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The activation of D₂ and D₃ receptor subtypes inhibits pathways mediating primary afferent depolarization (PAD) in the mouse spinal cord

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Abstract

Somatosensory information can be modulated at the spinal cord level by primary afferent depolarization (PAD), known to produce presynaptic inhibition (PSI) by decreasing neurotransmitter release through the activation of presynaptic ionotropic receptors. Descending monoaminergic systems also modulate somatosensory processing. We investigated the role of D₁-like and D₂-like receptors on pathways mediating PAD in the hemisectioned spinal cord of neonatal mice. We recorded low-threshold evoked dorsal root potentials (DRPs) and population monosynaptic responses as extracellular field potentials (EFPs). We used a paired-pulse conditioning-test protocol to assess homosynaptic and heterosynaptic depression of evoked EFPs to discriminate between dopaminergic effects on afferent synaptic efficacy and/or on pathways mediating PAD, respectively. DA (10 μ M) depressed low-threshold evoked DRPs by 43%, with no effect on EFPs. These depressant effects on DRPs were mimicked by the D₂-like receptor agonist quinpirole (35%). Moreover, by using selective antagonists at D₂-like receptors (encompassing the D₂, D₃, and D₄ subtypes), we found that the D₂ and D₃ receptor subtypes participate in the quinpirole depressant inhibitory effects of pathways mediating PAD.

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CRedit Author Statement

Milla-Cruz J: Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft. **Mena-Avila E:** Conceptualization, Methodology, Investigation. **Calvo J:** Investigation. **Hochman S:** Conceptualization, Writing - Review & Editing, Funding acquisition. **Villalón C:** Conceptualization, Writing - Review & Editing. **Quevedo J:** Conceptualization, Methodology, Resources, Writing - Review & Editing, Supervision, Funding acquisition

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Keywords

Dopamine; primary afferent depolarization; spinal cord; neuromodulation; presynaptic inhibition

Introduction

Somatosensory information is subject to pre- and postsynaptic inhibitory mechanisms at the spinal cord level. This type of PSI involves PAD generated by the activation of GABAA receptors in the intraspinal sensory terminals [1] leading to the efflux of Cl⁻ ions from afferent terminals [2]. PAD-related PSI modulates proprioceptive, cutaneous and visceral sensory information [1].

Descending monoaminergic systems play a fundamental role in modulating sensory afferent transmission, spinal neurons and glia in diverse spinal functions such as locomotion, tactile perception, nociception and processing of visceral information [3–8]. Moreover, some lines of evidence have demonstrated that biogenic monoamines reduce PAD evoked by stimulation of low-threshold cutaneous and proprioceptive afferents [1,3,8,9]. Interestingly, monoamines also modulate sensory integrative properties from heterogeneous deep dorsal horn interneurons in the neonatal rat spinal cord [5]. Nonetheless, it is unclear whether these monoamine's effects are exerted pre- or postsynaptically.

As to the dopaminergic system, the hypothalamic A11 area projections to the spinal cord have been identified as the primary source of dopamine (DA) [10,11,12]. This nucleus plays an important role as a neuromodulator of diverse sensory and motor functions [6,13]. DA has been shown to produce differential effects depending on the nature of the receptors involved. For example, in the spinal cord of rodents, DA induces a rhythmic and alternating activity between flexion and extension, stabilizing the locomotor pattern by activation of D₁-like receptors [13]. DA also produces depressant actions on group II dorsal horn interneurons mediated by activation of D₂-like receptors [14]. On the other hand, DA produces antinociception by depressing C fiber-evoked slow VRPs in neonatal rats, via D₁-like receptors [15,16].

There is evidence that DA produces no effect on low-threshold sensory synaptic transmission, but it inhibits the interneuronal pathways mediating PAD [8]. Notwithstanding, the specific role of the DA receptor subtypes involved in the modulation of synaptic transmission and PAD remains unexplored. The present study was designed to investigate in the hemisected mouse spinal cord the role of the D₂, D₃ and D₄ receptor subtypes in the modulatory actions of DA on low-threshold synaptic transmission and pathways mediating PAD. We demonstrated that DA depressing effects on PAD involve the specific activation of the D₂ and D₃ (but not of the D₄) receptor subtypes. Preliminary data of this investigation were published in abstract form [17].

Material and Methods

Ethical approval

All procedures described here comply with the guidelines contained in the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (USA), and were approved by the Institutional Committee for Animal's Use in the Center for Research and Advanced Studies (Mexico).

Preparation and dissection

Experiments were performed on neonatal BALB/c mice 6–7 days old (P6-P7) anaesthetized with 10% urethane (2 mg/kg i.p.) before decapitation at cervical level. The thoracic to lumbar cord was exposed in cooled high-sucrose solution. The preparation was pinned ventral side down in a Sylgard-coated Petri dish. A sagittal hemisection of the cord was performed by means of insect pins. Lumbar dorsal roots (DRs) and the sciatic nerve and branches were maintained in continuity. The mixed tibial (Tib), muscle deep peroneal (DP), cutaneous superficial peroneal (SP) and sural (SU) nerves, were dissected free and sectioned distally. The preparation was transferred to a 5 ml bath chamber with artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl 125; KCl 2.5; D-glucose 25; MgSO₄ 1.0; CaCl₂·2H₂O 2; NaH₂PO₄·H₂O 1.25; and NaHCO₃ 26. The oxygenated (95% O₂/ 5% CO₂) ACSF at room temperature (23°C) was perfused at 8–15 ml/min and recirculated. The preparation was then allowed to recover for about 1 h.

Stimulation and recording

The experimental setup is detailed in Figure 1A. Briefly, suction electrodes were used to stimulate electrically peripheral nerves, or a dorsal root (DR), with square pulses (0.1 Hz, 0.2 ms) 2 times the threshold (xT) for the most excitable afferent fibers. EFPs were recorded in the dorsal horn with micropipettes (1–2 MΩ) filled with 2 M NaCl. Micropipettes were placed perpendicularly through the cut surface of the L₄ spinal segments taking into account the EFPs with the largest amplitude (approximately between V–VI Rexed laminae).

PAD was inferred from dorsal root potentials (DRPs) recorded in DRs L₃ or L₄ by means of glass suction electrodes placed on the DR entry zone. EFPs were recorded with a MultiClamp 700B amplifier (Molecular Devices, USA), filtered at 2 KHz, and digitized at 20 KHz using a Digidata 1322A A/D card (Axon Instruments). DRPs were recorded with custom made AC-coupled amplifiers (band pass filter 0.1 Hz – 3 KHz), or with DC-coupled amplifiers (A-M Systems, USA; band pass filter DC – 3 KHz). Raw data were collected with pClamp software (v. 10.2, Molecular Devices, USA) and stored for off-line analysis.

Paired pulse protocol to assess homo- and heterosynaptic depression

The homosynaptic depression protocol was evaluated by means of the paired pulse protocol. Conditioning and test pulses were applied to homonymous low-threshold afferent fibers in the Tib nerve at 2 xT (Fig. 1C). We applied in alternation a sequence of conditioning (a), test (b) and conditioning + test pulses (c), with different inter-stimulus intervals (ISIs) at 0.05

Hz. Offline, the conditioning response was subtracted algebraically from paired responses (c - a) to obtain the test EFPs affected by conditioning stimulation.

For the heterosynaptic depression, the protocol was similar. In this case the conditioning pulse was applied to DR L₃ (2 xT) and the test pulse to heteronymous fibers in DR L₄ (2 xT), at the same ISIs (Fig. 1D).

Pharmacological studies

Stock solutions of drugs (1–100 mM) were prepared and stored at –20°C until needed. All drugs were dissolved in normal ACSF and superfused from separate gravity-fed reservoirs at known concentrations. The agonists DA (endogenous ligand), SKF38393 (D₁-like) and quinpirole (D₂-like), as well as the antagonists raclopride (D₂-like), L-741,626 (D₂), SB277011-A (D₃) and L-745,870 (D₄) (all purchased from Sigma-Aldrich) were superfused for 10–20 minutes each, at a concentration of 1–10 µM, depending on the protocol.

Data Analysis

Short-latency EFPs amplitude was measured from the onset to peak of the monosynaptic component and expressed as percentage of control. DRP amplitude was measured as the area under the curve (AUC) from the onset of depolarization up to 200 ms. All data are expressed as percentage of control values (mean ± SEM), and traces are averages of 12 samples. For statistical comparisons, we used the non-parametric Wilcoxon signed-rank test, unless otherwise stated. All differences were considered significant if $p < 0.05$.

Results

In 73 experiments, we tested the effect of DA both on low-threshold evoked EFPs and DRPs. EFPs and DRPs were evoked by stimulation of the Tib (n = 47), SU (n = 13) and DP (n = 13) nerves. The effect of DA on homosynaptic and heterosynaptic depression of EFPs was tested in 7 experiments for each protocol. The effects of the agonists SKF38393 (D₁-like) and quinpirole (D₂-like) on EFPs and DRPs were tested in 9 and 7 experiments, respectively. The depressant effect of DRPs by quinpirole was subject to prevention (blockade) experiments by: (i) the D₂-like receptor antagonist raclopride (n = 5); and (ii) the selective antagonists at D₂ (L-741,626; n = 4), D₃ (SB277011-A; n = 5) and D₄ (L-745,870; n = 5) receptor subtypes.

Effects of DA on EFPs and DRPs evoked by stimulation of myelinated low-threshold afferents from different nerves

Figure 2A–C shows representative examples of the effect DA (10 µM) on EFPs and DRPs evoked by stimulation of the Tib, SU and DP nerves, respectively. Figure 2D–F shows that DA produced no significant effect on EFPs (8 ± 2 , 5 ± 3 and 13 ± 3 % of control values, respectively) but significantly depressed DRPs by 43 ± 2 , 66 ± 4 , 43 ± 4 % of control values (n = 47, 13 and 13, respectively). The effects were fully reversible after wash.

Actions of DA on the homosynaptic depression used to assess modulation of neurotransmitter release

Homosynaptic depression of Tib-evoked EFPs at strength 2 xT was very robust (84% of control values at 25 ms ISI), and exhibited a long time course (> 10 s) with a time constant of 814 ms (Fig. 3Ai). DA (10 μ M) produced no significant changes in the magnitude of the homosynaptic depression of Tib-evoked EFPs at all tested ISIs (Fig. 3Ai and Aii; Table 1). Recordings from a representative experiment at 50 ms ISI (Fig. 3B) show that there is no significant difference in the magnitude of the homosynaptic depression between control (83%) and in the presence of DA (78%).

Actions of DA on heterosynaptic depression used to assess modulation of pathways mediating PAD

Fig. 4Ai and Aii shows that the magnitude of heterosynaptic depression is smaller and shorter (time constant = 542 ms) than homosynaptic depression (Fig. 3Ai). Bath application of 10 μ M DA produced a significant decrease of heterosynaptic depression at 25–200 ms ISIs (Table 2). Fig. 4B shows recordings from a representative experiment at 50 ms ISI, in which heterosynaptic depression was 32% in control conditions and 9% in the presence of DA.

Does the D₁-like or D₂-like receptor family mediate DA-induced depression of low-threshold evoked DRPs?

Fig. 5A and D shows that the D₂-like receptor agonist quinpirole (10 μ M) mimicked the effects of DA by depressing low-threshold evoked DRPs ($35 \pm 2\%$ of control values, $n = 7$) with no effect on the monosynaptic component of EFPs recorded simultaneously ($-2 \pm 3\%$ of control values; Fig. 5A and C). In contrast, the D₁-like receptor agonist SKF38393 produced no effect on DRPs ($8 \pm 6\%$ of control values, $n = 9$; Fig. 5B and D) or EFPs ($4 \pm 2\%$ of control values; Fig. 5B and C).

Specific activation of the D₂ and D₃ (but not D₄) receptor subtypes depressed low threshold-evoked DRPs

We performed a pharmacological dissection by preventing (blocking) the effect of the D₂-like agonist quinpirole on DRPs with a general and different subtype-selective antagonists. First, we tested the effect of 1 μ M quinpirole in the presence of the D₂-like receptor antagonist raclopride (1 μ M). Fig. 6A and Table 3 show that raclopride produced no significant effect *per se* ($15 \pm 3\%$ of control values, $n = 5$) but prevented the depressant action of quinpirole on DRPs ($20 \pm 4\%$), compared to the effect of quinpirole alone ($32 \pm 2\%$, $n=5$; Table 3).

In order to disclose in more detail the specific contribution of the D₂-like receptor subtypes, we employed selective antagonists (1 μ M) at D₂ (L-741,626), D₃ (SB277011-A) and D₄ (L-745,870) receptor subtypes on the depressant effects of DRPs by quinpirole. Fig. 6B–D shows that the three antagonists produced no significant effect *per se* on DRPs ($10 \pm 6\%$, $11 \pm 3\%$ and $19 \pm 6\%$ of control values; $n = 4$, 5 and 5, respectively). Fig. 6B and C show that the antagonists at D₂ (L-741,626) and D₃ (SB277011-A) receptor subtypes prevented the

DRP depression produced by quinpirole ($17 \pm 1\%$ and $19 \pm 5\%$; $n = 4$ and 5 , respectively). In marked contrast, the antagonist at D_4 (L-745,870) receptor subtype did not prevent the depressant actions of quinpirole on DRPs ($40 \pm 8\%$ of control values; $n = 5$; Fig. 6D). EFPs were not affected by all the antagonists. Table 3 shows the magnitude of the effects depicted in Fig. 6.

Discussion

DA depressed with a similar magnitude DRPs evoked by stimulation of the Tib, SU and DP nerves at strengths 2 xT (Fig. 2). This suggests that DA depressed DRPs evoked predominantly by activation of $A\beta$ /I-II afferents [18], and has a broad depressant action on pathways mediating PAD produced by different sensory modalities. For this reason, in the rest of the experiments we used the mixed nerve Tib, which is more suitable for dissection and stimulation.

DA or quinpirole produced no effect on the monosynaptic component of EFPs (Figs. 2 and 5) suggesting no modulation of synaptic transmission. Sometimes stimulation with strengths 2 xT recruited a second component in the EFPs, which tends to be more sensitive to DA (Fig. 2B) and quinpirole (Fig. 5A). However, this component seems to be oligosynaptic, since it exhibited jittering and it was abolished by high frequency stimulation (not shown).

In the rodent spinal cord homosynaptic depression produced by the stimulation of homonymous afferent fibers is presynaptic in origin, and a frequency- and calcium-dependent phenomenon [19]. We found that DA produced no significant effect on the magnitude and time course of homosynaptic depression as compared to control conditions (Fig. 3). This reinforces the hypothesis that DA produces no effect on synaptic efficacy of low threshold afferent fibers.

We found that DA depresses low-threshold evoked DRPs (Fig. 2) and reduces heterosynaptic depression of EFPs evoked by stimulation of heteronymous afferents (Fig. 4 and Table 2). Consequently, the depression of DRPs by DA suggests an inhibitory modulation of the interneuronal pathways mediating PAD. Heterosynaptic depression has been used to estimate PAD evoked on heteronymous afferents [20]. In fact, the time course of heterosynaptic depression and DRPs is very similar (Figs. 2 and 4), and both are depressed by DA and the $GABA_A$ receptor antagonist bicuculline (not shown). A reduction of heterosynaptic depression of EFPs is interpreted as a decrease in PAD and a relative facilitation of synaptic efficacy of afferent fibers, i.e. a reduction of PSI related to PAD.

Since the D_2 -like receptor agonist quinpirole mimicked the DA effects (Fig. 5), our results demonstrate that the depressant actions of DA on low-threshold DRPs involve the activation of D_2 -like receptors. Given that there are no selective agonists for the subtypes of the D_2 -like receptor family, we prevented the depressant actions of the D_2 -like agonist quinpirole on DRPs by using the general antagonist raclopride, and the subtype-selective antagonists at D_2 (L-741,626), D_3 (SB277011-A) and D_4 (L-745,870) receptors. We found that raclopride, L-741,626 and SB277011-A prevented significantly the depressant effect of quinpirole, suggesting that activation of D_2 and D_3 receptors inhibits interneuronal pathways mediating

PAD (Fig. 6B and C). In marked contrast, the D₄ receptor antagonist (L-745,870) failed to prevent the DRP depression (Fig. 6D). In view that L-745,870 is the only subtype-selective D₄ receptor antagonist available thus far, we cannot categorically discard the role -if any- of this receptor on DRP depression.

Hitherto, we demonstrated that DA depressant effects of DRPs occur most probably in the interneurons mediating PAD. Unfortunately, with our experimental approach, we cannot determine whether the D₂ and D₃ receptors are located on the somata or on interneuron terminals (Fig. 7). The evidence that the D₂-like receptor family modulates Ca²⁺ and K⁺ channels through Gβγ signaling pathways [21], supports the inhibitory role of these receptors in the excitability of interneurons mediating PAD.

Previous studies have revealed, by means of in situ hybridization and quantitative real-time PCR, the presence of all five DA receptor subtypes (D₁₋₅), with dominance of D₂ receptor subtype expression, in the mouse spinal cord [22]. Immunocytochemistry and in situ hybridization studies in rats have revealed expression of the D₂ receptor subtype throughout the neuronal cell bodies in the spinal dorsal horn, and around the central canal [23]. D₃ and D₄ receptor subtypes expression is low as compared to the presence of D₂ receptor subtype [22]. Although the expression of mRNA for D₂ receptor subtype has been described in the mouse DRG of low threshold mechanoreceptors [24], we did not observe significant effects of DA or quinpirole on the monosynaptic component of EFPs. It is not known to what extent the D₂ receptor subtypes are expressed in low-threshold afferent terminals.

In the neonatal rat, DA produces neuromodulatory actions on mammalian motor networks via D₁-like receptors [13]. In our experiments, notwithstanding, we found no effect of the D₁-like receptor agonist SKF38393 on low-threshold evoked EFPs or DRPs (Fig. 5B); this indicates that D₁-like receptors are not involved in such a modulation.

Presynaptic inhibition regulates transmission and processing of sensory information by directly gating sensory inputs on target neurons [25–28]. The amount of sensory feedback required to assist and adjust movements may be controlled by varying the level of PSI in primary afferents [29]. We found that DA reduces DRPs and in fact, this is reflected in a relative facilitation of synaptic transmission (Fig. 4). Therefore, by varying the level of presynaptic inhibition DA could be involved in a finer regulation of the sensory gating. It is known that DA induces and reinforces the locomotor activity in the isolated spinal cord of rodents [13]. It is expected that a reduction in PSI of cutaneous and proprioceptive sensory feedback would contribute even more to this reinforcement.

Conclusion

Altogether, our results suggest that DA modulates cutaneous and proprioceptive sensory information through the activation of raclopride-sensitive D₂-like receptors. The pharmacological profile of these receptors closely resembles that of the D₂ and D₃ receptor subtypes, which are located postsynaptically on the interneurons mediating PAD (Fig. 7). Activation of these receptors produces a relative facilitation of synaptic transmission associated to a decrease of PSI related to PAD.

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Highlights

- Dopamine depresses dorsal root potentials with no effect on afferent transmission
- Depressant effects are mimicked by the D₂-like receptor agonist quinpirole
- D₂ and D₃ receptors are located on primary afferent depolarization pathways
- Activation of D₂ and D₃ receptors leads to a decrease of presynaptic inhibition

Fig 1.

Schematic representation of the experimental setup. **A** Hemisectioned spinal cord and sciatic nerve with dissected nerve branches. Extracellular field potentials (EFPs) recorded at the deep dorsal horn L₄. Dorsal root potentials (DRPs) recorded in dorsal roots L₃/L₄. Peripheral nerves stimulated at strengths 2 xT. **B** Arrangement used for the heterosynaptic depression protocol. Dorsal roots L₃ (DR L₃) and L₄ (DR L₄) stimulated with strengths 2 xT. **C** and **D** Protocols to assess homo- and heterosynaptic depression, respectively (see text). *Tib*, tibial; *cond*, conditioning.

Fig 2.

DA inhibits neuronal pathways mediating PAD evoked by low-threshold afferents from different sensory modalities, with no effect on synaptic transmission. **A–C** EFPs (*upper traces*) and DRPs (*lower traces*) produced by the stimulation of Tib (A), SU (B) and DP (C) nerves. Traces during control conditions (*black*), in the presence of DA 10 μ M (*red*) and after 30 min wash (*blue*). Insets show an expanded segment of EFPs. **D–F** Summary plots of the effect of DA on EFPs (black bars) and DRPs (white bars). * $p < 0.05$ vs control (Wilcoxon test).

Fig 3.

DA produces no effect on homosynaptic depression suggesting that afferent transmitter release is not affected. **Ai** Homosynaptic depression of Tib-evoked EFPs in control conditions (black) and in the presence of 10 μ M DA (red) (n = 7). **Aii** Close view of the first two seconds. **B** Representative recordings of homosynaptic depression in control conditions (black) and in the presence of 10 μ M DA (red), at 50 ms ISI. *Left traces* correspond to test EFPs and *right traces* to EFPs affected by the conditioning stimulus. *p < 0.01 vs control (ANOVA and Bonferroni's test).

Fig 4.

DA inhibits pathways mediating PAD producing a relative facilitation of transmitter release.

Ai Heterosynaptic depression of DR L₄-evoked EFPs produced by conditioning stimulation of DR L₃, in control conditions (black), and in the presence of 10 μM DA (red) ($n = 7$). **Aii**

Close view of the first two seconds. **B** Representative recordings of heterosynaptic depression in control conditions (black) and in the presence of 10 μM DA (red), at 50 ms ISI. *Left traces* correspond to test EFPs and *right traces* to EFPs affected by the conditioning stimulus. * $p < 0.01$ vs control (ANOVA and Bonferroni's test).

Fig 5.

DA depressant effects on low threshold-evoked DRPs are mediated by the activation of the D₂-like receptor family. **A** and **B** EFPs (upper traces) and DRPs (lower traces) produced by the stimulation of the Tib nerve in control conditions (black), in the presence of 10 μ M quinpirole (**A**, red) and 10 μ M SKF38393 (**B**, red), and after wash (blue). Insets show magnified segments of EFPs. **C** and **D** Summary plots of quinpirole (black bars) and SKF38393 (white bars) effects on EFPs (**C**) and DRPs (**D**). *p < 0.01 vs control (Wilcoxon test).

Fig 6.

Activation of the D₂ and D₃ receptor subtypes inhibits neuronal pathways mediating PAD with no effect on low-threshold afferent synaptic transmission. Summary plots of preventive effects of the general D₂-like receptor antagonist raclopride (**A**), as well as the selective antagonists at D₂ (L-741,626; **B**), D₃ (SB277011-A; **C**) and D₄ (L-745,870; **D**) receptors, on the effects produced by quipirole on EFPs (*left*) and DRPs (*right*). *p < 0.05 vs control (Wilcoxon test).

Fig 7.

Schematic representation of the possible location of the D₂ and D₃ receptor subtypes that modulate interneuronal pathways mediating PAD. The PAD circuit comprises a tri-synaptic pathway: glutamatergic low-threshold afferents (blue), first-order glutamatergic interneurons (blue, Glut) and a last-order GABAergic interneurons (red, GABA), contacting afferent fiber terminals axoaxonically. Dopaminergic descending fibers are represented in green. PAD of low-threshold afferents is reduced by the activation of D₂ and D₃ receptor subtypes possibly expressed at the first- and/or last-order interneurons (see Discussion).

Table 1.

DA effects on the homosynaptic depression of low threshold-evoked EFPs at different ISIs.

Homosynaptic depression										
EFP depression (% of control)										
ISI (s)	.025	0.05	.1	.2	.5	1	2	5	10	n
Control	84 ± 2	78 ± 5	71 ± 5	57 ± 3	43 ± 5	35 ± 3	27 ± 2	13 ± 3	3 ± 2	7
DA	70 ± 7	68 ± 5	55 ± 8	48 ± 5	34 ± 8	26 ± 6	17 ± 5	9 ± 2	16 ± 11	7

Values are means ± SEM from the indicated number of experiments (n). There is no statistical difference at any ISI. $p < 0.01$ vs control (ANOVA and Bonferroni's test).

Table 2.

DA effects on the heterosynaptic depression of low threshold-evoked EFPs at different ISIs.

Heterosynaptic depression										
EFP depression (% of control)										
ISI (s)	.025	0.05	.1	.2	.5	1	2	5	10	n
Control	26 ± 2	18 ± 2	17 ± 5	15 ± 4	10 ± 1	5 ± 1	3 ± 1	-1 ± 1	0 ± 1	7
DA	17 ± 2 *	12 ± 2 *	7 ± 3 *	6 ± 3 *	4 ± 2	4 ± 2	1 ± 1	-1 ± 1	0 ± 1	7

Values are means ± SEM from the indicated number of experiments (n). Gray boxes show statistical difference at the corresponding ISI.

* p < 0.01 vs control (ANOVA and Bonferroni's test). DA, dopamine.

Table 3.

Effect of general and selective antagonists on quinpirole effects on EFPs and DRPs

EFP depression (% of control)					
Drugs	Quinpirole (D ₂ -like)	Raclopride (D ₂ -like)	L-741,626 (D ₂)	SB277011-A (D ₃)	L745,870 (D ₄)
Agonist (1 μ M)	-0.2% $\pm$ 2 (5)				
Antagonist (1 μ M)		4 $\pm$ 2 (5)	4 $\pm$ 6 (4)	4 $\pm$ 4 (5)	5 $\pm$ 6 (5)
Antagonist + quinpirole		8 $\pm$ 5 (5)	-4 $\pm$ 5 (4)	-4 $\pm$ 3 (5)	1 $\pm$ 5 (5)
Wash	10% $\pm$ 8 (5)	-1 $\pm$ 7 (5)	2 $\pm$ 5 (4)	-4 $\pm$ 9 (5)	1 $\pm$ 7 (5)
DRP depression (% of control)					
Agonist (1 μ M)	32 $\pm$ 2 (5)				
Antagonist (1 μ M)		15 $\pm$ 3 (5)	10 $\pm$ 6 (4)	11 $\pm$ 3 (5)	19 $\pm$ 6 (5)
Antagonist + quinpirole		20 $\pm$ 4 (5)	17 $\pm$ 1 (4)	19 $\pm$ 5 (5)	40 $\pm$ 8 [*] (5)
Wash	37% $\pm$ 5 (5)	25 $\pm$ 12 (5)	19 $\pm$ 5 (4)	26 $\pm$ 4 (5)	38 $\pm$ 7 (5)

Values are means $\pm$ SEM from the indicated number of experiments. Gray box indicates statistical difference. Experiments with quinpirole alone (first column) were performed separately because the effect is not reversible.

* p < 0.05 vs control (Wilcoxon test).