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REGULAR RESEARCH ARTICLE

The D2-like Dopamine Receptor Agonist Quinpirole Microinjected Into the Ventral Pallidum Dose-Dependently Inhibits the VTA and Induces Place Aversion

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Abstract

Background: The ventral pallidum (VP) is a dopaminoceptive forebrain structure regulating the ventral tegmental area (VTA) dopaminergic population activity. We have recently demonstrated that in the VP, the D2-like dopamine (DA) receptor agonist quinpirole dose dependently facilitates memory consolidation in inhibitory avoidance and spatial learning. According to our hypothesis, quinpirole microinjected into the VP can modulate the VTA DAergic activity and influence motivation and learning processes of rats.
Methods: Quinpirole was microinjected at 3 different doses into the VP of male rats, and controls received vehicle. Single unit recordings were employed to assess VTA DAergic activity. To investigate the possible reinforcing or aversive effect of quinpirole in the VP, the conditioned place preference paradigm was used.
Results: Our results showed that intra-VP quinpirole microinjection regulates VTA DAergic neurons according to an inverted U-shaped dose-response curve. The largest dose of quinpirole decreased the population activity and strongly reduced burst activity of the DAergic neurons in the first hour after its application. In contrast, the 2 smaller doses increased DA population activity, but their effect started with a delay 1 hour after their microinjection. The CPP experiments revealed that the largest dose of quinpirole in the VP induced place aversion in the rats. Furthermore, the largest dose of quinpirole induced an acute locomotor activity reduction, while the medium dose led to a long-duration increase in locomotion.
Conclusions: In summary, quinpirole dose dependently regulates VTA DAergic activity as well as the motivation and motor behavior of the rats at the level of the VP.

Keywords: Ventral pallidum, ventral tegmental area, D2 dopamine receptors, quinpirole, place aversion

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Significance Statement

The ventral tegmental area (VTA) plays an important role in motivational and learning processes. The ventral pallidum (VP) is an important structure in the regulation of the VTA dopaminergic activity. We show that the D2-like dopamine (DA) receptor agonist quinpirole microinjected into the VP dose dependently modulates the VTA DAergic activity. The largest dose of quinpirole decreased the population activity and strongly reduced burst activity in the first hour after its application. Furthermore, in harmony with these electrophysiological results, the largest dose of quinpirole induced place aversion in the experimental rats. Our results highlight the outstanding role of the VP D2 DA receptors in the remote feedback regulation of the VTA DAergic neuronal activity as well as in the control of the behavior.

Introduction

The mesencephalic dopaminergic (DA) ventral tegmental area (VTA) innervates numerous cortical and subcortical limbic structures, including the prefrontal cortex, hippocampus, nucleus accumbens (NAC), basolateral amygdala (BLA) and central amygdala, and the ventral pallidum (VP) (Oades and Halliday, 1987; Klitenick et al., 1992). D2-like dopamine receptors (D_2Rs) are 1 of the 2 subgroups of DA receptors and can be localized throughout the limbic system (Ariano et al., 1993). The D_2R subgroup (expressed as D2-like receptors) consists of D2, D3, and D4 DA receptors (Missale et al., 1998). It is well known that the D_2R-like agonist quinpirole mainly affects D2 and D3 DA receptors.

Several pieces of evidence support the role of DA and its D_2Rs in synaptic plasticity and memory consolidation (Jay, 2003). In addition, quinpirole microinjected into the NAC shell region induces place preference (White et al., 1991). The hippocampus regulates VTA DAergic population activity via the NAC-VP axis (Grace et al., 2007), and the NAC D_1 and D_2Rs jointly participate in control of the VTA (Rahman and McBride, 2000, 2001).

The VP is a basal forebrain limbic structure, the part of the loop controlling entry of information into long-term memory (Lisman and Grace, 2005), and via its innervation of the VTA it is the main regulator of DAergic population activity (Floresco et al., 2003). In the VP, both D2 and D3 as well as D4 DA D2-like receptors have been reported (Murray et al., 1994; Noain et al., 2006). Receptors of this subgroup can be located presynaptically, most probably on synaptic terminals of the BLA (Maslowski-Cobuzzi and Napier, 1994; Clark and Bracci, 2018) and the NAC (Robertson and Jian, 1995; Mengual and Pickel, 2002), as well as postsynaptically on the output neurons and perhaps on VP interneurons as well (Mengual and Pickel, 2002). Unfortunately, less is known about the exact distribution of the D2, D3, and D4 receptors within the VP. It has been shown that the D3 receptors are expressed on VP GABAergic neurons projecting to the lateral habenula and the VTA (Pribiag et al., 2021). It has been revealed that the VP D_2Rs are potential candidates for remote feedback control over VTA DAergic population activity. This is supported by the fact that a D_2R antagonist microinjected into the VP increases DA levels in the VP on a large scale (Melendez et al., 2005), even though the number of the D_2 autoreceptors in the VP is low (Mengual and Pickel, 2002).

Gong et al. have shown that DA release/uptake blockade by cocaine and amphetamine induces place preference when microinjected into the VP (Gong et al., 1996). In our recent paper, we posited that the formation of place preference (or aversion) requires the drug to have motivational/hedonic actions, which can be associated with environmental cues as well as memory consolidation-facilitating effects to consolidate the established association in the brain (Lénárd et al., 2018). We have recently shown that the D_2R-like agonist quinpirole microinjected into the VP dose dependently facilitates memory consolidation related to inhibitory avoidance (Lénárd et al., 2017) and spatial learning (Péczely et al., 2016) processes. These data suggest that DA and perhaps the activation of D_2Rs exert their effects in the VP via the modulation of VTA DAergic neurons.

In the present study, we aimed to clarify whether the D_2R-like agonist quinpirole microinjected into the VP has a rewarding or possibly aversive effect in the conditioned place preference (CPP) paradigm. According to our hypothesis, quinpirole microinjected into the VP can exert its effect indirectly via the modulation of the DAergic neurons of the VTA. For this reason, to unravel how intra-VP quinpirole modulates VTA DAergic activity, single-unit recordings were performed in the VTA.

METHODS

Subjects and Drug Administration

In the electrophysiological experiments, 30 male Sprague-Dawley rats were used, and 37 male Wistar rats were used in the behavioral experiments. Rats were pair-housed for electrophysiological experiments and single-housed for behavioral experiments to protect the cannula implant. Experiments with Sprague-Dawley rats were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh. Experiments with Wistar rats were carried out in accordance with institutional (BA02/2000-8/2012), national (Hungarian Government Decree, 40/2013. II. 14), and international standards (European Community Council Directive, 2010/63/EU). The rats were housed in a temperature-controlled room (22°C ± 1°C) under standard housing conditions with free access to food and water and a 12-hour-light/-dark cycle. The rats were tested during the lights-off cycle in behavioral experiments.

In electrophysiological experiments unilateral, while in behavioral experiments bilateral, microinjection of the D2-like dopamine receptor agonist (-)-quinpirole hydrochloride (Sigma-Aldrich Co., Q102, Burlington, MA, USA) dissolved in physiological saline into the VP was performed. Quinpirole was administered in 3 different doses (0.1 µg, 1.0 µg, or 5.0 µg, in 0.4 µL physiological saline; 0.98 mM, 9.77 mM, and 48.89 mM, respectively). Control animals received vehicle only. Solutions were kept at 4°C before administration. All drug doses represent the dose per side value. Drugs or vehicle were uni- or bilaterally microinjected through a stainless-steel injection tube inserted into the guide cannulas. The microinjection pipette was attached to a 10-µL Hamilton microsyringe via polyethylene tubing. All microinjections were delivered over a 60-second period. After completion of the microinjection, the pipette was left in place for an additional 60 seconds to allow diffusion into the surrounding tissue as well as to prevent the backflow of the solution along the insertion track.

Acute and Chronic Preparations

In the acute electrophysiological experiments, rats were anesthetized with chloral hydrate (400 mg/kg, i.p.; Sigma) and fixed in a stereotaxic frame. Stainless-steel guide cannulas (26 gauge) were implanted unilaterally dorsal to the VP (AP: –0.3 mm from bregma; ML: + 2.2 mm from midline; V: –5.8 mm from brain surface). The coordinates for the VTA single unit recording were AP: –5.3 to –5.7 mm from bregma, ML: +0.6 to 1.0 mm from midline, and V: 6.5 to 9.0 mm from the brain surface. In vivo extracellular recordings were performed using microelectrodes pulled from Omegadot 2.0-mm glass tubing on a vertical electrode puller (Narishige P-5, Japan) and the tip broken back under microscopic control to an impedance of 12–16 MΩ and filled with 2M NaCl containing 2% Chicago Sky Blue dye. During the acute experiments, body temperature was held constant at 37°C using a thermostatically controlled heating pad (Fintronics), and anesthesia was maintained by i.p. injection of chloral hydrate as needed to maintain suppression of the hindlimb compression reflex.

Chronic operations were carried out under anesthesia by intraperitoneal injection of a mixture of ketamine (Calypsol) and diazepam (Seduxen) mixed in a ratio of 4:1 (Calypsol, 80 mg/kg bwand Seduxen, 20 mg/kg bw, respectively; Richter Gedeon Ltd., Hungary). Then, 22-gauge stainless-steel guide cannulas were implanted bilaterally 0.5 mm above the VP (AP: –0.3 mm from bregma; ML: ±2.2 mm from midline; V: –7. 1 mm from dura) according to the Paxinos and Watson's stereotaxic atlas (Paxinos and Watson, 1997). Cannulas were fixed to the skull with self-polymerizing dental acrylic (Duracryl) anchored by 2 stainless-steel screws. The guide cannulas, except when being used for insertion of microinjection delivery cannulas, were occluded with stainless-steel obturators made of 27-gauge stainless-steel wire.

VP D_2R-Like Agonist Microinjections: VTA Extracellular Recordings

In the anesthetized rats, a stainless-steel guide cannula was implanted into the VP. Quinpirole was microinjected at different doses into the VP through the microinjection cannula inserted into the guide cannula. Control animals received vehicle. Ten to 15 minutes after the microinjection, a recording electrode was lowered through 6–9 vertical electrode tracks (following a meander line-shaped pattern the first track was the most anterior and medial, and the last one was the most posterior and lateral) in a predetermined pattern within the VTA of each rat as previously described (Valenti et al., 2011). Settings of these electrophysiological recordings were similar to those described above, with the electrical signals amplified 10 000× and band-pass filtered at 0.3–10 000 Hz. DA neurons were identified according to well-established electrophysiological criteria (Ungless and Grace, 2012). Three parameters were measured: population activity (i.e., the number of spontaneously active DA neurons per electrode track), average firing rate, and the percentage of action potentials occurring in bursts (Grace and Bunney, 1984). DA cell bursts are defined by increasing interspike intervals as the burst progresses; therefore, in the analysis, the burst onset was defined as the concurrence of 2 spikes with an interspike interval of ≤80 milliseconds, and burst termination was determined as an interspike interval >160 milliseconds. At the end of recordings, the recording sites were marked via electrophoretic ejection of Chicago Sky Blue dye from the tip of the electrode (20 μA constant negative current, 20 minutes) for histological confirmation of the recording sites.

VP D_2R-Like Agonist Microinjections: CPP Test

The CPP apparatus consisted of a circular open field with a diameter of 85 cm and 40 cm height. The apparatus (gray-colored walls and floor) was made of plastic. Black lines divided the floor into 4 quadrants of equal size, and surrounding visual cues helped to distinguish the quadrants and the spatial orientation of animals (Hasenohrl et al., 1989). The room was dimly lit by a 40-W bulb. The place preference procedure consisted of 1 habituation (first day), 2 conditioning (days 2–3), and 1 test (fourth day) trials, each lasting for 900 seconds (15 minutes). The apparatus was cleaned and dried after each session. All trainings and testing were conducted in a sound-isolated, well-separated experimental room. In the habituation trial, animals were placed into the apparatus and had free access to all quadrants for 900 seconds. We measured the time the rats spent in each of the 4 quadrants. The treatment quadrant was determined to be 1 of the 4 quadrants in which the animal had spent neither the longest nor the shortest time during habituation. Bilateral microinjections of the different dose of quinpirole or the vehicle solution were performed at the start of the conditioning trials. Subsequently, during each conditioning trial, rats were confined to the treatment quadrant for 15 minutes by means of a Plexiglas barrier. On the fourth day (test trial), animals had free access to all parts of the apparatus, and the time spent in each of the 4 quadrants was measured. The possible formation of place preference or aversion in the experimental animals was revealed by calculating the normalized time spent in the conditioning quadrant, that is, the difference between the time spent in the conditioning quadrant during the test and the habituation in the case of all animals. Locomotor activity of the animals was also recorded by a video camera. Data were stored and motion-analysis was made by the Noldus EthoVision Basic software (Noldus Information Technology b.v., Wageningen, the Netherlands).

Histology

At the end of electrophysiological recordings, a lethal dose of chloral hydrate (additional 400 mg/kg i.p.) was administered for euthanasia. The rats were decapitated and the brains removed before fixing the tissue in 8% paraformaldehyde for approximately 48 hours and then transferred to 25% sucrose solution for cryoprotection. The brains were frozen and sliced coronally (60 μm) and stained with a combination of neutral red and cresyl violet.

At the end of the behavioral experiments, rats received an overdose of urethane (i.p. injection of 40% urethane solution in a dose of 1.4 g/kg) and were perfused transcardially with isotonic saline followed by 10% formaldehyde solution (slow infusion rate: 500 mL/20 minutes). A week after fixation, brains were frozen, cut into 40-μm serial sections, and stained with cresyl violet.

Microinjection sites were reconstructed according to the rat brain stereotaxic atlas (Paxinos and Watson, 1997). Only data from animals with correctly placed cannulas were analyzed.

Statistical Analyses

For statistical analysis, mixed ANOVA and 1-way ANOVA followed by Bonferroni post hoc test were applied. Data are presented as mean ± SEM. To examine the possible correlations between the variables, the Pearson correlation test was used. Statistical significance was established as $P < .05$. In

mixed-ANOVA analysis, trial or time was regarded as the within-subject factor and treatment as the between-subject factor.

To preliminarily determine the appropriate sample size for the experiments, the software G*Power 3.1 was applied. Based on the results of our earlier experiments and the results of the pilot experiments, setting the power to 0.8, the estimated number of animals in the electrophysiological experiments was 24, and in the behavioral experiments it was 34 (in both cases we added 10%–15% of the estimated number of animals to the original number to compensate the loss of animals because of the histological analysis).

RESULTS

Effects of VP D_2R-Like Agonist Microinjection on VTA DA Neuron Population and Burst Activity

Cannulas for microinjection were placed into the VP and single-unit recording electrodes directed into the VTA. Histological examination showed that the microinjection cannulas were localized precisely to the VP and single-unit electrodes in the VTA in 27 of the 30 rats. Schematic illustration of cannula placements is shown in Figure 1A. The remaining 3 (3/30) rats were excluded from the statistical analysis based on incorrect placements.

One-way ANOVA analysis revealed that the microinjections into the VP significantly influenced the VTA DA neuron population activity ($F_{(3,23)}=13.529$, $P=.001$) (Fig. 1B). Bonferroni post hoc test indicated that the 0.1-µg quinpirole increased the population activity of VTA DA neurons compared with controls and 5.0 µg quinpirole-treated groups ($P=.026$, $P=.001$, respectively), whereas 1.0 µg increased it only compared with the 5.0-µg group ($P=.001$). To assess whether there was an effect along the anterior-posterior axis (Fig. 1C), the averages of first to third, fourth to sixth, and seventh to ninth electrode tracks were compared by mixed-ANOVA. Analysis indicated a significant treatment ($F_{(3,27.273)}=16.914$, $P=.001$), significant trial effect ($F_{(2,54.104)}=3.255$, $P=.046$), and significant treatment * trial interaction ($F_{(6,54.080)}=2.286$, $P=.049$). Post hoc test revealed that 5.0 µg quinpirole significantly decreased population activity compared with the control group ($P=.021$) in the first 3 electrode tracks. However, in the second 3 electrode tracks, there was no statistical difference between the control and the 5.0-µg quinpirole-treated groups, but the 0.1-µg quinpirole increased population activity compared with the controls and the 5.0-µg quinpirole-treated group ($P=.001$ in both cases); this effect persisted for the last 3 electrode tracks as well ($P=.021$, $P=.008$, respectively). In the last 3 electrode tracks, the 1.0-µg quinpirole also increased the population activity compared with the control and the 5.0-µg quinpirole-treated groups ($P=.016$, $P=.006$, respectively). The track and potential time-dependence are also represented in a cumulative population activity curve (Fig. 1D). Effect along the medio-lateral axis was analyzed (data not shown): the averages of first-sixth-seventh, second-fifth-eighth, and third-fourth-ninth electrode tracks were compared by mixed ANOVA. Analysis indicated a significant treatment effect ($F_{(3,27)}=17.288$, $P=.001$, a significant trial effect ($F_{(2,54)}=3.254$, $P=.046$), and a nonsignificant treatment * trial interaction ($F_{(6,54)}=0.716$, $P=.639$).

In addition to the population activity, both the frequency ($F_{(3,23)}=6.571$, $P=.002$) (Fig. 1E) and the burst activity ($F_{(3,23)}=13.716$, $P=.001$) (Fig. 1F) of the DA neurons were impacted by intra-VP quinpirole treatment. Post hoc test demonstrated that the intra-VP 5.0 µg quinpirole decreased the firing rate compared with the controls and 0.1 µg quinpirole treated group ($P=.026$, $P=.002$, respectively), and especially the burst activity of the DA neurons compared with all the other groups ($P=.001$ in all cases). Bursting activity distribution of the VTA DAergic neurons is also demonstrated (Fig. 1G). Thus, the 5.0-µg quinpirole microinjection into the VP shifts the bursting activity of the DA neurons from the high to the low bursting range.

Effects of VP D_2R-Like Agonist Microinjection on CPP

The effect of the D_2R-like agonist quinpirole in the CPP paradigm was investigated (Figs. 2 and 3). Histological examination showed that the microinjection cannulas were localized to the VP in 31 of the 37 rats (Fig. 2A). The remaining 6 (6/37) rats were excluded from the statistical analysis. In 5 of those rats, the cannula was too posterior, caudal to the VP, in the lateral hypothalamus (LH), and substantia innominata, and in 1 rat was caudal to the VP in the horizontal diagonal band.

One-way ANOVA revealed a statistically significant difference among the groups with respect to the normalized time spent in the conditioning quadrant (test-habituation) parameter ($F_{(3,27)}=3.372$, $P=.033$) (Fig. 2B). Bonferroni post hoc test demonstrated that the intra-VP 5.0 µg quinpirole decreased significantly the normalized time spent in the conditioning quadrant compared with the control group ($P=.045$), inducing place aversion.

To examine the impact of intra-VP quinpirole on locomotion, we analyzed and compared values recorded in 5-minute intervals (0–5 minutes, 5–10 minutes, and 10–15 minutes) of the trials (Figs. 3A, C). Intra-VP quinpirole microinjection influenced locomotor activity along the conditioning trials (Fig. 3A); mixed-ANOVA analysis demonstrated a significant treatment effect ($F_{(3,31.000)}=7.452$, $P=.001$), a significant trial effect ($F_{(1,\ 155.000)}=33.286$, $P=.001$), and a significant time effect ($F_{(2,155.000)}=84.292$, $P=.001$). Moreover, statistical analysis revealed a significant time*treatment interaction ($F_{(6,155.000)}=4.738$, $P=.001$) and a significant time*trial interaction ($F_{(2,155.000)}=5.734$, $P=.004$). Bonferroni post hoc test revealed that the 5.0-µg quinpirole treatment significantly decreased locomotor activity compared with all the other groups in the 0–5 min interval ($P=.001$ in all cases) and compared with the 0.1-µg and 1.0-µg quinpirole-treated group in the 5–10 minute interval ($P=.041$ and $P=.004$, respectively) of the conditioning trials. Pearson correlation test revealed a significant negative correlation between the anterior-posterior coordinates and the aversive effect of intra-VP 5.0 µg quinpirole ($R=-0.691$, $P=.043$); the more anterior the injection site, the more aversive was the treatment (see Fig. 2C). Furthermore, a positive correlation has been shown between the decrease in locomotor activity and the aversive effect of intra-VP 5.0 µg quinpirole ($R=0.825$, $P=.022$); the lower the activity during the first 5 minutes of the first conditioning, the more aversive the treatment (Fig. 3B). In addition to the acute effect, quinpirole treatment also had a delayed effect on the locomotor activity, which depended on time (Fig. 3C) within the test trial; a significant treatment ($F_{(3,31.000)}=3.495$, $P=.027$) and a significant trial effect ($F_{(2,62.000)}=58.530$, $P=.001$) and a significant treatment * trial interaction ($F_{(6,62.000)}=4.298$, $P=.001$) was observed. Bonferroni post hoc test indicated that 1.0 µg quinpirole microinjected into the VP just before the conditioning trials increased locomotor activity during the first 5 minutes of the test trial compared with the control and the 0.1 µg and 5.0 µg quinpirole-treated groups ($P=.001$ in all cases). Nevertheless, this effect decreased gradually over time in that significant differences were not observed during the second and third 5 minutes of the test trial among the groups.

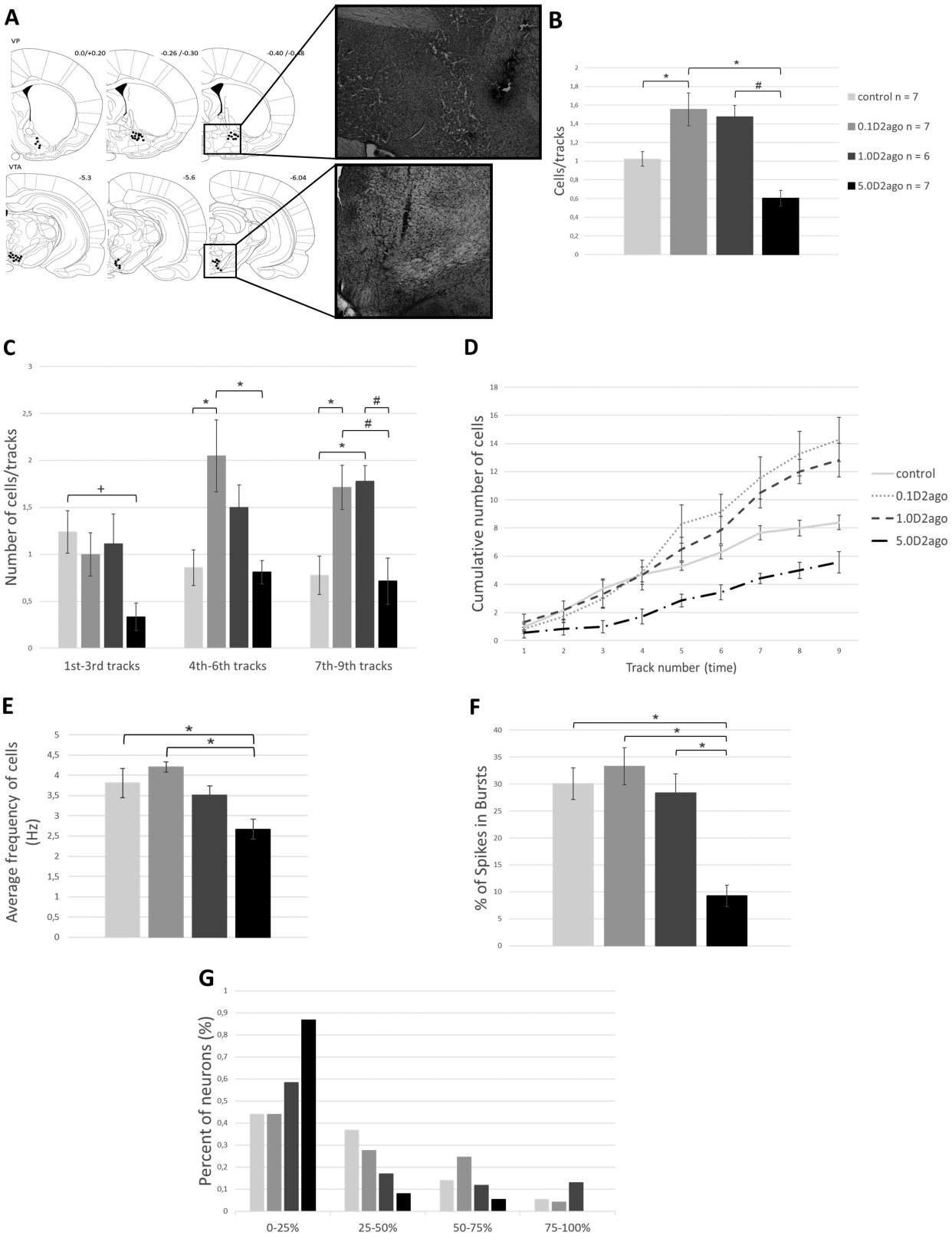

Figure 1. Intra-ventral pallidum (VP) quinpirole microinjection affected ventral tegmental area (VTA) neuronal activity states. (A) On the left side, schematic illustration of recording electrode placement in the VTA (upper part) and cannula in the VP (lower part) as shown in coronal sections of rat brain taken from Paxinos and Watson's atlas. The numbers refer to anterior–posterior distance from bregma in millimeters. On the right side, example images of slices showing the unilateral cannula and electrode placements. (B) Effect of intra-VP quinpirole on VTA population activity, that is, the number of spontaneously active dopamine (DA) neurons per electrode track. Intra-VP 0.1 μg and 1.0 μg quinpirole increased the population activity of VTA DA neurons compared with the 5.0-μg quinpirole-treated group, and 0.1 μg also increased it compared with the controls. (C) Effect of intra-VP quinpirole along the anterior–posterior axis of the VTA: means of the first to third, fourth to sixth, and

Discussion

The present experiments revealed dose-dependent effects of intra-VP quinpirole on the behavior of rats and modulation of VTA DAergic activity. It is well known that the VP can impact VTA DA neuron activity (Floresco et al., 2003; Grace et al., 2007). In this study, we found that the largest dose of the D2-like dopamine receptor agonist injected into the VP decreased VTA DA neuron population activity and firing frequency as well as potently reduced burst activity. In the present behavioral experiments, the 5.0-μg dose of quinpirole microinjected into the VP induced place aversion. It has been shown that phasic activation of VTA DAergic neurons induces place preference (Tsai et al., 2009), and direct optogenetic inactivation of these neurons evokes place aversion (Danjo et al., 2014). Based on these findings, a plausible explanation of our behavioral results can be that the 5.0 μg intra-VP quinpirole induces place aversion via decreasing VTA DAergic population and burst activity. Some data suggest that both phasic firing and population activity of VTA DAergic neurons correlates with the strength of place preference by increasing tonic DA level in the NAC shell (Boix et al., 1995; Morutto and Phillips, 1998). If this is accurate, then one would expect that the 0.1 μg and 1.0 μg intra-VP quinpirole should induce place preference, because they increase VTA population activity; however, this was not observed in this study. This apparent contradiction can be resolved if we consider that the formation of place preference (or aversion) obviously requires temporal overlap between the effect of the drug and the conditioning environment. The results of the DAergic activity along the anterior-posterior axis and the cumulative population activity can be interpreted as time-dependent changes in the population and burst activity. Considering these, we have shown that the intra-VP 5.0 μg agonist had an acute, immediate effect on VTA DAergic population and burst activity; in contrast, the 0.1-μg and 1.0-μg quinpirole doses had only a delayed effect on the population activity, appearing approximately 1–1.5 hours after their administration.

It is well known that the activation of the hippocampus–NAC axis increases (Floresco et al., 2001), and the stimulation of the BLA decreases (Chang and Grace, 2014) VTA population activity via the VP. Accordingly, one possible explanation for the delayed increase in VTA DAergic population activity can be that the 0.1 μg quinpirole attenuated the BLA input, whereas the 1.0 μg quinpirole facilitated the NAC shell input and thereby disinhibited VTA DAergic neurons. With respect to the NAC shell–VP pathway, this hypothesis is strongly supported by the fact that stimulation of D2 medium spiny neurons increases VTA population activity via the VP (Soares-Cunha et al., 2018). As discussed above in the Introduction, D_2Rs in the VP are localized presynaptically on the NAC and BLA terminals as well as on the output neurons and possibly on interneurons of the VP. It is therefore plausible that the effect on the synaptic plasticity and the consequent increased VTA DAergic population activity are caused by the activation of presynaptic D_2Rs, and the acute effect of the agonist is a postsynaptic one that can overcome the presynaptic effects over the VTA. The delayed increased locomotion induced by 1.0 μg quinpirole may therefore reflect the enhanced synaptic transmission in the NAC shell–VP fibers and the increased VTA DA neuron population activity.

Burst activity of VTA DAergic neurons is regulated primarily by the pedunculopontine tegmentum (PPTg), but not directly by the VP (Floresco et al., 2003). However, the VP innervates the PPTg (Swanson et al., 1984). We predict that the intra-VP 5.0-μg quinpirole treatment influences burst activity via the PPTg. This hypothesis is also supported by the fact that the burst distribution pattern in the present recordings was very similar to that of the intra-PPTg GABA agonist muscimol treatment (Floresco et al., 2003).

As the present results demonstrated, the 5.0-μg intra-VP quinpirole administration not only results in decreased VTA DAergic activity and place aversion, but it also causes an acute decrease in locomotor activity lasting approximately 10 minutes. This is consistent with the findings of Gong et al. (Gong et al., 1999), and we reproduced their result in the present study. Moreover, we have revealed a correlation between the locomotor reduction and the aversive effect of quinpirole action in the VP. D_2Rs play an important role in the regulation of reward and aversion and modulate locomotion in brain regions surrounding the VP. Quinpirole increases locomotor activity and induces place preference in the NAC shell region (White et al., 1991; Ikemoto, 2002). Inhibition of D_2Rs in the medial septum induces place aversion (Karami et al., 2003), whereas in the LH, in the perifornical area, it has rewarding effects and enhances locomotor activity (Morutto and Phillips, 1998). One potential confounding factor can be that quinpirole microinjected into the VP may have diffused into these structures and exerted its effect there. However, this option can be ruled out because in the NAC and the medial septum, quinpirole would have exerted the opposite effect from we observed with VP injection; moreover, in the anterior LH, close to the VP/LH border, quinpirole was not found to be aversive in the present experiments.

Gong and Neil demonstrated that psychostimulant-induced place preference in the VP is DA dependent (Gong et al., 1996, 1997). If this is the case, why would activation of VP D_2Rs be aversive? One possible explanation can be that the resultant effect of the D_1R and D_2R activation in the VP is rewarding. In this case, if the activation of the D_1Rs is potently rewarding in the VP, then this effect can compensate, or even overcome, the aversive effect of the D_2R activation. An analogous case can be made with respect to the locomotor effect in which intra-VP D_1R agonist increases while the large dose of the D_2R-like agonist decreases locomotion (Gong et al., 1999); as a result, the overall effect of DA is an increased locomotor activity (Napier and Chrobak, 1992).

Given the above-mentioned electrophysiological and behavioral findings, it is important to consider that quinpirole can exert its effect on both the D2 and D3 DA receptors from the D2R subgroup. The dose-dependent effects of quinpirole can be potentially explained by the differential localization of D2 and

seventh to ninth electrode tracks were compared with each other. In the first 3 electrode tracks, intra-VP 5.0 μg quinpirole decreased population activity, whereas during second 3 electrode tracks, 0.1 μg quinpirole increased population activity and the 5.0-μg quinpirole-treated group returned to the control level. In the third 3 electrode tracks, both 0.1 μg and 1.0 μg quinpirole led to an increase in population activity. (D) Cumulative changes in population activity are also represented in a cumulative population activity curve, where in every electrode track the cumulative number of DAergic neurons was divided by the track number. C and D can be interpreted as time-dependent changes in the population activity. (E) Average firing rate of DAergic neurons. In each animal, the mean of the DA neuron firing frequency was calculated, and the averages of these means within each group were used in the analysis. Intra-VP 5.0 μg quinpirole decreased the average firing rate of DA neurons. (F) The percentage of action potentials occurring in bursts. Intra-VP 5.0 μg quinpirole strongly reduced burst activity of DA neurons. In the figure, means ±SEM are represented, * and # indicate significant differences among the groups revealed by Bonferroni post hoc test ($P < .05$, see details in the text). (G) Bursting activity distribution of the VTA DAergic neurons. The 5.0-μg quinpirole microinjection into the VP shifts the bursting activity of the DA neurons from the high to the low bursting levels. The horizontal axis indicates the percentage of action potentials occurring in bursts.

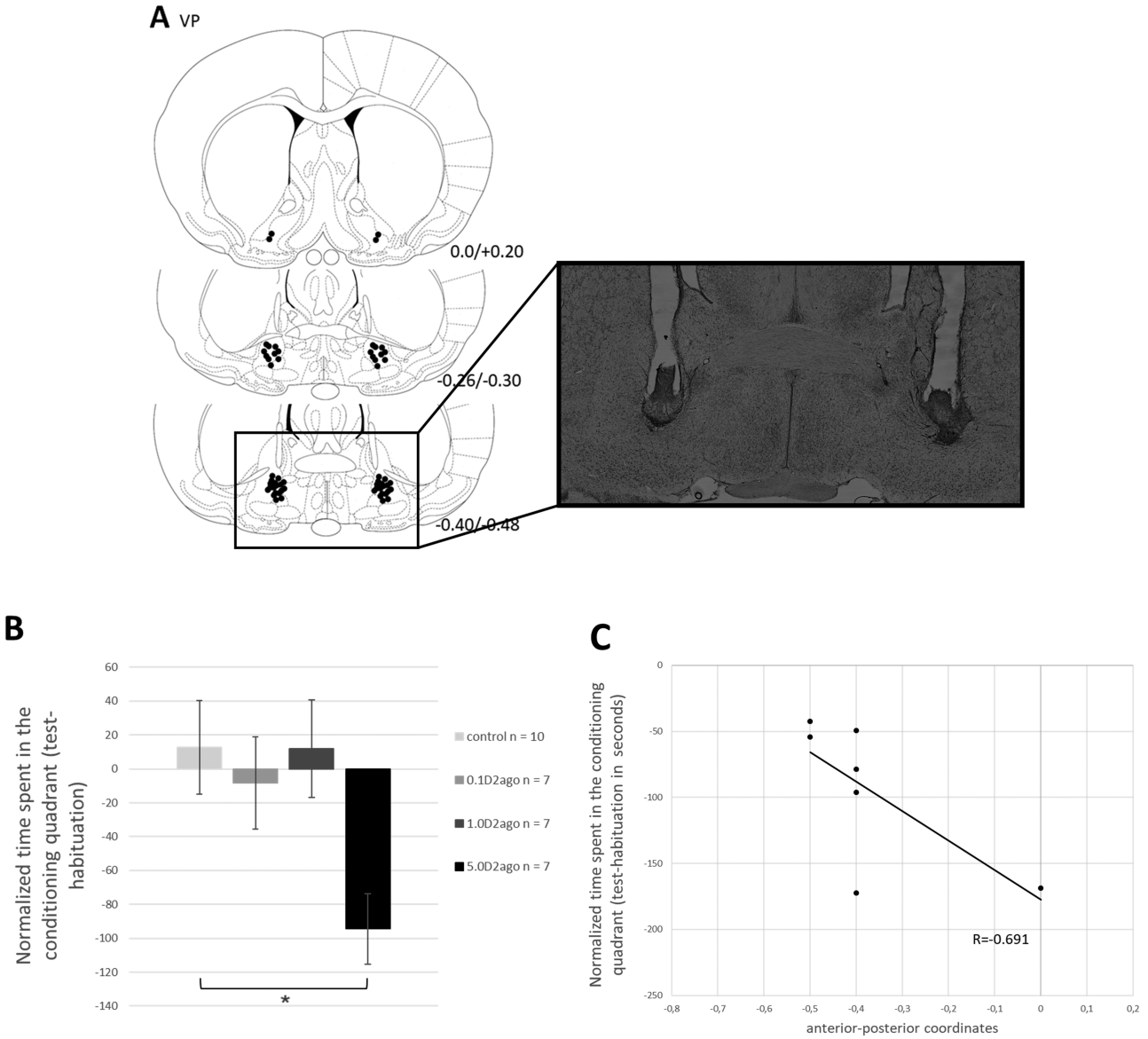

Figure 2. Intra-vental pallidum (VP) quinpirole microinjection produced aversive effect in conditioned place preference paradigm (CPP). (A) On the left side, schematic illustration of bilateral cannula placement in the VP as shown in coronal sections of rat brain taken from Paxinos and Watson's atlas. On the right side, example image of slice showing the bilateral cannulae placements. The numbers refer to anterior–posterior distance from bregma in mm. (B) The normalized time spent in the conditioning quadrant (test-habituation) was calculated for all rats. Microinjection of 5.0 µg quinpirole into the VP induced place aversion in the experimental animals. (C) A negative correlation between the anterior–posterior coordinates and the aversive effect of intra-VP 5.0 µg quinpirole ($R = -0.691$) was observed, with the more anterior injection site corresponding to the more aversive effect. In the figure, means ±SEM are represented; * indicates significant differences among the groups revealed by Bonferroni post hoc test ($P < .05$, see details in the text).

D3 receptors within the VP. Unfortunately, the distribution of D2 DA receptors is not known. However, it has been demonstrated that D3 receptors can be found on VP GABAergic neurons innervating the lateral habenula and within VP as well, and the optogenetic stimulation of these D3 receptor-expressing VP cells increases DA release in the NAC shell region (Pribiag et al., 2021). These findings and our present results suggest that perhaps the smaller doses of quinpirole activate the habenula or directly activate the VP neurons projecting to the VTA via D3 receptors, while the largest dose activates another pathway—for example, the proposed VP-PPTg-VTA pathway. Accordingly, the smaller doses activate the VTA, whereas the largest dose inhibits it, resulting in different behavioral effects. This model can also explain why cocaine exposure leads to place preference and not aversion: it has been shown that cocaine administration increases D3 receptor mRNA, but not D2 mRNA levels, in the VP (Campbell and Lobo, 2021; Pribiag et al., 2021). Nevertheless, the VP GABAergic neurons terminate on both DAergic and GABAergic neurons of the VTA (Faget et al., 2016); therefore, it is also possible that the different effects of the smaller doses and the largest dose can be due to the distinct sensitivity of the VP neurons innervating the VTA DAergic or GABAergic cells.

A potential limitation of the present study is that the behavioral and electrophysiological experiments were carried out using different rat strains. Nevertheless, it is well known that the Sprague-Dawley and Wistar rat strains are related in that Sprague-Dawley rats were derived from Wistar rats. Most of the investigations of rat CNS are performed in these 2 strains, and the theories constructed based on the experimental findings are frequently generalized from one to the other. This suggests that

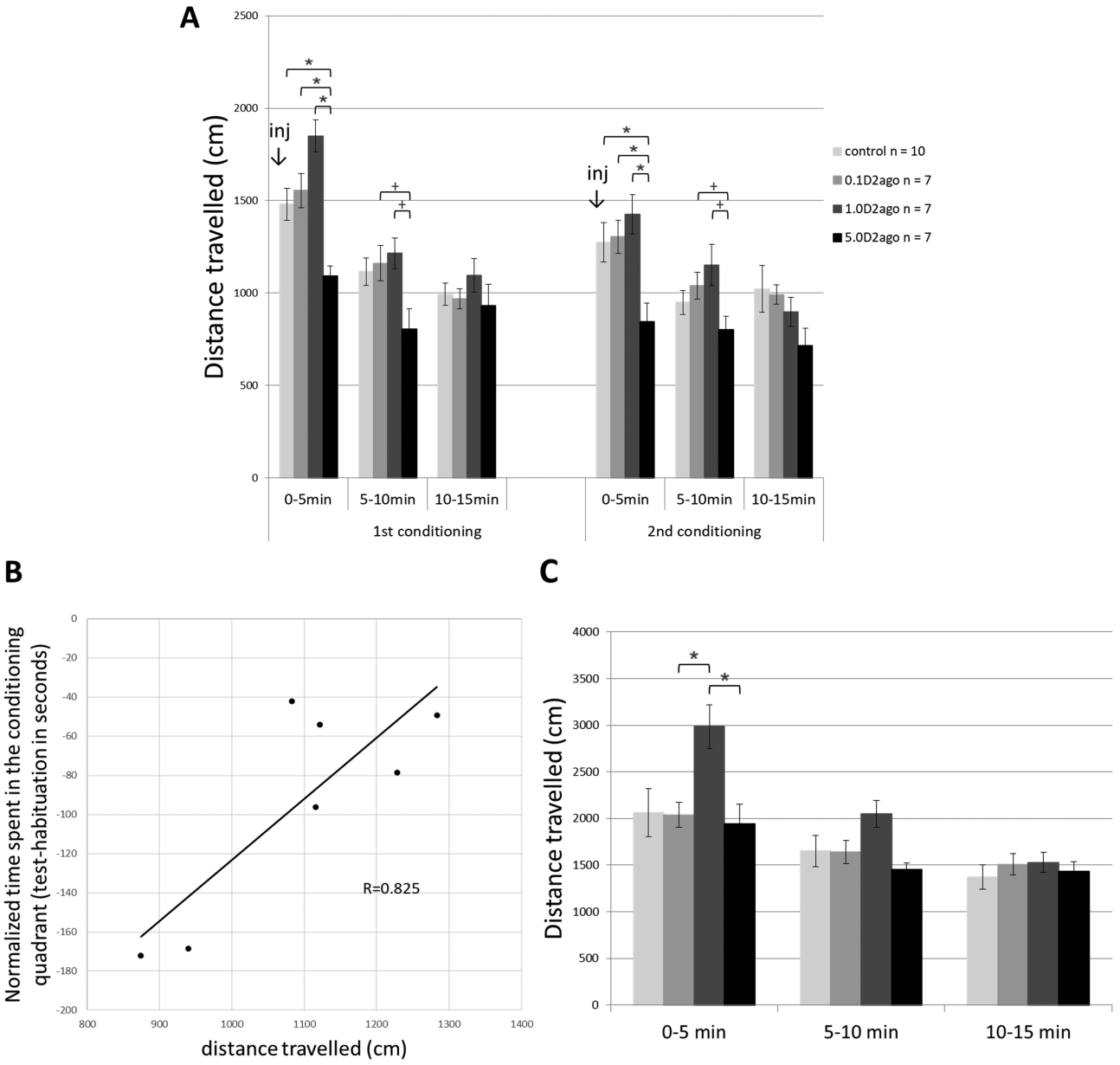

Figure 3. Effect of intra-ventral pallidum (VP) quinpirole microinjection on locomotion. Distance travelled was recorded and measured in centimeters. (A) Effect of quinpirole microinjection into the VP on locomotor activity of rats in the conditioning trials. In both trials, 5.0 μg quinpirole decreased locomotion, and this effect lasted approximately 10 minutes. (B) A correlation between the decreased locomotor activity and the aversive effect of intra-VP 5.0 μg quinpirole ($R = 0.825$) was observed, with the lowest activity observed during the first 5 minutes of the first conditioning during the most aversive treatment response. (C) The intra-VP 1.0 μg quinpirole induced an increased locomotor activity, which was seen after a delay only in the test trial of the conditioned place preference paradigm. In the figure, means ±SEM are represented; * and + indicate significant differences among the groups revealed by Bonferroni post hoc test ($P < .05$, see details in the text).

our present results can be incorporated in 1 model about the DAergic regulation of the behavior and the VP-VTA axis.

Conclusion

In conclusion, in the present study, we have shown that the largest dose of intra-VP quinpirole induces place aversion in the experimental animals. Furthermore, DAergic neurons of the VTA are modulated by intra-VP quinpirole along an inverted U-shaped dose-response curve. Our results may indicate that the low-dose agonist has a presynaptic effect possibly affecting the BLA or NAC shell inputs in the VP, whereas the largest dose modulates the VP neurons directly, decreasing the VTA DAergic activity. Or alternatively, it is possible that the inverted U-shaped dose-response curve is caused by the different location of the D2 and D3 receptors within the VP and their different affinity to quinpirole. In total, the suppressive effect of the large dose D_2R-like agonist is a logical negative remote feedback mechanism in terms of the control theory.

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Interest Statement

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References

Ariano MA, Fisher RS, Smyk-Randall E, Sibley DR, Levine MS (1993) D2 dopamine receptor distribution in the rodent CNS using anti-peptide antisera. Brain Res 609:71–80.

Boix F, Sandor P, Nogueira PJ, Huston JP, Schwarting RK (1995) Relationship between dopamine release in nucleus accumbens and place preference induced by substance P injected into the nucleus basalis magnocellularis region. Neuroscience 64:1045–1055.

Campbell RR, Lobo MK (2021) DRD3-dependent plasticity within the VP drives subcircuit activity critical for cocaine seeking. Neuron 109:2043–2044.

Chang CH, Grace AA (2014) Amygdala-ventral pallidum pathway decreases dopamine activity after chronic mild stress in rats. Biol Psychiatry 76:223–230.

Clark M, Bracci E (2018) Dichotomous dopaminergic control of ventral pallidum neurons. Front Cell Neurosci 12:260.

Danjo T, Yoshimi K, Funabiki K, Yawata S, Nakanishi S (2014) Aversive behavior induced by optogenetic inactivation of ventral tegmental area dopamine neurons is mediated by dopamine D2 receptors in the nucleus accumbens. Proc Natl Acad Sci USA 111:6455–6460.

Faget L, Osakada F, Duan J, Ressler R, Johnson AB, Proudfoot JA, Yoo JH, Callaway EM, Hnasko TS (2016) Afferent inputs to neurotransmitter-defined cell types in the ventral tegmental area. Cell Rep 15:2796–2808.

Floresco SB, Todd CL, Grace AA (2001) Glutamatergic afferents from the hippocampus to the nucleus accumbens regulate activity of ventral tegmental area dopamine neurons. J Neurosci 21:4915–4922.

Floresco SB, West AR, Ash B, Moore H, Grace AA (2003) Afferent modulation of dopamine neuron firing differentially regulates tonic and phasic dopamine transmission. Nat Neurosci 6:968–973.

Gong W, Neill D, Justice JB Jr (1996) Conditioned place preference and locomotor activation produced by injection of psychostimulants into ventral pallidum. Brain Res 707:64–74.

Gong W, Neill D, Justice JB Jr (1997) 6-Hydroxydopamine lesion of ventral pallidum blocks acquisition of place preference conditioning to cocaine. Brain Res 754:103–112.

Gong W, Neill DB, Lynn M, Justice JB Jr (1999) Dopamine D1/D2 agonists injected into nucleus accumbens and ventral pallidum differentially affect locomotor activity depending on site. Neuroscience 93:1349–1358.

Grace AA, Bunney BS (1984) The control of firing pattern in nigral dopamine neurons: burst firing. J Neurosci 4:2877–2890.

Grace AA, Floresco SB, Goto Y, Lodge DJ (2007) Regulation of firing of dopaminergic neurons and control of goal-directed behaviors. Trends Neurosci 30:220–227.

Hasenohrl RU, Oitzl MS, Huston JP (1989) Conditioned place preference in the corral: a procedure for measuring reinforcing properties of drugs. J Neurosci Methods 30:141–146.

Ikemoto S (2002) Ventral striatal anatomy of locomotor activity induced by cocaine, D-amphetamine, dopamine and D1/D2 agonists. Neuroscience 113:939–955.

Jay TM (2003) Dopamine: a potential substrate for synaptic plasticity and memory mechanisms. Prog Neurobiol 69:375–390.

Karami M, Zarrindast MR, Sepehri H, Sahraei H (2003) Sulpiride injections into the medial septum reverse the influence of intra-medial septum injection of L-arginine on expression of place conditioning-induced by morphine in rats. Brain Res 976:30–40.

Klitenick MA, Deutch AY, Churchill L, Kalivas PW (1992) Topography and functional role of dopaminergic projections from the ventral mesencephalic tegmentum to the ventral pallidum. Neuroscience 50:371–386.

Lénárd L, Ollmann T, László K, Kovács A, Gálosi R, Kállai V, Tóth A, Kertes E, Zagoracz O, Karádi Z, Péczely L (2017) Role of D2 dopamine receptors of the ventral pallidum in inhibitory avoidance learning. Behav Brain Res 321:99–105.

Lénárd L, László K, Kertes E, Ollmann T, Péczely L, Kovács A, Kállai V, Zagoracz O, Gálosi R, Karádi Z (2018) Substance P and neurotensin in the limbic system: their roles in reinforcement and memory consolidation. Neurosci Biobehav Rev 85:1–20.

Lisman JE, Grace AA (2005) The hippocampal-VTA loop: controlling the entry of information into long-term memory. Neuron 46:703–713.

Maslowski-Cobuzzi RJ, Napier TC (1994) Activation of dopaminergic neurons modulates ventral pallidal responses evoked by amygdala stimulation. Neuroscience 62:1103–1119.

Melendez RI, Rodd ZA, McBride WJ, Murphy JM (2005) Dopamine receptor regulation of ethanol intake and extracellular dopamine levels in the ventral pallidum of alcohol preferring (P) rats. Drug Alcohol Depend 77:293–301.

Mengual E, Pickel VM (2002) Ultrastructural immunocytochemical localization of the dopamine D2 receptor and tyrosine hydroxylase in the rat ventral pallidum. Synapse 43:151–162.

Missale C, Nash SR, Robinson SW, Jaber M, Caron MG (1998) Dopamine receptors: from structure to function. Physiol Rev 78:189–225.

Morutto SL, Phillips GD (1998) Interactions between sulpiride infusions within the perifornical region of the lateral hypothalamus and the nucleus accumbens on measures of locomotor activity and conditioned place preference. Behav Pharmacol 9:345–355.

Murray AM, Ryoo HL, Gurevich E, Joyce JN (1994) Localization of dopamine D3 receptors to mesolimbic and D2 receptors to mesostriatal regions of human forebrain. Proc Natl Acad Sci USA 91:11271–11275.

Napier TC, Chrobak JJ (1992) Evaluations of ventral pallidal dopamine receptor activation in behaving rats. Neuroreport 3:609–611.

Noain D, Avale ME, Wedemeyer C, Calvo D, Peper M, Rubinstein M (2006) Identification of brain neurons expressing the dopamine D4 receptor gene using BAC transgenic mice. Eur J Neurosci 24:2429–2438.

Oades RD, Halliday GM (1987) Ventral tegmental (A10) system: neurobiology. 1. Anatomy and connectivity. Brain Res 434:117–165.

Paxinos G, Watson C (1997) The rat brain in stereotaxic coordinates. 3rd ed. San Diego, CA: Academic Press.

Péczely L, Ollmann T, László K, Kovács A, Gálosi R, Kertes E, Zagoracz O, Kállai V, Karádi Z, Lénárd L (2016) Role of ventral pallidal D2 dopamine receptors in the consolidation of spatial memory. Behav Brain Res 313:1–9.

Pribiag H, Shin S, Wang EH, Sun F, Datta P, Okamoto A, Guss H, Jain A, Wang XY, De Freitas B, Honma P, Pate S, Lilascharoen V, Li Y, Lim BK (2021) Ventral pallidum DRD3 potentiates a pallido-habenular circuit driving accumbal dopamine release and cocaine seeking. Neuron 109:2165–2182 e2110.

Rahman S, McBride WJ (2000) Feedback control of mesolimbic somatodendritic dopamine release in rat brain. J Neurochem 74:684–692.

Rahman S, McBride WJ (2001) D1-D2 dopamine receptor interaction within the nucleus accumbens mediates long-loop negative feedback to the ventral tegmental area (VTA). J Neurochem 77:1248–1255.

Robertson GS, Jian M (1995) D1 and D2 dopamine receptors differentially increase Fos-like immunoreactivity in accumbal projections to the ventral pallidum and midbrain. Neuroscience 64:1019–1034.

Soares-Cunha C, Coimbra B, Domingues AV, Vasconcelos N, Sousa N, Rodrigues AJ (2018) Nucleus accumbens microcircuit underlying D2-MSN-driven increase in motivation. eNeuro 5:ENEURO.0386-18.2018.

Swanson LW, Mogenson GJ, Gerfen CR, Robinson P (1984) Evidence for a projection from the lateral preoptic area and substantia innominata to the “mesencephalic locomotor region” in the rat. Brain Res 295:161–178.

Tsai HC, Zhang F, Adamantidis A, Stuber GD, Bonci A, de Lecea L, Deisseroth K (2009) Phasic firing in dopaminergic neurons is sufficient for behavioral conditioning. Science 324:1080–1084.

Ungless MA, Grace AA (2012) Are you or aren’t you? Challenges associated with physiologically identifying dopamine neurons. Trends Neurosci 35:422–430.

Valenti O, Lodge DJ, Grace AA (2011) Aversive stimuli alter ventral tegmental area dopamine neuron activity via a common action in the ventral hippocampus. J Neurosci 31:4280–4289.

White NM, Packard MG, Hiroi N (1991) Place conditioning with dopamine D1 and D2 agonists injected peripherally or into nucleus accumbens. Psychopharmacology (Berl) 103:271–276.