gear et al., 1999, Gear and Levine, 2011). Thus, intra-accumbens injection of quinpirole (a D2 receptor agonist) dose-dependently inhibited nociception preferentially during the late phase of formalin-induced pain. It seems that the agonism of D2 receptors in the NAc regulates C-fibre mediated pain signals (Taylor et al., 2003). Recent studies have indicated that the NAc has a potential role in setting up nociceptive sensitivity and there is also evidence that both opioid and dopamine links in the nucleus accumbens are involved in chemical or thermal noxious stimulation induced during pain modulation (Gear et al., 1999; Schmidt et al., 2002; Gear and Levine, 2011). Current knowledge suggests that the descending pathway for

**Figure 6** Area under the curves (AUCs) calculated separately for pain scores in the (A) early (5 min), and (B) late (15–60 min) phases of formalin-induced pain, during 60-min period of the test. Animals received different doses of Sulpiride (0.25, 1 and 4 μg/0.5 μl dimethyl sulfoxide (DMSO) per rat) or 0.5 μl DMSO (as control) into the nucleus accumbens (NAc), 5 min before the forced swim stress test (6 min). Data are represented as mean ± SEM in each group. In Sulpiride-control group (hatched bar), animals received the maximum dose of Sulpiride (4 μg/0.5 μl DMSO per rat) alone into the NAc before the formalin test. *p < 0.05; **p < 0.01 and ***p < 0.001 compared to formalin-control group (no forced swim stress).

**Figure 7** Diagram showing percentage decrease in the area under the curve (AUC) values calculated for the dimethyl sulfoxide (DMSO)-control and treatment groups (groups that received Sulpiride) compared to the AUC value calculated for formalin-control group. Animals received Sulpiride (0.25, 1 and 4 μg/0.5 μl DMSO per rat) or 0.5 μl DMSO (control group) into the nucleus accumbens, 5 min before the forced swim stress test (6 min). Data are shown separately for either the early (left panel) or late (right panel) phases of nociceptive responses. *p < 0.05; **p < 0.01 and ***p < 0.001 compared to formalin-control group (no forced swim stress).
antinociception mediated by the NAc might include the PAG, RVM and the spinal cord pain modulation system. In this study, intra-NAc injection of SCH-23390 or Sulpiride alone had no effect during the two phases of formalin test compared to the formalin-control group. In agreement with present study, Haghparast et al. (2012) showed that the administration of the maximum doses of SCH-23390 or Sulpiride alone into the NAc did not have significant effect on formalin and tail flick tests (Haghparast et al., 2012). Therefore, it appears that D1/D2-like dopamine receptors within the NAc in normal situations are not relevant in pain modulation of persistent inflammatory pain.

The reciprocal connections between the NAc and other structures show that there is a possible role for the NAc in regulation of some functions such as emotional reactions and motivational processes. (Scott et al., 2006). In this context, it has been demonstrated that dopamine transmission in the NAc modulates the behavioural responses to stress (Scornaiencki et al., 2009). For instance, extracellular dopamine levels in the NAc are increased by the administration of corticosterone at doses that induce an increase in the levels of the hormone identical to those induced by stress (Piazza et al., 1996). On the other hand, evidence suggests that the activation of the dopaminergic system that occurs during stressful situations is modulated by direct or indirect projections from the prefrontal cortex (PFC), hippocampus and amygdala (Mora et al., 2012). For example, corticofugal projections mediate PFC modulation of the NAc dopamine response to stress, whereas this action in the NAc is mediated at least in part by D1 receptors located in the PFC (Stevenson and Gratton, 2003; Scornaiencki et al., 2009).

In this study, pretreatment with SCH-23390 or Sulpiride led to decreased in the inhibition of FSS during two phases of formalin test, and it was more prominent during the late phase than the early phase. Considering that there are different mechanisms underlying the development of both phases of pain-related behaviours after formalin injection, it was opined that accumbal dopaminergic system involves more in SIA mechanisms which are principal in the development of the late phase of formalin-induced nociception. In a similar vein, Altier and Stewart’s (1998) findings have shown that activation of the VTA-NAc dopamine pathway also plays a historic role in mediating the stress-induced suppression of tonic pain in such way that bilateral intra-NAc infusions of either the mixed dopamine receptor antagonist flupenthixol or the dopamine D1-like receptor antagonist SCH-23390 attenuated SIA. Similarly, blockade of dopamine D2 receptors in the NAc by raclopride prevented the analgesic effects induced by infusions into the VTA of the SP analog DiMe-C7 (Altier and Stewart, 1998). It has been revealed that intra-acccumbens microinjection of a dopamine antagonist (flupentixol) or an opioid antagonist (naloxone) block stress-induced analgesia. Based on these considerations, chemical or thermal noxious stimulation as a stressor causes pain modulation by an ascending nociceptive control and that this effect depends on both opioid and dopamine links in the nucleus accumbens (Gear et al., 1999). In conclusion, the present results confirmed the involvement of accumbal dopaminergic system in FSS-induced analgesia in both early and late phases of the formalin test and further implicate both D1/D2-like dopamine receptors that play a crucial role in SIA, although it is more prominent during the late phase.

Author contributions

A.H. substantially contributed to the conception and design of the study. G.F. and A.H. were responsible for acquisition, analysis and interpretation of data. G.F. and M.Z. drafted the article. A.H. and G.F. revised the article critically for important intellectual content. A.H. was responsible for the final approval of the version to be published.

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References


Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

**Figure S1.** (A) A photomicrograph image of the nucleus accumbens (NAc) showing the cannula placement (stereotaxic surgery was done on both sides of the midline in which some rats were randomly underwent surgery on the left and some on the right side); (B) Schematic representation of coronal sections showing the microinjection sites (● = saline- or DMSO-control; ○ = SCH-23390 and ▲ = Sulpiride). cc, corpus callosum; NAc, nucleus accumbens; AcbC, accumbens nucleus, core; AcbSh, accumbens nucleus, shell; CPU, caudate putamen (striatum); acA, anterior commissure, anterior part; DEn, dorsal endopiriform nucleus; LV, lateral ventricle; VP, ventral pallidum. The numbers show AP coordinate relative to Bregma. Scale bar is 1 mm.