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Mohanna Akbarin Mayvani

Shahid Beheshti University of Medical Sciences

Sara Arvand

Shahid Beheshti University of Medical Sciences

Farzaneh Nazari-Serenjeh

Payame Noor University

Roghayeh Mozafari

Shahid Beheshti University of Medical Sciences

Abbas Haghparast

haghparast@sbmu.ac.ir

Shahid Beheshti University of Medical Sciences

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Functional interaction between D2-like dopamine and μ -opioid receptors in the hippocampal dentate gyrus modulates inflammatory pain in male rats

**Mohanna Akbarin Mayvani ¹, Sara Arvand ², Farzaneh Nazari-Serenjeh ³,
Roghayeh Mozafari ^{1,4,**}, Abbas Haghparast ^{1, 4, 5, 6,*}**

¹ *Neuroscience Research Center, Institute of Neuroscience and Cognition, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

² *Neurobiology Research Center, Institute of Neuroscience and Cognition, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

³ *Department of Biology, Payame Noor University (PNU), Tehran, Iran*

⁴ *School of Cognitive Sciences, Institute for Research in Fundamental Sciences, Tehran, Iran*

⁵ *Department of Basic Sciences, Iranian Academy of Medical Sciences, Tehran, Iran*

⁶ *National Brain Mapping Laboratory (NBML), Tehran, Iran*

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*** Corresponding author**

Abbas Haghparast, PhD

Neuroscience Research Center, Shahid Beheshti University of Medical Sciences

P.O. Box 19615-1178, Tehran, Iran

Email: Haghparast@yahoo.com; Haghparast@sbmu.ac.ir

**** Co-corresponding author**

Roghayeh Mozafari, PhD

Email: mozafariorkide@gmail.com; r.mozafari@sbmu.ac.ir

Abstract

While the hippocampal dentate gyrus (DG) is recognized as a supraspinal region involved in pain modulation, the specific receptor-mediated mechanisms underlying its role in persistent inflammatory pain remain poorly understood. This study investigated the functional interaction between the D2-like dopamine receptor (D2R) and the μ -opioid receptor in the DG in the formalin model of inflammatory pain in rats. Male Wistar rats received unilateral intra-DG microinjections of morphine (5, 10, 25, and 50 mmol), quinpirole (4, 8, 16, and 32 mmol), sulpiride (3, 6, 12, and 24 mmol), or naloxone. To confirm receptor involvement, naloxone (0.5, 1.5, 5, and 15 mmol) was administered before the effective dose of morphine (50 mmol), and sulpiride before the effective dose of quinpirole (16 mmol). To assess receptor interaction, sulpiride was given before morphine, whereas naloxone (1.5, 5, 15, and 45 mmol) was given before quinpirole. Nociceptive behavior was evaluated during the early and late phases of the formalin test, and locomotor activity was assessed in the open field. Morphine produced dose-dependent antinociception in both phases, and this effect was blocked by naloxone, supporting μ -opioid receptor involvement. Quinpirole also reduced formalin-evoked nociceptive behavior in a dose-dependent manner, with greater efficacy in the late inflammatory phase; this effect was blocked by sulpiride, confirming D2R mediation. In addition, sulpiride attenuated morphine-induced antinociception, whereas naloxone reduced quinpirole-induced antinociception. None of the treatments altered locomotor activity. These findings reveal that D2R and μ -opioid receptors converge functionally within the DG to regulate inflammatory pain, positioning this hippocampal subregion as a candidate site for opioid-sparing analgesic approaches.

Keywords: Nociception; D2-like dopamine receptor; μ -opioid receptor; Dentate gyrus; Formalin test; Rats

1. Introduction

Chronic inflammatory pain remains a debilitating condition for which current pharmacological interventions provide insufficient relief in many cases, highlighting the necessity of investigating supraspinal pain-modulatory mechanisms at the circuit and receptor levels. Although the spinal and peripheral components of nociception have been studied extensively, the affective and emotional dimensions of persistent pain depend critically on limbic structures. Among these, the hippocampus has emerged as a significant contributor to pain modulation in rodent models, with disruption of hippocampal function altering nociceptive behavior and affective pain responses [1]. Within the hippocampus, the dentate gyrus (DG) serves as a principal gateway, receiving convergent projections from pain-related pathways and integrating sensory and emotional aspects of nociception [2, 3]. Nevertheless, the neurochemical mechanisms by which the DG modulates persistent inflammatory pain, as produced experimentally by the formalin test, remain incompletely characterized.

Traditionally associated with reward processing and motivated behavior [4], the dopaminergic system is increasingly recognized as a key modulator of the affective dimension of pain, including in hippocampal circuitry [5]. Among dopamine receptor subtypes, the D2-like dopamine receptors (D2R)—comprising D2, D3, and D4 receptors—mediate inhibitory effects on neuronal excitability, and the magnitude of their influence on nociceptive thresholds is determined by local receptor density and anatomical context [6]. Concurrently, μ -opioid receptors represent the primary substrate for opioid analgesia. However, systemic opioid administration is associated with tolerance, dependence, and opioid-induced hyperalgesia, motivating the search for

localized receptor targets that may achieve analgesia with fewer adverse consequences [7]. Evidence from the nucleus accumbens (NAc) [8] and the CA1 subregion of the hippocampus [9] indicates that dopaminergic and opioidergic systems interact functionally to regulate pain-related behavior in rodents, raising the possibility, yet to be tested, that comparable interactions occur within the DG.

Previous research has demonstrated that dopaminergic–opioidergic interactions within the DG modulate acute nociception [10] and that orexinergic–opioidergic signaling in the same region influences inflammatory pain responses [11]. Within the CA1 subregion, a functional interaction between D1-like dopamine receptor (D1R) and μ -opioid receptors has been shown to modulate formalin-induced pain in rats [12]. However, whether D2R, coupling to Gi/o rather than Gs proteins and consequently engaging distinct intracellular signaling cascades relative to D1R [13], participates in an analogous interaction within the DG remains unexplored, leaving the potential for a reciprocal or synergistic modulation of persistent pain by these receptors in the DG an open question.

Male rats were selected to eliminate the confounding influence of estrous cycle–driven hormonal variability on nociceptive sensitivity, consistent with the standard approach in preclinical pain research [14]. Accordingly, the present study was designed to investigate the functional interaction between intra-DG D2R and μ -opioid receptors in a rat model of inflammatory pain. By microinjecting selective agonists and antagonists into the DG, this study sought to determine whether these receptor systems act synergistically or antagonistically to modulate both the early and late phases of formalin-induced nociception. These findings contribute to a mechanistic understanding of hippocampal involvement in pain processing and may identify a neurochemical target

for the development of localized analgesic strategies that bypass the systemic limitations of conventional opioid therapy.

2. Materials and methods

2.1. Animals

All experimental procedures were conducted in strict accordance with the ethical guidelines of the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (8th edition, 2011) and were approved by the Institutional Animal Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran (Ethical Protocol No: IR.SBMU.PHNS.REC.1403.079). Every effort was made to minimize animal suffering and to use the minimum number of subjects necessary to achieve statistical significance.

Male Wistar rats ($n = 225$; weight range: 230–260 gr at the time of surgery) were obtained from the Pasteur Institute (Tehran, Iran). The animals were housed in groups of three or four per cage under a controlled environment with a standard 12-h light/dark cycle (lights on at 07:00). Food and water were provided *ad libitum*. All behavioral experiments were performed during the light phase, between 08:00 and 14:00, to minimize the circadian rhythm's influence on nociceptive responses.

2.2. Stereotaxic surgery and cannula implantation

Rats were deeply anesthetized using an intraperitoneal injection of a ketamine/xylazine cocktail (100 and 10 mg/kg, respectively) and secured in a Stoelting stereotaxic frame (USA). After reflecting the scalp and exposing the cranium, the

surgical site was sanitized. A single 23-gauge stainless-steel guide cannula (8 mm length) was unilaterally positioned above the DG according to the Paxinos and Watson stereotaxic atlas [15]. The coordinates were defined as: AP: -3.8 ± 0.15 mm posterior to bregma, ML: ± 0.9 mm from the midline, and DV: -3.1 ± 0.2 mm below the skull surface. The cannula was anchored to the skull with stainless-steel miniature screws and secured in place with dental acrylic resin to ensure structural stability. A postoperative recovery window of 5–7 days was provided before commencing behavioral assays.

2.3. Pharmacological agents

The D2Rs selective agonist quinpirole, the D2R antagonist sulpiride, and the μ -opioid receptor antagonist naloxone hydrochloride were sourced from Tocris Bioscience (Bristol, UK). Morphine sulfate, acting as a μ -opioid receptor agonist, was provided by Temad (Tehran, Iran). Morphine and naloxone were prepared in sterile 0.9% saline. Quinpirole and sulpiride were dissolved in 12% dimethyl sulfoxide (DMSO; Sigma-Aldrich, Germany). All pharmacological solutions were freshly prepared on the day of testing. For the induction of inflammatory pain, a 2.5% formalin solution was prepared by diluting a 37% formaldehyde stock (Merck, Germany) in physiological saline.

2.4. Intracerebral microinjection protocol

The site-specific microinjection of D2R and μ -opioid receptor agonists/antagonists into the DG region was performed as schematically illustrated in Figure 1A. Specifically, the procedure involved a 30-gauge injector coupled to a 1- μ l Hamilton syringe via PE-20 polyethylene tubing. A total volume of 0.5 μ l—containing either the pharmacological agents or their respective vehicles—was administered

unilaterally to each rat. The injection was maintained at a steady rate for 60 seconds to ensure high precision. Following the procedure, the needle was left in situ for an additional min to facilitate localized drug diffusion and preclude any potential reflux along the cannula track.

2.5. Assessment of inflammatory pain (formalin test)

This research utilized the formalin test [16], a robust and validated paradigm for persistent inflammatory pain to quantify nociceptive modulation. Nociceptive responses were elicited by a subcutaneous injection of 50 μ l of 2.5% formalin into the plantar surface of the rat's hind paw, localized contralateral to the DG microinjection site (the procedure is depicted in Figure 1B). Immediately after the formalin challenge, rats were placed in a transparent Plexiglas observation chamber (35 $\times$ 35 $\times$ 35 cm). To facilitate an unobstructed and comprehensive view of the animal's paw posture, a mirror was strategically positioned at a 45° angle behind the enclosure [17], as shown in Figure 1C. Nociceptive behaviors were monitored and quantified for 60 min post-formalin injection. Pain intensity was calculated in 5-min intervals. Specifically, behaviors were categorized into four distinct levels (Figure 1, Pain score):

Grade 0: The injected paw is placed firmly on the floor, and weight is distributed evenly.

Grade 1: The injected paw is rested lightly on the floor, or weight-bearing is significantly reduced.

Grade 2: The injected paw is completely elevated and does not contact any surface.

Grade 3: The rat intensely licks, bites, or shakes the injected paw.

The aggregate pain score for each 5-min block was derived using the following formula:

$$\text{Pain score} = \frac{(t_0 \times 0) + (t_1 \times 1) + (t_2 \times 2) + (t_3 \times 3)}{t_0 + t_1 + t_2 + t_3}$$

In this equation, t_0 , t_1 , t_2 , and t_3 denote the time (in seconds) the animal spent in each respective behavioral category during the 300-second block. The temporal evolution of the response, characterized by an early neurogenic phase (0–5 min) and a late inflammatory phase (20–50 min), is summarized in Figure 1C. As previously documented [8], these phases are separated by a distinct 15-min quiescent interphase, reflecting the transition from direct nociceptor activation to central sensitization and peripheral inflammation.

2.6. Experimental design

This study investigated the intricate functional interactions between D2R and μ -opioid receptors within the DG region and their modulation of inflammatory pain in male rats. A series of experiments was meticulously designed to elucidate these mechanisms, employing the formalin-induced pain model. The experimental protocols, including group allocations, dosage regimens, and drug administration sequences, are detailed in Table 1.

2.6.1. Formalin injection as a pain stimulus

The foundational experiment established a baseline for pain perception. Animals were allocated into two primary cohorts: A saline-treated control group and a formalin-treated control group. The objective was to quantify and contrast the behavioral manifestations of pain following formalin administration. To induce nociceptive behavior, 50 μ l of 2.5% formalin was administered subcutaneously into the plantar

surface of the rat's hind paw. The saline-control group received an equivalent volume of saline via the same route. This experimental setup allowed for a comparative analysis of the behavioral manifestations of acute and tonic inflammatory pain.

2.6.2. Antinociceptive potential of intra-DG μ -opioid receptor agonism

To ascertain the pain-relieving efficacy of the μ -opioid receptor within the DG, the second experimental phase involved unilateral microinjections of varying doses of morphine, a potent μ -opioid receptor agonist, directly into the DG. Rats ($n = 7-8$ per group) were assigned to five distinct treatment arms. These included four morphine dosage levels (5, 10, 25, and 50 mmol, delivered in 0.5 μ l) and a vehicle control group receiving 0.5 μ l of saline ($n = 7$). Following the intra-DG microinjections, all subjects underwent the formalin test, as described previously, to assess antinociception.

2.6.3. Modulation of pain perception by intra-DG μ -opioid receptor antagonism

The third experiment was designed to test the hypothesis that μ -opioid receptor antagonism in the DG significantly influences pain perception. Four experimental groups ($n = 7-8$ per group) were established. A vehicle control group received a microinjection of saline followed by an effective dose of morphine (50 mmol) into the DG. Concurrently, four treatment groups received escalating doses of naloxone (0.5, 1.5, 5, and 15 mmol), a selective μ -opioid receptor antagonist, administered prior to the intra-DG microinjection of the established effective dose of morphine. The formalin test was subsequently performed on all subjects to evaluate the impact of naloxone on morphine-induced analgesia.

2.6.4. Influence of intra-DG D2R agonism on pain responses

To investigate the role of D2R in pain modulation, the fourth experimental series examined the effects of activating these receptors within the DG. Five distinct groups of rats ($n = 7-8$ per group) underwent unilateral microinjections into the DG. Treatments consisted of four escalating doses of quinpirole (4, 8, 16, and 32 mmol, delivered in 0.5 μ l), a selective D2Rs agonist, or its vehicle (0.5 μ l DMSO, $n = 7$). Following these interventions, all animals received the standard formalin injection (50 μ l, 2.5%) to elicit pain, after which their behavioral responses were recorded.

2.6.5. Effect of intra-DG D2R antagonism on D2R agonist-induced antinociception

The fifth experiment aimed to confirm the specificity of D2R actions by assessing the impact of D2R antagonism on the antinociceptive effects of a D2R agonist. Four groups of rats ($n = 7-8$ per group) were studied. A vehicle control group received DMSO prior to the intra-DG administration of an effective dose of quinpirole (16 mmol). Three treatment groups received varying doses of sulpiride (3, 6, 12, and 24 mmol), a selective D2R antagonist, prior to the intra-DG injection of the same effective quinpirole dose. Subsequently, all rats underwent the formalin test to determine if D2R antagonism could attenuate D2R agonist-mediated antinociception.

2.6.6. Impact of D2R antagonism on morphine-induced analgesia

In the sixth experimental phase, the potential influence of D2R on the analgesic properties of the μ -opioid receptor was explored. Five groups of rats ($n = 7-8$ per group) were subjected to intra-DG microinjections. The vehicle control group received DMSO prior to an effective intra-DG dose of morphine (50 mmol). Four treatment groups received escalating doses of sulpiride (3, 6, 12, and 24 mmol) before the same effective

morphine dose. The formalin test was conducted to assess whether D2R antagonism alters the antinociceptive response to morphine.

2.6.7. Assessment of μ -opioid receptor blockade on D2R agonist-induced analgesia

The seventh and final experimental component investigated the interaction between the D2R and μ -opioid receptor regarding analgesia. Four groups of rats were utilized ($n = 6-7$ per group) to evaluate how blocking μ -opioid receptors in the DG affects the analgesia produced by intra-DG D2R agonism. The vehicle group received saline prior to the intra-DG injection of an effective dose of quinpirole (16 mmol). Four treatment groups received varying doses of naloxone (1.5, 5, 15, and 45 mmol) administered intra-DG before the effective quinpirole dose. A formalin test was performed to determine the interaction between these receptor systems.

2.6.8. Evaluation of locomotor activity

To control for potential confounding effects of the experimental manipulations on general motor function, a separate experiment assessed locomotor activity. Rats were placed individually in an open-field arena ($60 \times 60 \text{ cm}^2$) for 60 min. The open-field test is a widely accepted paradigm for evaluating exploratory behavior and general locomotion in rodents. The present study analyzed parameters of locomotor activity following administration of various agents: Intact controls, sham procedures, vehicle, morphine (50 mmol), quinpirole (32 mmol), sulpiride (24 mmol), and naloxone (45 mmol). All movements were recorded using a 3CCD video camera and subsequently analyzed with Ethovision software (Noldus Information Technology, the Netherlands; Version 7).

2.7. Histological verification of cannula placement

Following the completion of all behavioral experiments, rats were deeply anesthetized with ketamine and xylazine. Brains were carefully extracted and post-fixed in 4% formaldehyde. To verify the anatomical accuracy of the microinjection sites, the brains were sectioned coronally (50 μ m thickness) using a microtome. These sections were then meticulously examined and mapped against the Paxinos and Watson rat brain atlas [15]. Nine animals (4 in the D2R experiment and 5 in the D2R and μ -opioid receptor interaction experiment) were excluded from the final data analysis due to inaccurate cannula placement (indicated by green triangles in Figure 2).

2.8. Statistical analysis

Data were analyzed using GraphPad Prism (version 8.0) and expressed as the mean $\pm$ standard error of the mean (SEM). The assumptions of normality and homogeneity of variance were verified using the Kolmogorov–Smirnov test. Behavioral responses in the formalin test were evaluated via a two-way repeated-measures ANOVA, with ‘treatment’ and ‘time’ as factors, followed by Bonferroni’s post-hoc test for multiple comparisons. To provide a comprehensive assessment of total nociceptive response, the area under the curve (AUC) was calculated for both the early (0–5 min) and late (20–50 min) phases. These AUC datasets were subsequently compared across treatment groups utilizing a one-way ANOVA, followed by Dunnett’s multiple comparison test. Differences between means were considered statistically significant at a threshold of $P < 0.05$.

3. Results

3.1. Nociceptive response characterization following formalin administration

This study monitored behavioral responses following a 50 μ l intraplantar injection of 2.5% formalin into the rat's hind paw to evaluate the induction of inflammatory pain. A longitudinal analysis using repeated-measures two-way ANOVA demonstrated a robust divergence in pain profiles between the experimental groups. Specifically, significant main effects were observed for treatment [$F(1, 121) = 71.19, P < 0.0001$] and time [$F(11, 121) = 13.05, P < 0.0001$]. Furthermore, a significant treatment and time interaction effect [$F(11, 121) = 10.17, P < 0.0001$] confirmed that the nociceptive trajectory in the formalin-control group was distinctly different from the stable, low-level activity seen in the saline-control group (Figure 3A).

The temporal analysis revealed the classic biphasic pattern of the formalin test. Post-hoc comparisons indicated a sharp increase in pain scores immediately following injection, followed by a sustained late phase of hyperalgesia. This was further validated by quantifying the AUC for both phases. Unpaired *t*-test analysis revealed a substantial elevation in the mean AUC of the formalin group during the early phase (0–5 min) compared to saline-treated counterparts [$t(11) = 8.973, P < 0.0001$; Figure 3B]. Similarly, the late-phase (20–50 min) AUC, which reflects inflammatory processes and central sensitization, showed a marked increase in the formalin-control group [$t(11) = 10.47, P < 0.0001$; Figure 3C]. Collectively, these data confirm that the formalin model successfully induced a profound, statistically significant nociceptive state across both the acute and tonic phases of the assay.

3.2. Antinociceptive modulation via intra-DG morphine administration

Behavioral responses to varying morphine doses (5, 10, 25, and 50 mmol/0.5 μ l) were assessed to elucidate the modulatory influence of morphine within the DG on formalin-evoked pain. The findings revealed a clear, dose-dependent antinociceptive profile, as evidenced by a comprehensive one-way ANOVA followed by Dunnett's post hoc comparisons.

In the early phase of the formalin test, morphine administration significantly attenuated nociceptive behaviors compared to the saline-treated vehicle group [$F(4,37) = 15.36, P < 0.0001$]. Notably, this inhibitory effect was dose-responsive, achieving statistical significance at 25 ($P < 0.01$) and 50 ($P < 0.001$) mmol (Figure 4A). Similarly, during the late phase, traditionally associated with inflammatory hyperalgesia, morphine maintained its suppressive efficacy. Analysis of the late-phase responses confirmed a significant reduction in pain scores across the experimental cohorts [$F(4,37) = 10.87, P = 0.0006$], with significant relief observed at the 10 ($P < 0.05$), 25 ($P < 0.05$), and 50 ($P < 0.001$) mmol doses (Figure 4B). Collectively, these behavioral data underscore the potent role of DG-opioid signaling in effectively dampening both phases of formalin-induced nociception.

3.3. Pharmacological validation of μ -opioid receptor mediation

A pharmacological challenge using the opioid antagonist naloxone was conducted to verify that the observed antinociceptive effects were specifically mediated via μ -opioid receptors. Rats were pre-treated with varying doses of naloxone (0.5, 1.5, 5, and 15 mmol) 5 min prior to the administration of the pre-established effective dose of morphine (50 mmol).

A one-way ANOVA indicated that naloxone pretreatment dose-dependently reversed morphine-induced analgesia. In the early phase, administration of naloxone resulted in a significant restoration of pain behaviors, with the 5 mmol dose achieving statistical significance ($P < 0.01$) and the 15 mmol dose demonstrating a more robust reversal effect ($P < 0.001$) compared to the vehicle group [$F(4,35) = 6.728$, $P = 0.0005$; Figure 5A]. Consistent with these findings, the late phase also exhibited a significant blockade of morphine's analgesic efficacy. In this phase, both the 5 ($P < 0.01$) and 15 ($P < 0.01$) mmol doses of naloxone significantly attenuated the pain-suppressing action of morphine [$F(4,35) = 7.049$, $P = 0.0004$; Figure 5B]. Collectively, these results provide robust pharmacological evidence that morphine exerts its antinociceptive effects in the DG primarily via activating the μ -opioid receptor.

3.4. Antinociceptive profile of DG D2R activation

To investigate the contribution of D2R within DG to inflammatory pain modulation, the selective D2R agonist quinpirole was microinjected at doses of 4, 8, 16, and 32 mmol/0.5 μ l, while the vehicle control group received DMSO.

A one-way ANOVA demonstrated that intra-DG administration of quinpirole exerted a significant, phase-specific suppressive effect on formalin-induced pain behaviors. In the early phase of the nociceptive response, quinpirole treatment yielded a significant reduction in mean pain scores [$F(4,37) = 5.916$, $P = 0.0011$]. Post-hoc analysis showed that this antinociceptive effect was restricted to the higher dose range, where only the 16 and 32 mmol doses reached statistical significance ($P < 0.01$; Figure 6A).

In contrast, during the late phase, the therapeutic window of quinpirole expanded, demonstrating greater potency against inflammatory pain [$F(4,37) = 9.299$,

$P < 0.0001$]. In this phase, Dunnett's multiple comparison tests revealed that quinpirole significantly attenuated pain behaviors not only at the 16 and 32 mmol doses ($P < 0.01$) but also at the lower dose of 8 mmol ($P < 0.05$; Figure 6B). These observations reveal a robust, dose-dependent role for DG-localized D2R in regulating pain, with notably higher sensitivity during the late inflammatory phase of the formalin assay.

3.5. Impact of intra-DG sulpiride on quinpirole-induced antinociception

To evaluate the receptor-specificity of quinpirole-mediated pain modulation, the ability of the selective D2R antagonist, sulpiride, to attenuate the antinociceptive effects of quinpirole was investigated. Sulpiride (3, 6, 12, and 24 mmol) was administered into the DG 5 min prior to the previously established effective dose of quinpirole (16 mmol).

The obtained findings indicate that intra-DG D2R antagonism dose-dependently reversed the suppressive effect of quinpirole on formalin-evoked pain behavior. One-way ANOVA followed by Dunnett's post-hoc analysis confirmed that while the lowest dose of 3 mmol failed to elicit a significant response, higher doses of sulpiride (6, 12, and 24 mmol) effectively diminished the antinociceptive potency of quinpirole. This reversal effect was statistically robust in both the early phase [$F(4,39) = 13.23$, $P < 0.0001$; Figure 7A] and the late phase [$F(4,39) = 12.19$, $P < 0.0001$; Figure 7B]. These data provide compelling evidence that the pain-relieving action of quinpirole in the DG is specifically mediated by D2R activation.

3.6. Modulation of morphine-induced antinociception by intra-DG D2R antagonism

To elucidate the functional interaction between D2R and μ -opioid receptor within the DG, it was evaluated whether pharmacological blockade of D2R could

attenuate morphine-induced antinociception. Sulpiride (3, 6, 12, and 24 mmol) was microinjected into the DG 5 min prior to the administration of an effective dose of morphine (50 mmol).

Statistical analysis via one-way ANOVA followed by Dunnett's post-hoc tests indicated that sulpiride treatment interfered with morphine's suppressive effect on pain behaviors in a dose-dependent manner. In the early phase, this interference reached statistical significance only at the highest dose of 24 mmol [$F(4,36) = 4.558$, $P = 0.005$; Figure 8A]. Conversely, in the late phase, the antagonistic impact of sulpiride was more pronounced, with both 12 ($P < 0.05$) and 24 ($P < 0.01$) mmol doses significantly curtailing the antinociceptive effect of morphine [$F(4,36) = 5.257$, $P = 0.0023$; Figure 8B]. Collectively, these results suggest that D2R signaling within the DG acts as a crucial upstream or downstream component in the modulation of opioid-mediated antinociception, with a distinct temporal sensitivity to receptor blockade.

3.7. Interaction between DG D2R and μ -opioid receptor in pain modulation

The current study investigated whether the antinociceptive effect induced by quinpirole (16 mmol) in the DG depends on μ -opioid receptor activation to further characterize the underlying neurochemical circuitry. To this end, this research administered the non-selective opioid receptor antagonist, naloxone (1.5, 5, 15, and 45 mmol), 5 min prior to the injection of quinpirole.

The obtained results demonstrate that naloxone significantly attenuated quinpirole's analgesic action in a dose-dependent manner. As illustrated in the early phase, the reversal effect of naloxone was restricted to the higher doses of 15 ($P < 0.01$) and 45 ($P < 0.001$) mmol [$F(4,35) = 7.66$, $P = 0.0002$; Figure 9A]. In contrast, the

inhibitory effect of naloxone was more potent during the late phase, where administration of 5 ($P < 0.05$), 15 ($P < 0.01$), and 45 ($P < 0.01$) mmol doses all led to a significant reduction in quinpirole-induced antinociception [$F(4,35) = 5.96$, $P = 0.0011$; Figure 9B]. These data provide substantial evidence for a functional interaction between DG D2R and μ -opioid receptor, suggesting that the dopamine-mediated modulation of nociceptive processing may involve a downstream opioid mechanism.

3.8. Evaluation of locomotor activity

To exclude the possibility that motor deficits confounded the observed antinociceptive effects, this study assessed the locomotor activity of the animals following intra-DG microinjections. Locomotor performance was quantified as the total distance traveled during a 60-min open-field session.

This study compared several experimental groups, including intact, sham, vehicle, morphine (50 mmol), quinpirole (32 mmol), sulpiride (24 mmol), and naloxone (45 mmol). One-way ANOVA followed by Tukey's post-hoc test revealed no significant differences in the total distance traveled across all groups [$F(6,52) = 0.9818$, $P = 0.4485$; Figure 10]. These data confirm that intra-DG microinjection of the tested compounds at the specified doses did not alter the rats' spontaneous locomotor activity, ensuring that the analgesic findings reported in the previous sections reflect genuine antinociceptive modulation rather than changes in motor capacity.

4. Discussion

The present study systematically characterized the antinociceptive roles of D2R and μ -opioid receptors in the hippocampal DG and provides evidence for a functional

interaction between these receptors in modulating formalin-induced inflammatory pain in male Wistar rats. Four principal findings emerged from the data: Intra-DG morphine produced dose-dependent antinociception in both the early and late phases of the formalin test, an effect reversed by naloxone; intra-DG quinpirole attenuated formalin-evoked nociceptive behaviors in a dose-dependent manner, with greater sensitivity during the late inflammatory phase, an effect blocked by sulpiride; D2R antagonism with sulpiride reduced morphine-induced antinociception; and μ -opioid receptor blockade with naloxone attenuated quinpirole-induced antinociception. Collectively, these findings indicate that the DG is an essential site of dopaminergic-opioidergic convergence and suggest that D2R- and μ -opioid receptor-dependent processes are not completely independent.

To establish the physiological relevance of these receptor interactions, it was first essential to validate our experimental approach. The formalin test was selected as the primary behavioral assay because it elicits a well-characterized biphasic nociceptive response, allowing dissociation of peripheral neurogenic activation from centrally mediated inflammatory hyperalgesia within a single experimental session [16]. The early phase (0–5 min) reflects direct chemical activation of peripheral nociceptors [18], while the late phase (20–50 min) is driven by central sensitization and sustained peripheral inflammation [19]. This dual-phase structure is methodologically advantageous, as pharmacological manipulations that selectively attenuate the late phase are more likely to reflect actions on central, supraspinally mediated mechanisms rather than peripheral nociceptor activation [20]. The results of the present study confirmed the validity of this model: Animals receiving subcutaneous formalin exhibited a robust biphasic pain profile with statistical separation from saline-injected controls in both

phases, consistent with the paradigm's well-established sensitivity to detect supraspinal antinociceptive interventions.

Selecting the DG as the target region was grounded in its anatomical position as the principal input gateway of the hippocampal formation, through which the trisynaptic hippocampal circuit begins [3]. Accumulating preclinical evidence indicates that hippocampal subregions, including the DG, participate in the modulation of both acute and persistent pain-related behaviors [5]. Activating DG neurons in response to nociceptive stimuli has been evidenced by upregulating c-Fos expression in the DG following formalin injection, indicating that noxious inputs engage this subregion [21]. The DG receives convergent projections from pain-related limbic and cortical regions, and its intrinsic neuromodulatory systems, including dopaminergic terminals originating in the ventral tegmental area and endogenous opioidergic neurons, position it as an essential interface between the sensory and affective components of nociception [22]. The present pharmacological findings, demonstrating that direct manipulations of receptor systems within the DG reliably and reproducibly modify formalin-evoked pain behavior, are consistent with this anatomical framework and extend the evidence base for DG involvement from indirect stimulation paradigms to direct receptor-level investigation in a model of persistent inflammatory pain [23, 24].

Having validated the DG as a critical locus for nociceptive modulation, we first examined the contribution of its local opioidergic system. Intra-DG microinjection of morphine produced dose-dependent attenuation of formalin-evoked nociception, with significant effects at 25 and 50 mmol during the early phase and at 10, 25, and 50 mmol during the late phase. The broader efficacy of morphine in the late phase compared to the early phase is consistent with evidence that supraspinal opioid systems contribute

more substantially to the modulation of centrally sensitized, inflammation-driven pain than to the brief neurogenic activation that characterizes the early phase [17, 25]. The pharmacological specificity of these effects was confirmed by their dose-dependent reversal following naloxone pretreatment.

These observations align directly with recent data from Sotoudeh et al. [10], who established that intra-DG injections of D1R and μ -opioid receptor agonists increase acute pain thresholds in the tail-flick test and demonstrated a functional interaction between the D1R and μ -opioid receptor in the DG for acute nociception. The present study extends this opioidergic mechanism to persistent inflammatory pain, providing evidence that the μ -opioid receptor system within the DG is engaged not only during acute stimuli but also during the sustained, centrally mediated phase of the formalin test. Previous studies have similarly demonstrated that orexin receptors within the DG modulate inflammatory pain responses in the formalin model [24], suggesting that the DG harbors multiple, convergent neuromodulatory systems capable of attenuating persistent nociception.

In addition to the opioidergic network, the dopaminergic system within the hippocampus represents another major modulatory pathway. Evidence from hippocampal studies further supports the contribution of dopaminergic signaling to pain modulation. For example, pharmacological blockade of D1R and D2R in the CA1 region of the hippocampus significantly attenuated restraint-stress-induced analgesia in rats, indicating that hippocampal dopamine receptors participate in the regulation of nociceptive processing [26]. Within this broader framework, the robust antinociception produced by quinpirole in the present study, together with its reversal by sulpiride,

supports a functionally significant inhibitory role of D2R in the DG during inflammatory pain.

Our previous studies have established the involvement of dopaminergic receptors in pain modulation. Consistent with this notion, intra-DG administration of both D1R and D2R antagonists attenuated antinociception induced by chemical stimulation of the lateral hypothalamus during persistent inflammatory pain in male Wistar rats, indicating that dopaminergic circuits within the DG contribute to descending pain modulatory pathways [2]. Likewise, intra-DG blockade of both D1R and D2R suppressed antinociceptive responses elicited by lateral hypothalamic stimulation in an orofacial pain model, further indicating the involvement of DG dopaminergic receptors in pain modulation [21]. The current data extend these findings from indirect pharmacological stimulation paradigms to direct agonist activation within the DG, providing cleaner pharmacological evidence that DG D2Rs are intrinsically capable of generating antinociception when activated by an exogenous selective agonist.

Intra-DG quinpirole produced antinociception that was dose-dependent and phase-selective, with efficacy extending to the 8 mmol dose during the late inflammatory phase but limited to higher doses (16 and 32 mmol) during the early neurogenic phase. This phase selectivity mirrors observations from dopaminergic pharmacology in adjacent hippocampal subregions: In the CA1 region, quinpirole-induced analgesic responses were attenuated by sulpiride and blocked by naloxone, indicating that D2R-mediated analgesia in the CA1 also exhibits preferential sensitivity during the inflammatory phase of persistent pain [9]. The quantitative differences in threshold doses between the DG and CA1 likely reflect differences in regional D2R density, local circuit architecture, and afferent input patterns, underscoring the need to

examine each hippocampal subregion independently rather than to extrapolate findings across the structure.

The sulpiride-induced reversal of quinpirole-mediated antinociception confirmed receptor specificity and excluded non-specific actions of quinpirole at the doses employed. A similar pharmacological profile has been reported in the NAc, where bilateral quinpirole microinjection dose-dependently suppressed the late phase of formalin-induced nociception; the selective D2R antagonist raclopride blocked this effect, whereas the D1R agonist SKF38393 had no effect, suggesting a preferential role for D2R in limbic antinociception during persistent inflammation [27]. The present findings demonstrate that a parallel relationship exists within the DG, extending this principle to the hippocampal formation. Importantly, the preferential sensitivity of the late inflammatory phase to D2R-mediated modulation in the DG may reflect upregulation of D2R coupling efficiency or enhanced sensitivity of inhibitory circuits under inflammatory conditions, analogous to the well-characterized upregulation of supraspinal μ -opioid receptor sensitivity during sustained inflammation [5].

The core conceptual contribution of the present study, however, lies in demonstrating that these two receptor systems do not operate in isolation but rather participate in a bidirectional functional interaction. Sulpiride pretreatment dose-dependently attenuated morphine-induced antinociception, with significant effects at 12 and 24 mmol during the late phase. Conversely, naloxone pretreatment dose-dependently reduced quinpirole-induced antinociception, with significant effects observed from 5 mmol during the late phase and from 15 mmol during the early phase. This reciprocal pharmacological profile strongly argues that D2R and μ -opioid receptor

are functionally linked within DG nociceptive circuits, rather than operating in parallel or independently.

Similar evidence for such cross-talk in adjacent hippocampal territories further contextualizes these findings. In the CA1 region, opioidergic and dopaminergic (D2R) mechanisms have been shown to interact synergistically during inflammatory pain, where quinpirole-induced antinociception was blocked by sulpiride and by naloxone, and sulpiride attenuated morphine-evoked analgesia [9]—a pharmacological profile closely paralleling the DG interactions observed in the present study. However, an essential distinction should be noted. In a previous CA1 study on orofacial pain, sulpiride reversed morphine-induced analgesia only during the early phase, and the effect did not reach statistical significance [12]. In contrast, in the present DG study, sulpiride significantly attenuated the late-phase antinociception produced by morphine at 12 and 24 mmol. This divergence suggests that D2R–opioid receptor coupling may be more robust in the DG during persistent hind-paw inflammatory pain, potentially reflecting the greater engagement of supraspinal inhibitory neuromodulatory circuits during the late inflammatory phase compared with the early neurogenic phase.

A behaviorally interpretable mechanistic possibility is that D2R activation in the DG recruits an endogenous opioid-dependent modulatory process. In this framework, locally released opioid peptides may act on the μ -opioid receptor to alter granule cell excitability and reshape DG output, thereby reducing nociceptive transmission through hippocampal–limbic circuits [28, 29]. This interpretation is consistent with naloxone’s partial blockade of quinpirole-induced antinociception in the absence of exogenous opioid administration. Alternatively, D2R and μ -opioid receptor may share downstream Gi/o-linked effector pathways, such that activation of either receptor converges on a

common inhibitory signaling cascade. D2R signaling via Gi/o can reduce adenylyl cyclase activity and intracellular cyclic adenosine monophosphate (cAMP), which may interact with μ -opioid receptor-mediated Gi/o signaling to modulate DG network excitability [6, 30, 31]. Overall, the behavioral data support a functional interaction between dopaminergic and opioidergic systems within the DG.

Importantly, when interpreting these sensory effects, one must rule out the possibility of non-specific behavioral confounds. Pharmacological pain studies always risk the interpretation that observed changes in nociceptive behavior reflect altered locomotion or sedation rather than genuine antinociception. The open-field test conducted in the present study confirmed that none of the agents employed—morphine (50 mmol), quinpirole (32 mmol), sulpiride (24 mmol), or naloxone (45 mmol)—significantly altered spontaneous locomotion compared to intact, sham, or vehicle controls. This absence of motor effects is consistent with prior findings from the NAc D2R– μ -opioid interaction study, in which intra-accumbal quinpirole and morphine at analgesically effective doses did not impair rotarod performance [32], supporting the interpretation that the observed pain-score reductions represent genuine antinociceptive modulation rather than global behavioral suppression.

While these findings provide robust evidence of localized antinociceptive interactions, certain methodological parameters warrant discussion. To maintain consistency with previously reported methodologies [10, 26] concerning hippocampal pain modulation and to avoid the confounding effects of hormonal variability associated with the estrous cycle, male Wistar rats were utilized throughout the study. Sex and genotype interact to determine analgesic responsiveness in rodents, and hormonal fluctuations across the estrous cycle can modulate nociceptive thresholds [33, 34]—a

source of variance controlled for by restricting the study to male animals. The predominant use of male rodents in preclinical pain research has historically reflected the challenge of controlling for estrous cycle effects, though this restriction imposes recognized limitations on generalizability [14, 35]. The primary scientific objective of the present study—to determine whether D2R– μ -opioid receptor interactions modulate persistent inflammatory pain within the DG—was most efficiently addressed under conditions of minimal hormonal variability. Future studies that systematically incorporate female animals and account for the estrous cycle phase will be essential to determine whether the DG D2R– μ -opioid receptor interaction is conserved across sexes [36].

The behavioral approach adopted here was deliberately restricted to quantitative pain-behavior scoring using a validated graded scale, consistent with the translational objective of characterizing receptor-level analgesic interactions at a defined hippocampal site. Future studies incorporating *in vivo* microdialysis to detect endogenous opioid peptide release in response to quinpirole administration within the DG would directly test the hypothesis that D2R activation mobilizes the endogenous opioid system at this site [37].

In conclusion, the present study demonstrates a bidirectional functional interaction between D2R and the μ -opioid receptor in the hippocampal DG, modulating persistent inflammatory pain in male rats. Both receptor systems independently produced dose-dependent antinociception, particularly during the late inflammatory phase of the formalin test, and blockade of either receptor attenuated the analgesic efficacy of the other. These findings establish the DG as a site of dopaminergic–opioidergic convergence in supraspinal pain control, complementing and extending

evidence from the CA1 subregion and the NAc. By delineating this interaction at the level of a defined hippocampal subregion using a behaviorally validated inflammatory pain model, the present study advances a mechanistic understanding of limbic involvement in persistent nociception, identifying the DG D2R– μ -opioid receptor axis as a potential target for future development of localized analgesic strategies capable of circumventing the systemic adverse effects of conventional opioid pharmacotherapy.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Figure Legends

Figure 1. Schematic representation of the experimental design and behavioral

assessment. (A) Intra-dentate gyrus (DG) microinjection protocol.

Pharmacological agents—specifically agonists and antagonists for μ -opioid receptor (morphine/naloxone) and D2-like dopamine receptor (D2R;

quinpirole/sulpiride)—were administered via microinjection into the DG. **(B)**

Formalin-induced inflammatory pain model. Inflammatory pain was induced by

the subcutaneous injection of 50 μ l of 2.5% formalin into the plantar surface of

the rat's hind paw. **(C)** Formalin test apparatus and scoring system. Behavioral

observations were conducted in a 35 $\times$ 35 $\times$ 35 cm Plexiglas chamber equipped

with a 45° angled mirror for comprehensive pain observation. The temporal

phases of nociceptive response are categorized as the early phase (0–5 min),

interphase (5–20 min), and late phase (20–50 min). The pain intensity for each

5-min epoch was quantified using a 4-point scale: 0, normal weight-bearing; 1,

light resting of the paw; 2, elevation of the paw; and 3, intense licking, biting, or

shaking of the injected paw.

Figure 2. Schematic representations of coronal brain sections illustrate the

microinjection sites within the DG. (A) D2-like dopamine receptor

experiments: The colored symbols indicate the targeted microinjection sites for

the vehicle group (white circles; n=7), the quinpirole-treated group (blue circles;

n=31), and the sulpiride + quinpirole-treated group (red circles; n=32). **(B)** D2-

like dopamine and μ -opioid receptor interaction experiments: Injection sites are depicted for the vehicle group (white circles; n=8), the sulpiride + morphine-treated group (blue circles; n=30), and the naloxone + quinpirole-treated group (red circles; n=28). Cannula placements located outside the defined boundaries of the DG were identified as misplacements (green triangles; n=9 total) and were systematically excluded from the final behavioral data analysis. Scale bar: 1 mm.

Figure 3. Validation of the formalin-induced inflammatory pain model and longitudinal characterization of nociceptive behavior. (**A**) Time-course analysis of pain scores over the 60-min observation period. The formalin-control group (solid circles) exhibits a characteristic biphasic nociceptive response compared to the stable baseline observed in the saline-control group (open circles). The temporal divergence was confirmed by a repeated-measures two-way ANOVA, followed by Bonferroni's post hoc test. (**B, C**) Total pain intensity quantified as the area under the curve (AUC) for the (**B**) early phase (0–5 min) and (**C**) late phase (20–50 min) of the formalin test. Formalin injection resulted in a significant elevation of AUC values in both phases compared to saline-treated controls (unpaired *t*-test; $P < 0.0001$). Data are presented as mean $\pm$ SEM (n=6-7 rats per group). Significant differences relative to the saline-control group are denoted by: * $P < 0.05$ and *** $P < 0.001$.

Figure 4. Dose-dependent antinociceptive effects of intra-DG morphine

administration on formalin-evoked pain. Microinjection of morphine (5, 10, 25, and 50 mmol/0.5 μ l) into the DG significantly attenuated pain scores in a dose-dependent manner across both phases of the formalin test. **(A)** During the early phase, one-way ANOVA followed by post hoc Dunnett's analysis confirming significant antinociception at 25 ($P < 0.01$) and 50 ($P < 0.001$) mmol doses. **(B)** In the late phase, morphine demonstrated sustained suppressive efficacy, with significant reductions in pain scores observed at 10 ($P < 0.05$), 25 ($P < 0.05$), and 50 ($P < 0.001$) mmol. Data are expressed as mean $\pm$ SEM (n=7-8 rats per group). Statistical significance relative to the vehicle (saline) group is denoted by: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

Figure 5. Pharmacological antagonism of morphine-induced antinociception by

intra-DG naloxone administration. Pre-treatment with the selective μ -opioid receptor antagonist naloxone (0.5, 1.5, 5, and 15 mmol) dose-dependently reversed the morphine (50 mmol)-induced suppression of pain behaviors. **(A)** In the early phase, one-way ANOVA followed by post hoc Dunnett's analysis confirming significant reversal at 5 ($P < 0.01$) and 15 ($P < 0.001$) mmol doses. **(B)** Similarly, during the late phase, naloxone administration effectively antagonized morphine's antinociceptive profile, with statistical significance reached at 5 ($P < 0.01$) and 15 ($P < 0.01$) mmol. Data are presented as mean $\pm$ SEM (n=7-8 rats per group). Significant differences compared to the vehicle (saline) + morphine (50 mmol) group are indicated by: ** $P < 0.01$ and *** $P < 0.001$.

Figure 6. Dose-dependent antinociceptive effects of intra-DG quinpirole on formalin-induced pain behaviors. Unilateral microinjection of the selective D2R agonist quinpirole (4, 8, 16, and 32 mmol/0.5 μ l) into the DG significantly reduced formalin-evoked nociceptive scores in a dose-dependent manner. The vehicle group received 0.5 μ l DMSO. **(A)** In the early phase (0–5 min), one-way ANOVA followed by Dunnett’s post hoc test showed significant antinociception at the 16 (** $P < 0.01$) and 32 (** $P < 0.01$) mmol doses. **(B)** In the late phase (20–50 min), quinpirole produced a stronger suppressive effect on pain behavior, with significant reductions at 8 mmol (* $P < 0.05$), 16 mmol (** $P < 0.01$), and 32 mmol (** $P < 0.01$) compared with vehicle (DMSO). Data are presented as mean $\pm$ SEM (n=7-8 rats per group).

Figure 7. Pharmacological blockade of quinpirole-induced antinociception by intra-DG sulpiride administration. Pre-treatment with the selective D2R antagonist sulpiride (3, 6, 12, and 24 mmol) dose-dependently reversed the antinociceptive effect of the effective dose of quinpirole (16 mmol). The control group received vehicle (DMSO) prior to quinpirole administration. **(A)** In the early phase, one-way ANOVA showed a significant reversal of quinpirole’s suppressive effect, with significant antagonism observed at 6 ($P < 0.01$), 12 ($P < 0.001$), and 24 ($P < 0.01$) mmol doses. **(B)** Similarly, in the late phase, sulpiride administration significantly attenuated quinpirole’s antinociceptive efficacy, with statistically significant reversal noted at 6 ($P < 0.05$), 12 ($P < 0.001$), and 24 ($P < 0.001$) mmol doses. Data are expressed as mean $\pm$ SEM (n=7-8 rats per

group). Significant differences relative to the vehicle (DMSO) + quinpirole (16 mmol) group are denoted by: $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

Figure 8. Functional interaction between D2R and μ -opioid receptor in DG-

mediated antinociception. To evaluate the potential interaction between D2R and μ -opioid receptor, the D2R antagonist sulpiride (3, 6, 12, and 24 mmol) was administered prior to an effective dose of morphine (50 mmol). **(A)** In the early phase, one-way ANOVA indicated that D2R blockade significantly interfered with morphine-induced analgesia, with the reversal effect reaching statistical significance at the 24 mmol ($P < 0.01$) dose. **(B)** In the late phase, sulpiride demonstrated a more pronounced antagonistic impact on morphine's efficacy, with significant attenuation observed at both 12 ($P < 0.05$) and 24 ($P < 0.01$) mmol doses. Data are presented as mean $\pm$ SEM (n=7-8 rats per group). Statistical significance relative to the vehicle (DMSO) + morphine (50 mmol) group is indicated by: $*P < 0.05$ and $**P < 0.01$

Figure 9. Pharmacological antagonism of quinpirole-induced antinociception by

intra-DG naloxone. To investigate whether the antinociceptive effect of D2R activation depends on the activation of μ -opioid receptors within the DG, the non-selective μ -opioid receptor antagonist naloxone (1.5, 5, 15, and 45 mmol) was microinjected 5 min prior to an effective dose of the D2R agonist quinpirole (16 mmol). **(A)** In the early phase, one-way ANOVA revealed a dose-dependent reversal of quinpirole's analgesic effect, with significant attenuation observed at

15 ($P < 0.01$) and 45 ($P < 0.001$) mmol doses. **(B)** During the late phase, naloxone administration exerted a more pronounced inhibitory effect, significantly counteracting quinpirole-induced antinociception at doses of 5 ($P < 0.05$), 15 ($P < 0.01$), and 45 ($P < 0.01$) mmol. Data are expressed as mean $\pm$ SEM (n=6-8 rats per group). Significant differences relative to the vehicle (saline) + quinpirole (16 mmol) group are denoted by: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

Figure 10. Assessment of locomotor activity following intra-DG microinjections.

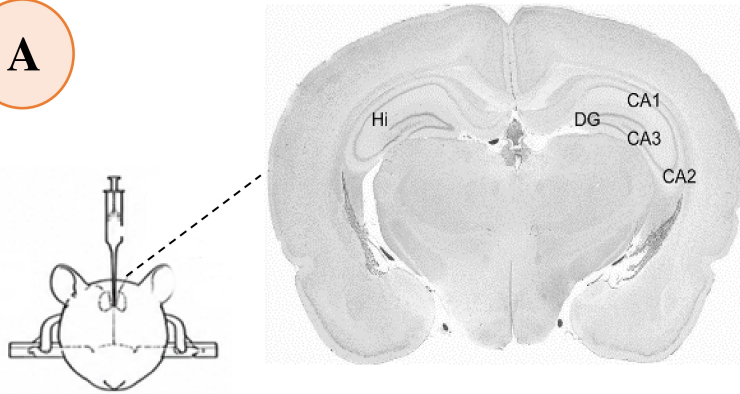
To verify that the antinociceptive effects observed in the formalin test were not confounded by motor impairments, general locomotor activity was quantified in an open-field arena over a 60-min session. The groups included intact, sham, vehicle, morphine (50 mmol), quinpirole (32 mmol), sulpiride (24 mmol), and naloxone (45 mmol). One-way ANOVA revealed no significant differences in the total distance traveled across all experimental groups [$F(6,52)=0.9818$, $P=0.4485$], confirming that intra-DG microinjections of these agents at the specified doses did not alter spontaneous locomotor performance. Data are presented as mean $\pm$ SEM (n=7-8 rats per group).

Intra-DG microinjection of

μ -opioid receptors

Pain score

A



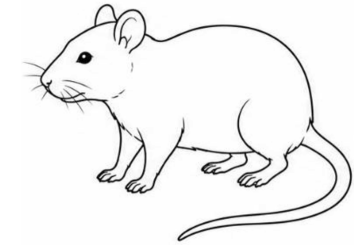
Agonist: Morphine

Antagonist: Naloxone

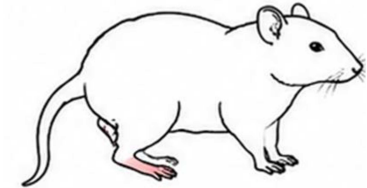
D2-like dopamine receptors

Agonist: Quinpirole

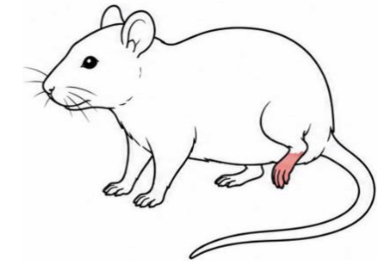
Antagonist: Sulpiride



0



1



2



3

Subcutaneous administration of 50 μ l formalin (2.5%)

B



Time (min):

0' 5' 10' 15' 20' 25' 30' 35' 40' 45' 50' 55' 60'

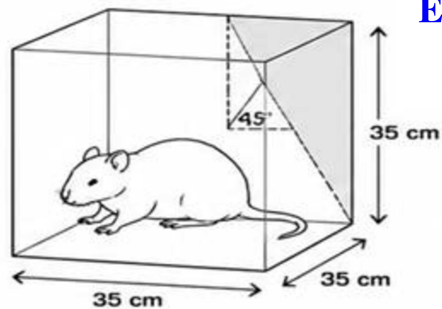
C

Formalin test

Early Phase
(0-5 min)

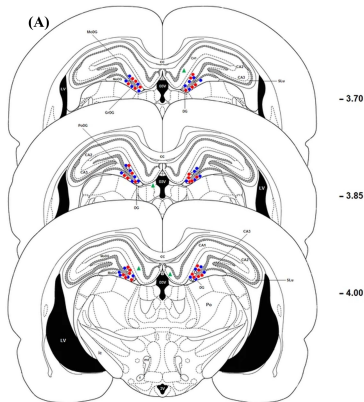
Interphase
(5-20 min)

Late Phase
(20-50 min)



D2-like dopamine receptors experiment

(A)

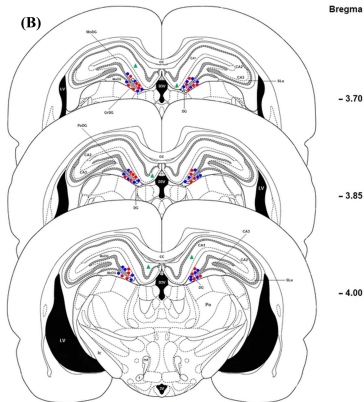


- Vehicle (DMSO) n=7
- Treatment (Quinpirole) n=31
- Treatment (Sulpiride+ Quinpirole) n=32
- ▲ Misplacement (n=4)

1 mm

D2-like dopamine and μ -opioid receptors interaction experiment

(B)



- Vehicle (DMSO) n=8
- Treatment (Sulpiride+ Morphine) n=30
- Treatment (Naloxone+ Quinpirole) n=28
- ▲ Misplacement (n=5)

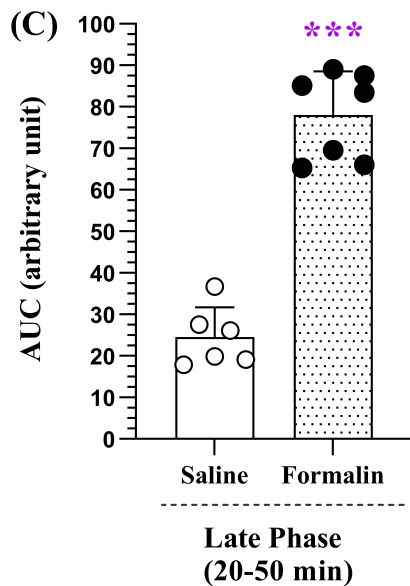
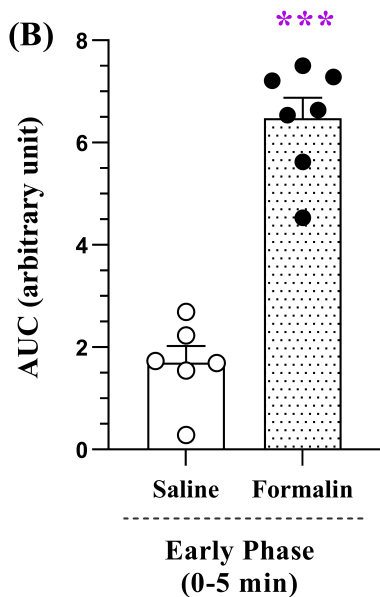
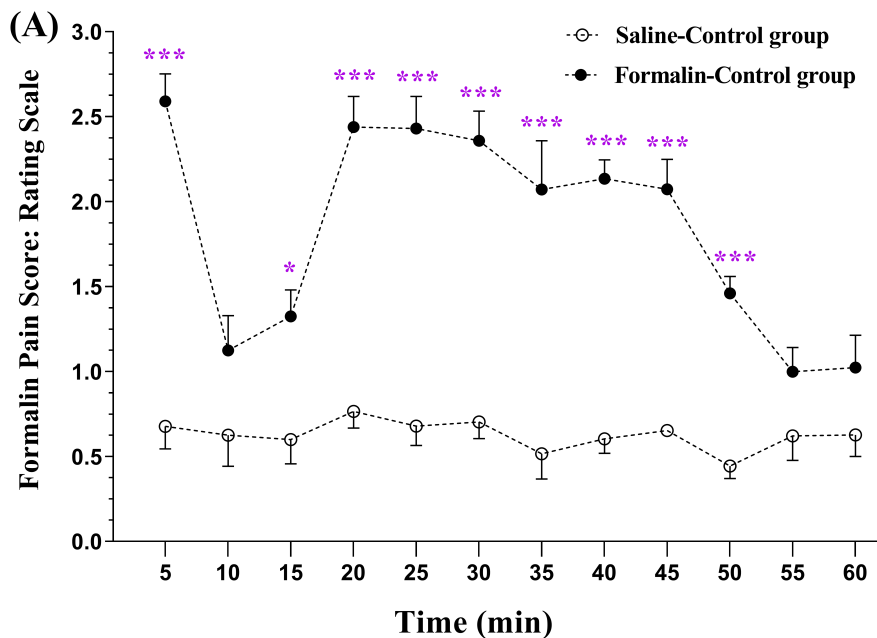
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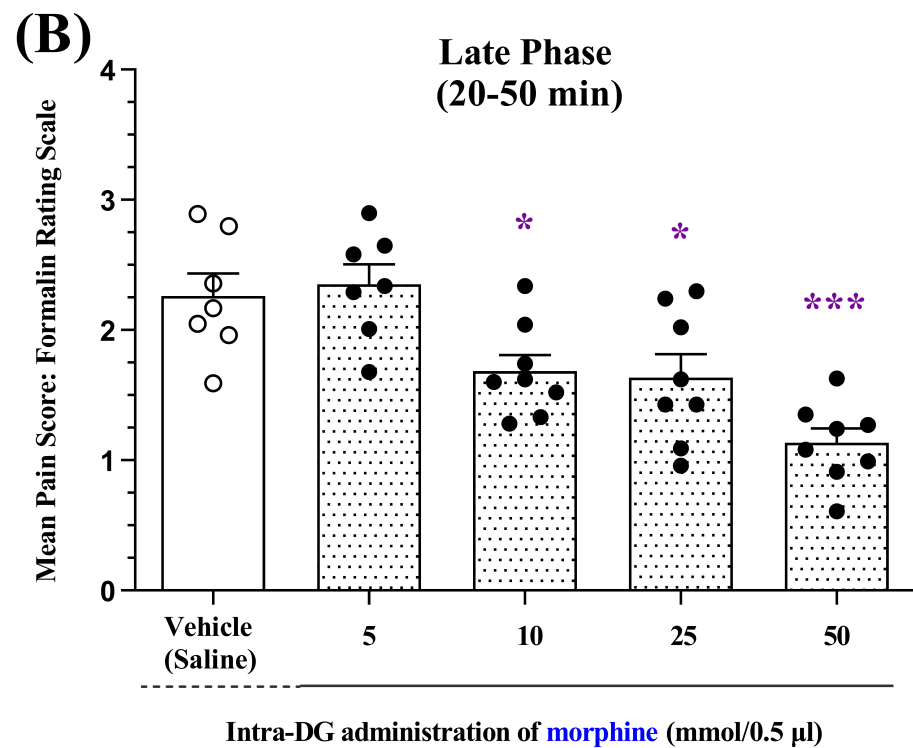
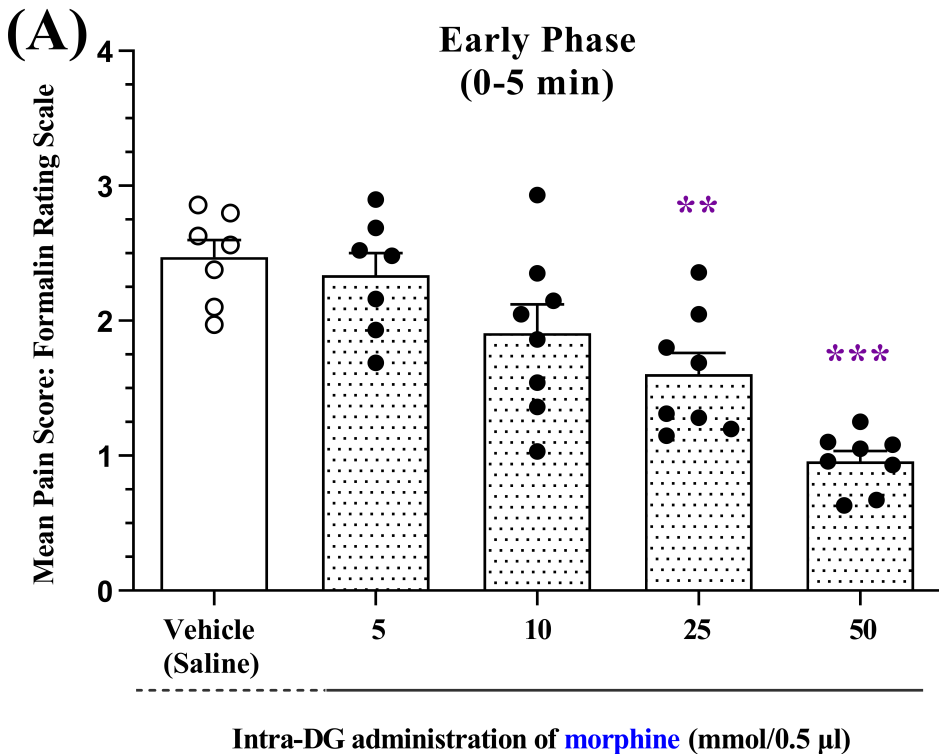
Bregma

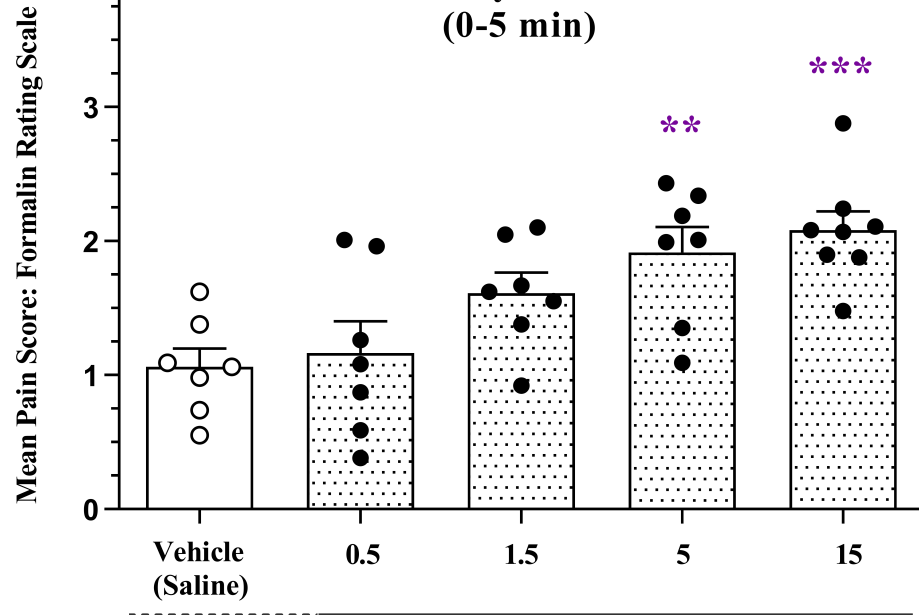
- 3.70

- 3.85

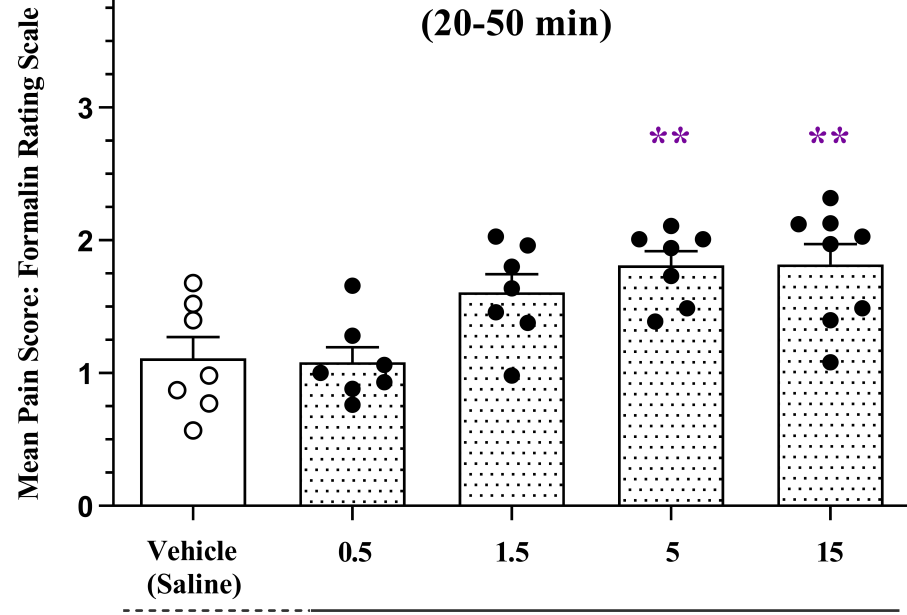
- 4.00



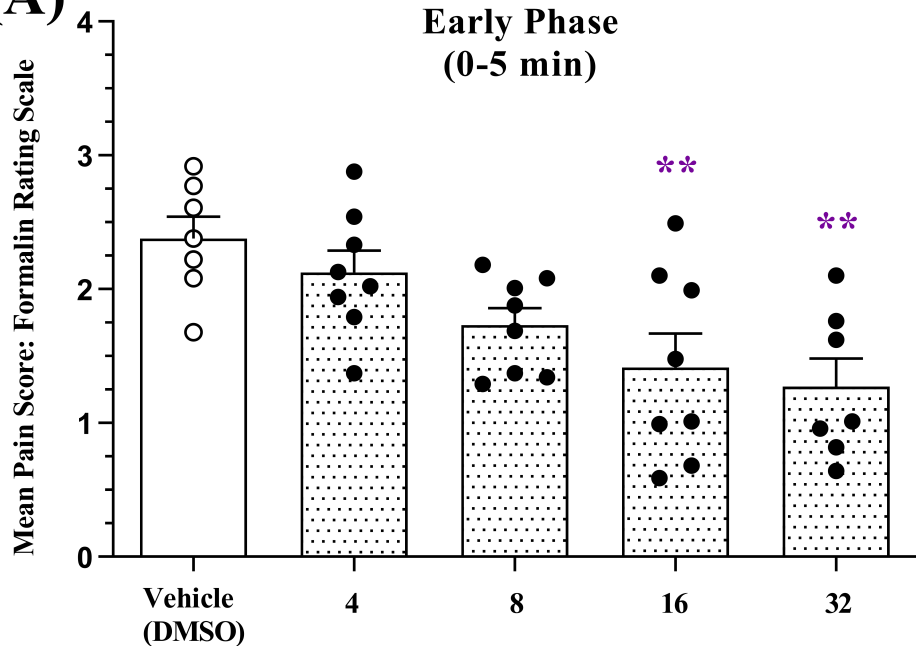


(A)

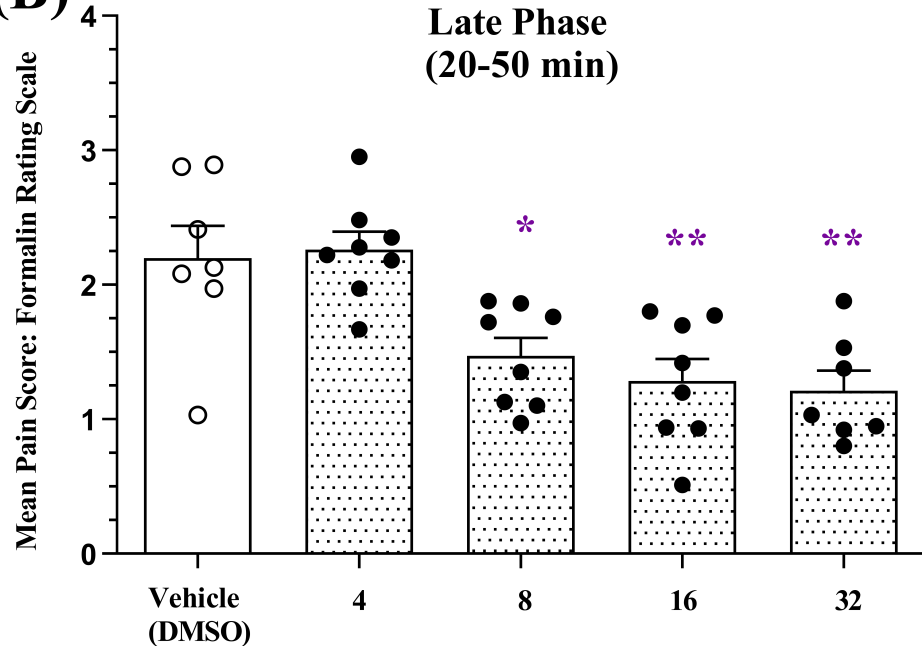
Intra-DG administration of **naloxone** (mmol)
+ **morphine** (50 mmol)/0.5 µl

(B)

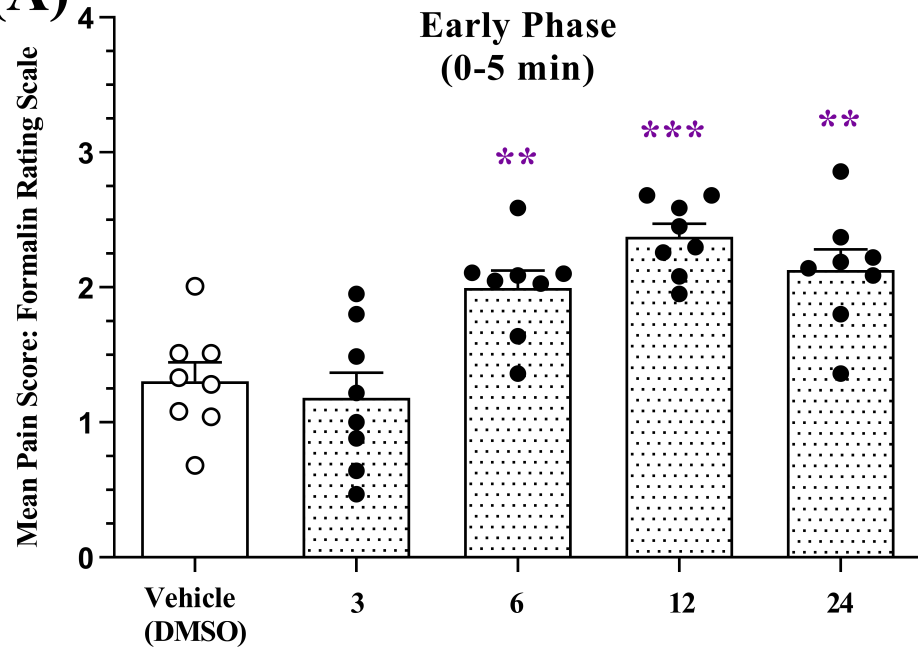
Intra-DG administration of **naloxone** (mmol)
+ **morphine** (50 mmol)/0.5 µl

(A)

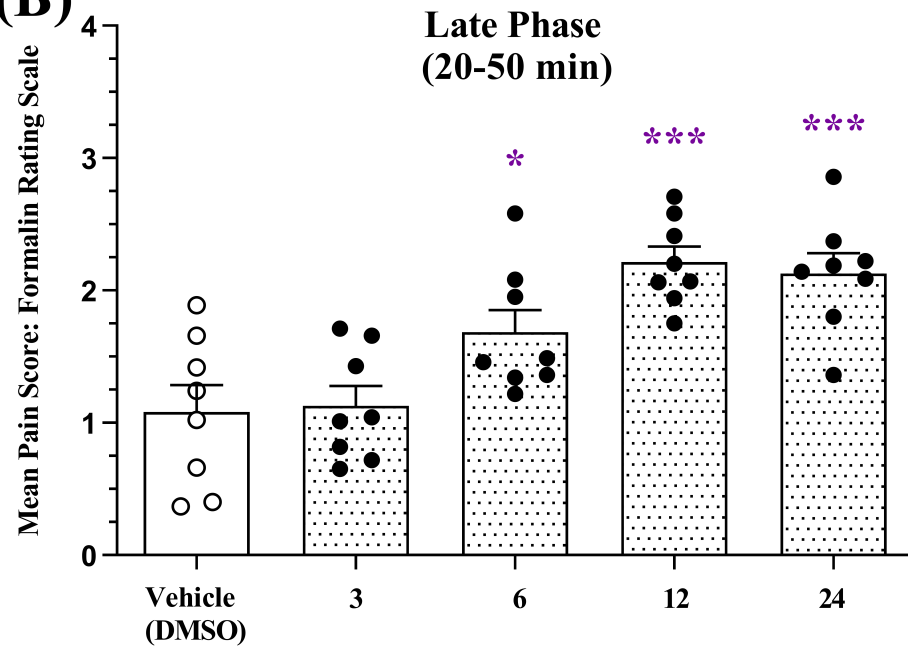
Intra-DG administration of **quinirole** (mmol/0.5 µl)

(B)

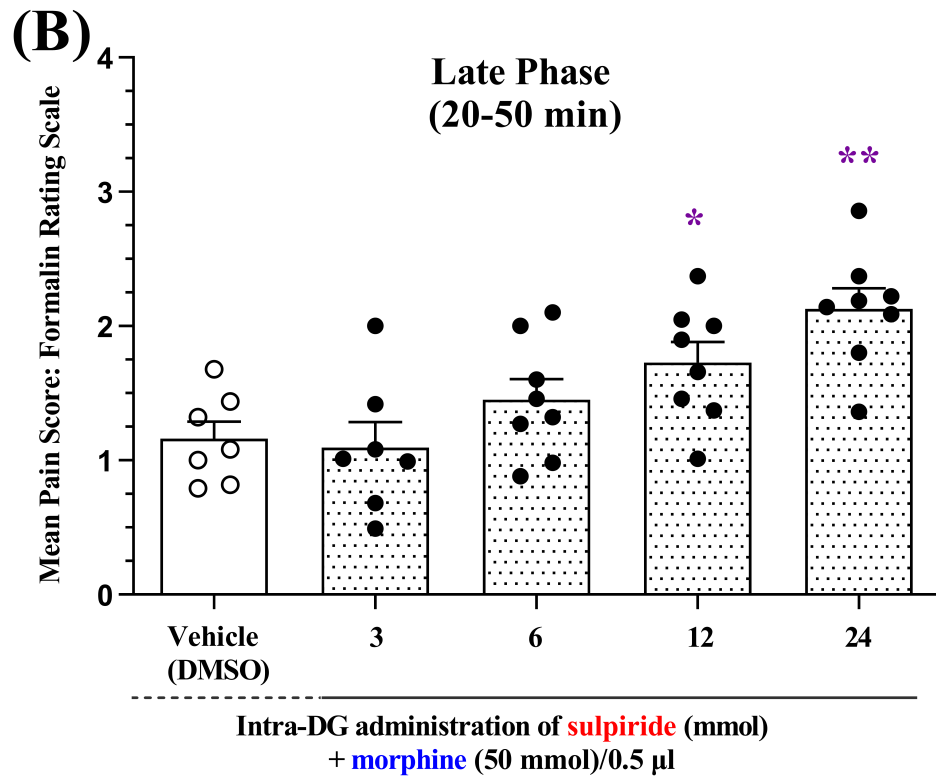
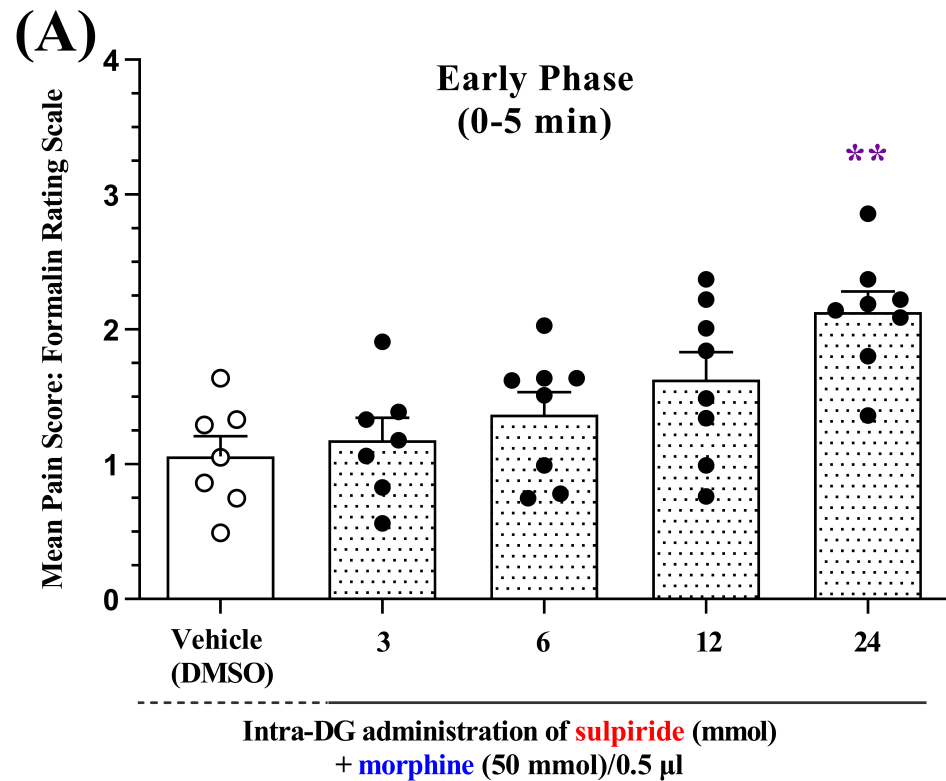
Intra-DG administration of **quinirole** (mmol/0.5 µl)

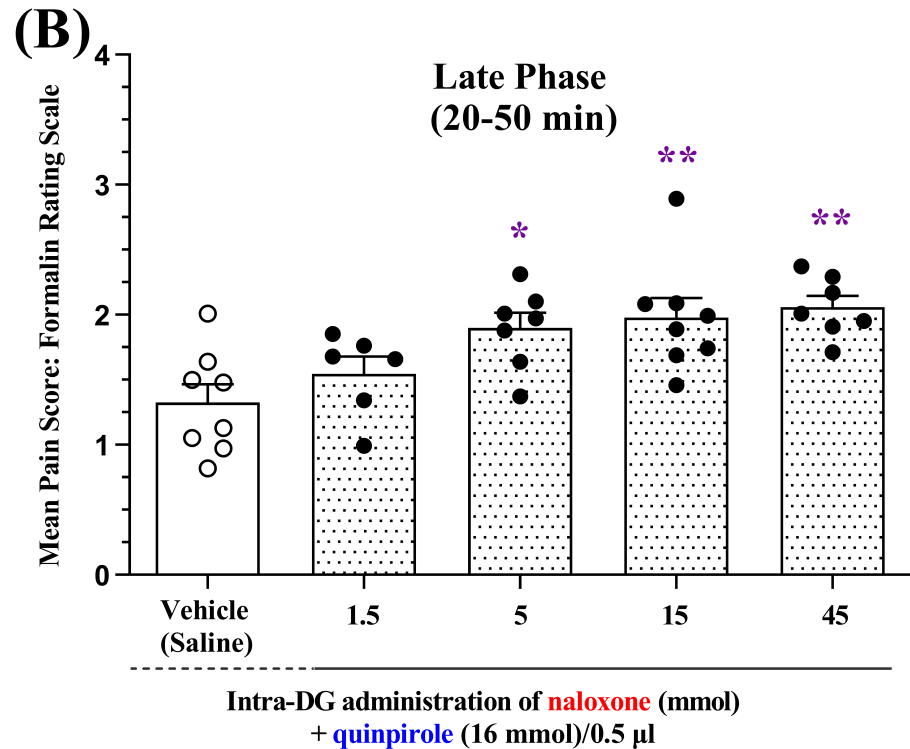
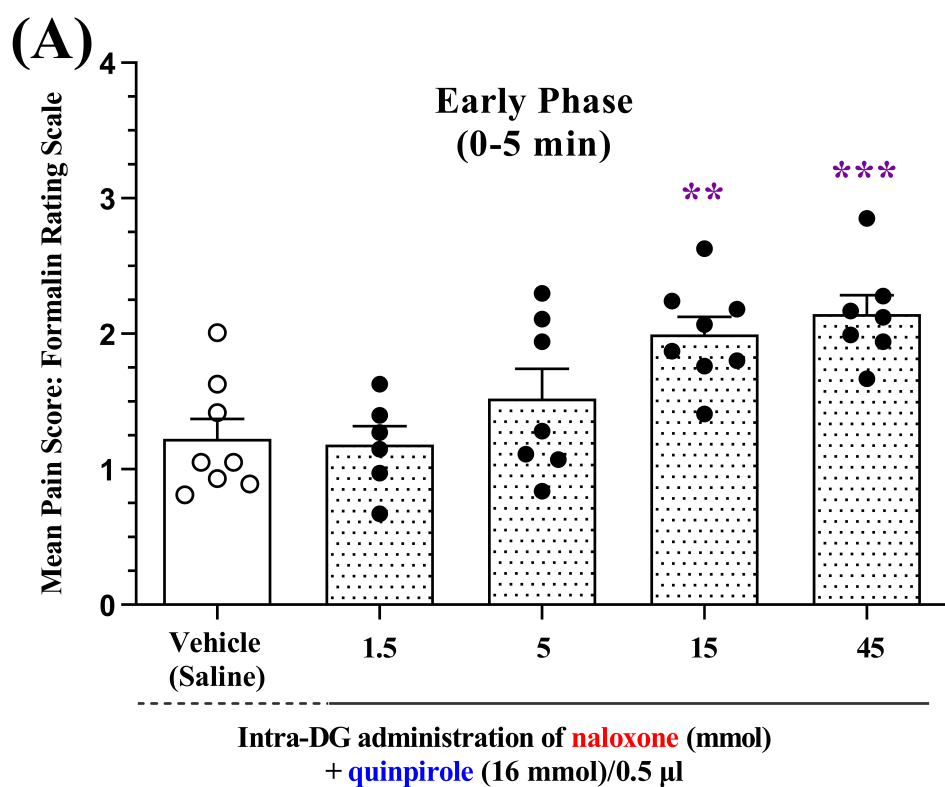
(A)

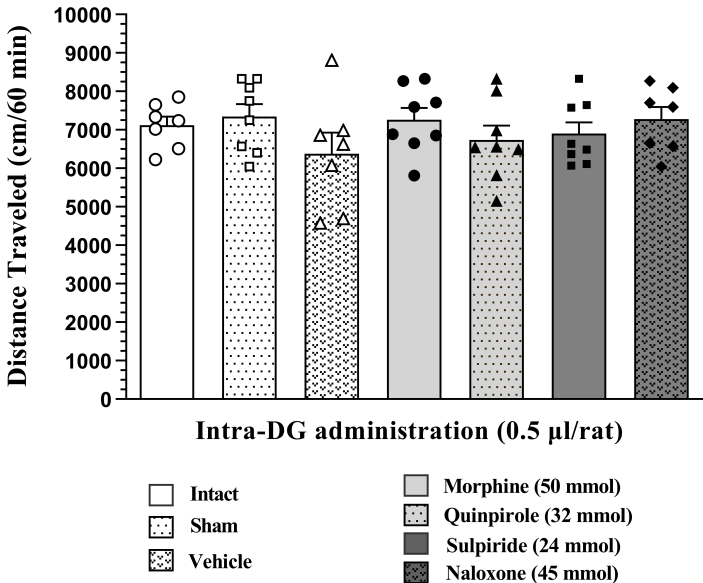
Intra-DG administration of **sulpiride** (mmol)
+ **quinpirole** (16 mmol)/0.5 μ l

(B)

Intra-DG administration of **sulpiride** (mmol)
+ **quinpirole** (16 mmol)/0.5 μ l







Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table1.docx](#)