Opposing modulatory effects of D1- and D2-like receptor activation on a spinal central pattern generator

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Clemens S, Belin-Rauscent A, Simmers J, Combes D. Opposing modulatory effects of D1- and D2-like receptor activation on a spinal central pattern generator. J Neurophysiol 107: 2250–2259, 2012. First published January 18, 2012; doi:10.1152/jn.00366.2011.—The role of dopamine in regulating spinal cord function is receiving increasing attention, but its actions on spinal motor networks responsible for rhythmic behaviors remain poorly understood. Here, we have explored the modulatory influence of dopamine on locomotory central pattern generator (CPG) circuitry in the spinal cord of premetamorphic Xenopus laevis tadpoles. Bath application of exogenous dopamine to isolated brain stem-spinal cords exerted divergent dose-dependent effects on spontaneous episodic patterns of locomotory-related activity recorded extracellularly from spinal ventral roots. At low concentration (2 μM), dopamine reduced the occurrence of bursts and fictive swim episodes and increased episode cycle periods. In contrast, at high concentration (50 μM) dopamine reversed its actions on fictive swimming, now increasing both burst and swim episode occurrences while reducing episode periods. The low-dopamine effects were mimicked by the D2-like receptor agonists bromocriptine and quinpirole, whereas the D1-like receptor agonist SKF 38393 reproduced the effects of high dopamine. Furthermore, the motor response to the D1-like antagonist SCH 23390 resembled that to the D2 agonists, whereas the D2-like antagonist raclopride mimicked the effects of the D1 agonist. Together, these findings indicate that dopamine plays an important role in modulating spinal locomotor activity. Moreover, the transmitter’s opposing influences on the same target CPG are likely to be accomplished by a specific, concentration-dependent recruitment of independent D2- and D1-like receptor signaling pathways that differentially mediate inhibitory and excitatory actions.

dopamine neuromodulation; D1/D2 receptors; locomotion

MONOAMINES EXERT potent modulatory influences on neural networks within the spinal cord, including central pattern generator (CPG) circuits responsible for locomotion (see, e.g., Baldissera et al. 1981; Bras et al. 1989; Jankowska et al. 1997; Machacek and Hochman 2006; MacLean et al. 1998). Typically, D1-like receptors mediate excitatory effects on spinal CPG function, whereas D2-like receptor agonists bromocriptine and quinpirole have no effects on spontaneous locomotor activity (Cowley and Schmidt 1994; Garraway and Hochman 2001; Kiehn et al. 1999; Schotland et al. 1995). While the actions of serotonin (5-HT) and norepinephrine (NE) on spinal circuitry are well documented (Cowley and Schmidt 1994; Garraway and Hochman 2001; Kiehn et al. 1999; Machacek and Hochman 2006; MacLean et al. 1998), there is strong evidence for the presence of both receptor subtypes according to the levels of amine present in the spinal cord (Barriere et al. 2004; Clemens and Hochman 2004; Garraway and Hochman 2001; Han et al. 2007; Han and Whelan 2009; Schotland et al. 1995; Svensson et al. 2003; Wang et al. 2011). This may be attributable to the fact that dopamine has been found to elicit differential and even opposing responses within related components of the same spinal network via the involvement of different dopamine receptor subtypes (Abramets and Samoilovich 1991; Clemens and Hochman 2004; Gallagher et al. 1980; Kemnitz 1997). Furthermore, the activation of spinal CPG circuitry in vitro often requires additional sensory or neurochemical stimulation (Cazalets et al. 1992; Elin et al. 2010; Gabbay et al. 2002; Gordon and Whelan 2006; Juvin et al. 2005). Consequently, pinpointing dopamine-specific effects on spinal CPG function becomes difficult.

Dopamine’s actions are mediated by different receptor subfamilies: D1-like (D1, D5) and D2-like (D2, D3, D4), based on similarities in pharmacology, biochemistry, and structure, and there is strong evidence for the presence of both receptor families in the spinal cord (Barraud et al. 2010; Dubois et al. 1986; Gladwell et al. 1999; Hasegawa et al. 1990; Levant and Hochman 2004; McCarson 2001; Wamsley et al. 1989; Zhu et al. 2007). Typically, D1-like receptors mediate excitatory effects on neurons (Gallagher et al. 1980; Smith et al. 1995), including spinal neurons and circuitry involved in locomotion (Barriere et al. 2004; Han and Whelan 2009; Lapointe et al. 2009), whereas the activation of D2-like receptors hyperpolarizes dorsal root ganglia neurons (Abramets and Samoilovich 1991) and depresses spinal sensorimotor network excitability through inhibitory actions on synaptic transmission (Carp and Anderson 1982; Clemens and Hochman 2004; Gajendiran et al. 1996; Garraway and Hochman 2001; Tamae et al. 2005). Furthermore, D1 and D2 receptors are not only present in the ventral horn of the mammalian lumbar spinal cord, the area in which locomotory CPG circuitry is located, but they may even coexpressed in the same cell types, including α-motoneurons (Zhu et al. 2007). As dopamine has a significantly higher binding affinity for D2-like than D1-like receptors (http://pdp.med.unc.edu/pdp.php), the possibility arises that dopamine’s modulatory effects on the spinal locomotory CPG might be achieved through a differential activation of these receptor subtypes according to the levels of amine present in the extracellular space. Interestingly, a precedence for such divergent extrasynaptic dopaminergic modulation has been identified in the nematode worm Caenorhabditis elegans, in which dopamine can instruct the continuation or inhibition of locomotion via D1- and D2-like receptors that through their differing affinities and opposing actions can mediate antago-
nistic effects on swimming behavior (Chase et al. 2004). However, whether such opposing dopaminergic effects are also exerted on the locomotory CPG circuitry of vertebrates remains unknown.

In premetamorphic *Xenopus laevis* tadpoles, the rhythmic motor output patterns that drive episodes of undulatory tail-based swimming movements continue to be spontaneously expressed by the underlying pattern-generating network in the spinal cord of the isolated central nervous system (CNS) in vitro (Combes et al. 2004; Sillar et al. 2008). Although the modulatory influences of the monoamines 5-HT and NE on axial CPG circuitry in these animals have been described (Rauscent et al. 2006, 2009), the role of dopamine in shaping tadpole locomotory behavior has not been reported. In larval zebrafish, recent evidence has suggested that dopamine, acting via D2-like receptors, selectively suppresses the initiation of spontaneous fictive swimming episodes (Thirumalai and Cline 2008), although the locus of dopaminergic action appears to be supraspinal rather than at the level of the swim CPG circuitry itself. Given that dopamine-containing neurons and their projections are present in the brain and spinal cord of *Xenopus* larvae from embryonic and early postembryonic development (Dulcis and Spitzer 2008; Gonzalez et al. 1994, 1995; Heathcote and Chen 1994; Sanchez-Camacho et al. 2001; Velazquez-Ulloa et al. 2011), we asked what the amine’s functional impact might be on spinal locomotor network operation in vitro.

Here, by recording extracellularly from ventral roots of isolated brain stem-spinal cord preparations of late-stage premetamorphic animals, we provide evidence that exogenously applied dopamine exerts distinct concentration-dependent actions on locomotory central pattern generation, with low doses mediating inhibitory effects on the spontaneous expression of fictive swimming episodes while higher doses promote an increase in episodic CPG activity. Furthermore, D1-like and D2-like receptor agonists mimicked the actions of high and low dopamine doses, respectively, while corresponding receptor antagonists had the opposite effects. Thus our data are consistent with the intriguing possibility that dopamine modulation in the larval *Xenopus* spinal cord varies oppositely according to the amine’s endogenous levels of release, enabling the differential recruitment of suppressive D2-like and permissive D1-like receptor pathways.

**METHODS**

*Animals and dissections.* All experimental procedures were approved by the local ethics committee of the Université de Bordeaux and complied with European Community Directive 86/609/EEC. The general methods used have been described in detail previously (Rauscent et al. 2009) and therefore are only summarized here. Experiments were conducted on a total of 73 stage 50–55 (Nieuwkoop and Faber 1956) larvae of the South African clawed toad, *Xenopus laevis*, bred from an in-house colony to the required stages of oop and Faber 1956) larvae of the South African clawed toad, *Xenopus laevis*, bred from an in-house colony to the required stages of development. Animals were anesthetized in ice-chilled frog Ringer solution (in mM: 120 NaCl, 2.5 KCl, 5 CaCl2, 1 MgCl2, 15 NaHCO3, pH 7.4) containing 230 µg/ml 3-aminobenzoic acid ethyl ester (MS222, “Schlafen powder”; Sigma-Aldrich) and subsequently secured with insect pins under fresh saline in a Sylgard-lined petri dish. After the dorsal part of the brain was exposed, the forebrain was removed and the remaining brain stem together with the spinal cord was dissected out and transferred to a second petri dish for electrophysiological experimentation. For the latter, preparations were placed under circulating saline (see below) equilibrated with 95% O2-5% CO2 and kept at 17°C with a Peltier cooling system and a feedback temperature controller system (HCC-100A; Dagan, Minneapolis, MN).

*Electrophysiology and pharmacology.* For the in vitro experiments, extracellular recordings of spontaneous locomotor-related activity were made with glass suction electrodes placed on selected ventral motor roots at different segmental levels along the spinal cord. The nerve signals were amplified, displayed, and stored on a PC equipped with a data acquisition system (1401 CED; Cambridge Electronic Design) and analyzed off-line with home-written scripts running under Spike2 software (script language, CED).

After stable baseline levels of spontaneous fictive swimming activity were established, which was generally recorded overnight in automated 30-min epochs, selected drugs were bath-applied to the isolated spinal cord for durations of 30–60 min, followed by washout with standard saline. Tests of individual drug carrier substances [ethanol, DMSO, and the antioxidant sodium metabisulfite (Na2S2O5)] at their final concentrations used in the experiments did not lead to any of the effects on locomotory activity that were observed with the additional presence of their respective drug.

All dopaminergic drugs were initially bath-applied through an open-loop peristaltic pump supply that was switched to closed-loop circulation after 10–15 min. By this time, the volume of the bath had been exchanged three to five times and corresponded to the time frame in which the onset of any drug-induced effects (including those of dopamine) on spontaneous locomotor-related activity was found to occur. Recordings were made throughout the ensuing 30- to 60-min period of drug exposure. Dopamine was applied in concentrations of 2 or 50 µM (cf. Clemens and Hochman 2004), while the dopamine D2 receptor-selective agonists bromocriptine (RBI, Sigma) and quinpirole (Tocris) were applied at a concentration of 10 µM. Raclopride (Tocris), a D2-like receptor antagonist (and an inverse agonist of the D3 receptor subtype), was bath-applied at a concentration of 25 µM. The D1-like receptor agonist SKF 38393 and antagonist SCH 23390 (both from Tocris) were administered at final concentrations of 100 and 50 nM, respectively.

The effects of several dopaminergic substances were often compared in the same experiment. In these cases, the preparations were washed thoroughly for at least 30–60 min with normal saline between individual drug applications to ensure that spinal motor activity had returned to control levels. To exclude potential lingering effects of the sequential drug applications on spinal activity, we also tested whether the order in which the drugs were applied might play a role in the modulatory effects observed. However, we found no correlation between the order of application of the drugs tested and the modulatory effects they induced.

*Data analysis.* SigmaPlot 11 (SPSS Science, Chicago, IL) was used to analyze data and test for significant differences in the course of an experiment, using parametric or nonparametric tests where appropriate. All values are given as means ± SE. Differences were considered significant when *P* < 0.05.

**RESULTS**

*Spontaneous fictive locomotion in vitro.* Extracellular recordings from spinal ventral roots of premetamorphic *Xenopus* stage 50–55 tadpoles were consistent with previously reported findings (Combes et al. 2004; Rauscent et al. 2009) that the isolated brain stem-spinal cord of these animals is capable of spontaneously expressing intermittent episodes of fictive swimming that are appropriate for driving undulatory axial swimming movements in the intact animal. In the present study, to target the specific modulatory actions of dopamine in the spinal cord in vitro, a Vaseline wall was placed across the junction of the brain stem and cord (at the level of the obex) to allow differential
perfusion of these two regions with normal saline or saline plus dopaminergic drugs, respectively (Fig. 1A), while enabling access of descending brain stem pathways necessary for the production of spontaneous fictive swimming. Such spinal motor activity, which consisted of repeating episodes of alternating bursts in ventral roots on opposite sides of the cord (Fig. 1B1), was recorded in 30-min epochs, during which the number of episodes and the interval between the onsets of consecutive episodes (episode cycle period) were determined (Fig. 1B2).

Under initial control conditions, an aggregate analysis of such episodic patterns of fictive swimming revealed a considerable variability between preparations. Overall, burst durations within episodes ranged from 0.05 to 3.8 s (mean 0.072 ± 0.01 s), while episode lengths varied from 0.02 to 40.4 s (mean 0.27 ± 0.1 s). For a given preparation, however, spontaneous baseline swimming activity in the absence of drug application was usually very stable for up to 12–15 h, although the number of swim episodes expressed per 30-min epoch could range from 5 to as many as 30–40 episodes in different preparations (n = 73). Consequently, the effects of subsequently applied

**Fig. 1.** Experimental setup and parameters of fictive swimming measured in the study’s raw data analysis. A: isolated brain stem-spinal cord preparation in vitro. A Vaseline wall positioned between the brain stem and spinal cord allowed selective superfusion of the cord with dopaminergic drugs, while the brain stem was exposed to normal saline. Suction electrodes were placed on spinal ventral roots (vrs) on opposite sides of the cord to record spontaneous left (L)-right (R) alternating fictive locomotion. B: typical swimming-related activity recorded from a spontaneously active preparation under normal saline application to both the brain stem and spinal cord. B1: a continuous 30-min epoch recorded from the left and right vrs of segment 16. Note the regular periodic expression of brief episodes of fictive swimming interspersed with periods of silence. B2: time-expanded recording of a typical fictive swim episode in the 30-min epoch showing the onset of alternating bursting activity in the recorded bilateral vrs, which could last for several seconds before terminating spontaneously. Parameters analyzed in this study were 1) no. of episodes per 30-min epoch, 2) no. of bursts per epoch, 3) durations of individual fictive swim episodes, and 4) overall cycle periods of episodes (i.e., interval between onsets of successive swim episodes).

**Fig. 2.** Opposing effects of bath-applied low (A–C) and high (D–F) dopamine (DA) concentrations to the spinal cord on the expression of spontaneous fictive swimming. A: representative 30-min epoch of spontaneous locomotor-related activity recorded in ipsilateral vrs of spinal segments 16 and 20 (i) and a time-base expanded example of a single swim episode (ii). Tilted dashed line indicates the rostro-caudal delay in vr activation that typifies fictive swimming. B: application of 2 μM dopamine to the spinal cord of the same preparation reduced the occurrence of swim episodes and increased episode durations and cycle periods. C: effects of low dopamine were reversed by normal saline washout. D: 30-min epoch recording (i) and expanded traces of a single episode (ii) of spontaneous fictive swimming in a different preparation under control saline conditions. Here, the recorded left and right vr16 displayed typical burst alternation (see dashed line). E: bath application of 50 μM dopamine increased episodic occurrences of central pattern generator (CPG) activity and decreased episode durations and cycle periods. F: as with low dopamine, the effects were completely reversible with normal saline washout. Note that neither low (B) nor high (E) dopamine caused consistent changes in the durations or intensities of bursts within episodes. G: group analysis from 9 preparations showing that 2 μM dopamine significantly decreased the mean number (expressed as % change from control fictive swimming, indicated as 0%) of bursts per 30-min epoch, whereas 50 μM dopamine significantly increased the overall burst number per epoch. H: application of 2 μM dopamine also significantly decreased the mean number of swim episodes per epoch, while application of 50 μM dopamine increased episode number. I: 2 μM and 50 μM dopamine tended to increase and decrease episode durations, respectively, although not significantly. J: 2 μM dopamine significantly increased the cycle periods of episodes, while 50 μM dopamine significantly decreased episode periods. Note that here and in subsequent figures, the SE ranges for the respective controls are denoted by dashed horizontal lines on either side of the 0% baseline, while significant differences (P < 0.05) from this baseline are indicated by asterisks.
exogenous dopamine or dopamine receptor agonists and antagonists on fictive swimming were assessed in equivalent 30-min recording epochs by normalizing data to the respective control saline measurements for each preparation.

**Differential dopamine modulation of spinal CPG activity.** The influence of dopamine on swim circuit output was tested by bath-applying the amine at different low (2 μM) and high (50 μM) concentrations. This distinct two-tier administration was motivated by previous findings that endogenous dopamine at <1 μM is present in the extracellular space of the rat CNS (Smith et al. 1992) and bath-applied dopamine at 1–5 μM is sufficient to selectively recruit high-affinity D2-like receptor pathways (Clemens and Hochman 2004; also see DISCUSSION). Moreover, since exogenous dopamine concentrations >50 μM have been found to activate low-affinity D1 receptors in the rodent spinal cord (e.g., Barriere et al. 2004; Han et al. 2007), we applied a similar elevated concentration in order to distin-

![Graphs and charts](http://jn.physiology.org/)

**A i** Control  
**B i** 2 μM DA  
**C i** Wash  
**D i** Control  
**E i** 50 μM DA  
**F i** Wash  

**A ii**  
**B ii**  
**C ii**  
**D ii**  

**G** Bursts / 30 min  
**H** Episodes / 30 min  
**I** Episode durations  
**J** Episode cycle periods  

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guish D1-like from D2-like receptor-mediated actions in our spontaneously active *Xenopus* preparations.

Application of low dopamine to the spinal cord (n = 9) consistently led to an overall decrease in the episodic occurrences of spontaneous bursting activity and a corresponding increase in the cycle periods of burst episodes. As illustrated in the representative ventral root recordings from a single experiment in Fig. 2, A–C, and the group analyses of Fig. 2, G–J, there was a significant decrease in the total number of bursts (by 63.6 ± 18.2% of control, P = 0.005; Fig. 2G) and episodes (by 70.9 ± 8.7%, P = 0.002; Fig. 2H) per 30-min epoch. Furthermore, although there was a tendency toward an increase in the durations of individual episodes (by 58% ± 42.3%, P = 0.34; Fig. 2I; see also Fig. 2B), the cycle periods of episodes were significantly increased (by 183.8 ± 57.5%, P < 0.001; Fig. 2J). These effects, which were reversed by subsequent washout with normal saline (Fig. 2), therefore indicated that the presence of low levels of dopamine in the tadpole spinal cord exerts an overall inhibitory effect on the spontaneous expression of episodic swimming activity.

In direct contrast, bath application of 50 μM dopamine to spinal cords (n = 10) led in nine preparations to an overall increase in rhythmic ventral root bursting activity (see single experiment in Fig. 2, D and E, and group analysis in Fig. 2, G–J). This enhancement, which was also reversible (Fig. 2F), included a significant increase in both the total number of individual bursts (by 97 ± 31.5%, P = 0.026; Fig. 2G) and episodes per epoch (by 272.9 ± 68.2%, P < 0.001; Fig. 2H) as well as a concomitant decrease in episode cycle period (by 52.8 ± 6.2%, P < 0.001; Fig. 2J). However, as with 2 μM dopamine exposure, episode durations were not significantly altered (P = 0.35; Fig. 2I), nor were consistent changes observed in either the duration or the intensity of impulse bursts within episodes (see Fig. 2, B and E; cf. Fig. 2, A and C and D and F, respectively). These effects were thus in direct opposition to those observed with low dopamine levels, suggesting that at elevated concentrations dopamine’s modulatory actions on the same spinal CPG circuitry switched in a concentration-dependent manner from functional inhibition to excitation of the swimming CPG network.

*D2-like receptor agonists mimic effects of low dopamine.* We next assessed whether the intervention of different dopamine receptor subtypes might be responsible for the divergent dose-dependent influences of exogenous dopamine application. Since the D2 receptor subfamily has a relatively high affinity for dopamine (Clemens et al. 2006) and is known to mediate inhibitory influences in the spinal cord (e.g., Clemens and Hochman 2004; Tamae et al. 2005), we first examined whether this subfamily was also involved in dopamine’s depressant effects on tadpole fictive swimming. To this end, D2-like receptor-prefering agonists and antagonists (Fig. 3) were applied to the spinal cords of isolated CNS preparations. Bath application of bromocriptine (10 μM; n = 4) or quinpirole (10 μM; n = 2) consistently mimicked the effects of low exogenous dopamine application, leading to a significant decrease in the numbers of both bursts per episode (by 48.8 ± 19.3% of control, P = 0.045; Fig. 3A) and fictive swim episodes within epochs (by −40.0 ± 23.4%, P < 0.001; Fig. 3B). In contrast, while episode durations were again not modified by the D2 agonists (P = 0.88; Fig. 3C), there was a tendency, albeit statistically insignificant, for episode cycle periods to increase (by 21.2 ± 7.5%, P = 0.075; Fig. 3D).

We next asked whether blocking D2 receptors could occlude the effects of low dopamine on spontaneous CPG activity. Bath application of the D2-like receptor antagonist raclopride (25 μM) led to a slight, but insignificant, increase in the expression of fictive swimming in four of five preparations (data not shown), consistent with a suppressive effect of the antagonist on a basal release of endogenous dopamine that was presumably occurring spontaneously within the isolated spinal cord. To better reveal the consequences of D2 receptor inactivation, therefore, we applied raclopride in combination with 2 μM dopamine, which, as seen above (Fig. 2), exerted a global inhibitory influence on spinal CPG activity.

Application of raclopride in the presence of low exogenous dopamine reversed the latter’s inhibitory actions, leading instead to a significant increase in the overall number of ventral root bursts (by 101.4 ± 34.7% of control, P = 0.027; Fig. 3A) and a slight increase in the number of episodes (by 55.9 ± 25.5%, P = 0.071; Fig. 3B). Additionally, while the D2 antagonist did not significantly modify episode durations (P = 0.075; Fig. 3C), it caused a significant decrease in episode cycle periods (by 36.6 ± 6.5%, P < 0.001; Fig. 3D). Again, these effects were reversed by washout with control saline. These data therefore indicated that the application of low dopamine concentrations during the concomitant blockade of D2 receptors at least partly replicated the effects of high dopamine levels whereby the amine’s action on spinal CPG activity became inverted from global inhibition to excitation. This switch in the absence of functional D2 receptors was presumably due to the continued activation of a sufficient number of D1 receptors to exert a facilitatory action.

**D1-like receptor agonists mimic effects of high dopamine application.** Are the effects of high dopamine levels (50 μM) mimicked by agonist activation of D1-like receptors, and alternatively, blocked by the latter’s inactivation? Bath exposure of spinal cords to the D1-like receptor agonist SKF 38393 (100 nM) consistently led to a significant increase in the number of bursts per epoch in all four preparations tested (by 1,103 ± 200% of control, P = 0.005; Fig. 4A) and a tendency for the number of fictive swim episodes to increase (by 284.6 ± 129.8%, P = 0.09; Fig. 4B). As with high dopamine application, the D1 receptor agonist did not significantly modify episode durations (P = 0.017; Fig. 4C), but it significantly decreased episode cycle periods (by 54.7 ± 5.2%, P < 0.001; Fig. 4D).

In contrast, when the D1-like receptor antagonist SCH 23390 (at 50 nM) was applied in the absence of any additional dopamine (n = 3), the number of bursts per epoch decreased significantly (by 82 ± 14% of control, P = 0.004; Fig. 4A), as did the number of swim episodes (by 89.4 ± 4.4%, P < 0.001; Fig. 4B). In contrast, both episode durations (Fig. 4C) and cycle periods (Fig. 4D) showed a tendency to increase. Moreover, in one additional preparation (data not shown) SCH 23390 caused a complete suppression of spontaneous fictive swimming throughout the ensuing 30-min recording. These data indicate that the modulatory actions of high dopamine levels in the spinal cord are indeed mediated by D1-like receptor pathways, and that a blockade of these pathways leads to a decline or even a shutdown of locomotor CPG activity.

**Blockade of opposing receptor pathways does not alter actions of D1- or D2-like receptor agonists.** Finally, to further assess the specificity of the modulatory effects of D1-like versus D2-like receptor activation on fictive swimming we...
conducted a series of experiments in which spinal cords were subjected to either D1 or D2 receptor activation while the other receptor subfamily was blocked. Application of the D2-like receptor agonist quinpirole in the presence of the D1-like receptor antagonist SCH 23390 (\(n = 3\) experiments) led to a sharp decline in the total number of burst cycles per 30-min epoch (to 40%, 37%, and 7% of control activity, respectively). Conversely, administration of the D1-like receptor agonist SKF 38393 in the presence of the D2 antagonist raclopride had effects on burst cycles similar to those observed during application of the D1 agonist alone. This drug mix led in all experiments (\(n = 4\)) to a strong increase in burst number per 30-min epoch, in three cases reaching from \(\sim 500\)% up to \(1,500\)% of control values.

Moreover, although episode cycle periods were apparently unaffected by the presence of the “inhibitory” D2 agonist-D1 antagonist mixture (\(P = 0.9\); possibly because of the near-complete loss of bursting activity during this treatment), the

![Fig. 3. Effects of bath-applied D2 agonists (gray bars) and an antagonist (black bars) on fictive swimming. A: the D2 receptor agonists bromocriptine and quinpirole (each at 10 \(\mu M\)) significantly reduced the number of bursts per 30-min epoch. In contrast, in the presence of low dopamine (2 \(\mu M\)), the D2 receptor antagonist raclopride strongly increased the number of bursts per epoch. B: application of both D2 agonists also significantly decreased the number of episodes per epoch, while raclopride slightly increased episode cycle periods. C and D: the D2 agonists did not significantly alter episode durations (\(C\)) or episode cycle periods (\(D\)), although the receptor antagonist raclopride had a tendency to increase episode periods (\(C\)), in contrast to raclopride, which significantly decreased the cycle periods of episodes (\(D\)).](http://jn.physiology.org/)

![Fig. 4. Effects of bath application of the D1 receptor agonist SKF 38393 (100 nM; gray bars) and the antagonist SCH 23390 (50 nM; black bars) on fictive swimming. A: the number of bursts per 30-min epoch increased significantly under agonist application but decreased under the antagonist application. B: SKF 38393 increased, albeit insignificantly, the mean number of swim episodes per epoch, whereas application of SCH 23390 significantly decreased the mean number of swim episodes from control. C and D: as with high dopamine, the D1 agonist did not significantly modify episode durations (\(C\)) but significantly decreased episode cycle periods (\(D\)). In direct contrast to the agonist’s effects, the D1 receptor antagonist SCH 23390 showed a tendency to increase both episode durations and cycle periods, although not significantly.](http://jn.physiology.org/)
“excitatory” D1 agonist-D2 antagonist pairing not only increased overall bursting but also led to a corresponding and significant decrease in episode cycle periods (from 247 ± 74 s to 74 ± 9 s; \( P = 0.004 \)). Thus a concomitant blockade of either the D1- or D2-like receptor-mediated pathways did not interfere with the activation of the other receptor subtype and its ability to produce corresponding overall changes in spontaneous fictive swimming.

Taken together, therefore, our findings provide strong pharmacological evidence that dopamine can act in an opposing and bimodal manner on spontaneously expressed locomotor output from the tadpole spinal cord. At low exogenous levels dopamine exerts inhibitory influences on the swim CPG through the specific activation of D2-like receptor-mediated signaling pathways, whereas at higher concentrations the amine’s modulatory actions switch to become excitatory via the recruitment of independent D1-like receptor-mediated pathways.

**DISCUSSION**

The multifaceted interplay between neuromodulatory influences and the central pattern-generating networks they control can give rise to a wide variety of rhythmic motor outputs (Harris-Warrick and Marder 1991; Marder 2000; Marder and Calabrese 1996; Nadim et al. 2008). Different neuromodulators can alter a CPG’s output differently and in a systemwide manner (Hasselmo 1995; Heckman et al. 2008; Katz et al. 1994; Pfliiger 1999; Viemari and Tryba 2009), but there is also evidence that they can exert their influence with a high spatial and temporal resolution according to the dynamically changing needs of the system in question (Clemens et al. 1998, 2007; Fadool and Ache 1992; Kiehn and Katz 1999; Kiehn and Kjaerulff 1996; Marder and Bucher 2007). The different consequences of neuromodulation generally arise from the actions of different transmitters on the same target network (see, e.g., Coleman et al. 1992; Marder 2000; Marder and Calabrese 1996; Marder and Rehm 2005; Nusbaum et al. 2001) or from the actions of the same transmitter on different components of a given circuit (Fickbohm and Katz 2000; Katz and Frost 1996). However, it is less well known whether a single neuromodulator can alone exert distinct actions on the same neural circuit as a function of its concentration. Moreover, how this can be achieved and what the behavioral consequences of such differential actions might be remain unclear.

In this study we provide evidence for dose-dependent modulatory influences of dopamine and related selective ligands on spontaneously generated, episodic fictive swimming in the spinal cord of late premetamorphic *X. laevis*. Specifically, we found that 1) low levels of exogenously applied dopamine (2 \( \mu \)M) to the spinal cord exert overall suppressive effects on swim episode expression, while high levels of dopamine (50 \( \mu \)M) produce permissive effects; 2) D2-like receptor-specific agonists mimic the low-concentration dopamine actions, while D1-like receptor agonists replicate the actions of high amine levels; and 3) antagonists for D1-like or D2-like receptors have effects opposite to their respective agonists. Our data therefore suggest that in *Xenopus* tadpoles changes in intraspinal dopamine concentrations are capable of conferring opposite behavioral outcomes, and that these opposite actions on the spinal CPG for swimming are mediated by the differential activation of D1 or D2 receptor pathways, respectively. These bimodal neuromodulatory actions of dopamine are summarized in Fig. 5. According to this proposal, a relatively modest activation of dopamine-containing pathways, presumably descending from the brain stem, preferentially activates high-affinity D2-like receptor pathways that in turn mediate inhibitory actions on the spinal swim CPG network. In contrast, with the presence of higher magnitudes of dopamine in the spinal cord, which would presumably result from stronger descending pathway activation, lower-affinity D1-like receptor pathways in the same CPG circuitry become additionally recruited, and their engagement now overrides the depressive actions mediated by the D2-like receptors and leads to a switch to an overall facilitatory CPG response.

Previous anatomical evidence indicates that dopaminergic innervation in the mammalian spinal cord is both synaptic and nonsynaptic, suggesting that dopaminergic signaling may occur via both conventional synaptic and volume transmission (Ridet et al. 1992). It is not yet known, however, whether such dual-release mechanisms are also present in the larval *Xenopus*
spinal cord. Furthermore, the differential modulatory effects we observed in vitro derived from the bath application of exogenous dopamine at two distinct concentrations. We are therefore unable to predict whether the amine’s normal physiological actions might be to instruct abrupt on/off transitions in locomotor behavior or to produce progressive behavioral changes through the detection of a continuum of effective dopamine concentrations at spinal synapses.

Dopamine is already known to exert differential modulatory influences on spinal sensorimotor and locomotor circuitry. For example, low dopamine levels (1–5 μM) depress the amplitudes of excitatory synaptic currents in the deep dorsal horn (Garraway and Hochman 2001) and of spinal reflexes (Clemens and Hochman 2004), with both actions being mediated primarily by the preferential recruitment of high-affinity D2-like receptor pathways. In contrast, higher levels of exogenous dopamine (50–100 μM) have been found to increase spinal motoneuron excitability and premotor synaptic transmission in mice (Han et al. 2007; Han and Whelan 2009) and, at still higher concentrations (100–1,000 μM), induce locomotor CPG rhythmogenesis in newborn rats (Barriere et al. 2004; Kiehn and Kjaerulff 1996), in all cases via the activation of D1- rather than D2-like pathways (Han et al. 2007; Han and Whelan 2009). Thus while divergent dose-dependent effects of dopamine in the spinal cord have been reported, these influences have tended to be both receptor- and circuit specific, i.e., either inhibitory (D2-mediated modulation of sensory input pathways) or excitatory (D1-mediated modulation of motor output commands). Our results from spontaneously active preparations of the tadpole spinal cord suggest that a dose-dependent modulatory shift can also occur within a single neuronal assemblage, enabling the CPG network for rhythmic swimming movements to react to different dopamine levels by switching oppositely between decreased or increased locomotory responses. Although opposing amnergic modulatory influences on spinal locomotory activity patterns in larval Xenopus have been reported previously (Rauscent et al. 2009), these divergent actions are ascribed to two different amines (5-HT and NE), unlike the opposite effects described here, which are accomplished by the same transmitter molecule.

It is also noteworthy that we did not observe long-lasting excitatory effects of exogenous dopamine as reported in the isolated neonatal rat spinal cord (Barriere et al. 2004). This may be due to the very different “high” levels of dopamine applied in the two studies (see above) or to species differences in the intrinsic properties and dopamine sensitivities of the underlying CPG networks themselves, which in the case of rodents (and in contrast to Xenopus) are unable to operate spontaneously in vitro without extrinsic neurochemical stimulation.

Application of the D2-like receptor agonists bromocriptine and quinpirole to a large extent mimicked the modulatory responses induced by low dopamine levels, whereas superfusion of the isolated tadpole cord with the D1-like receptor agonist SKF 38393 replicated the effects observed with high dopamine levels. The consistency of the effects induced by the different levels of exogenous dopamine, D1-like and D2-like receptor agonists, and their respective receptor antagonists, therefore further supports the conclusion that endogenous dopamine release can recruit different second messenger signaling pathways as a function of changing levels of neuromodulator to which the spinal locomotory circuitry is exposed.

The extracellular recording techniques used in our study did not allow the intracellular pathways involved in changing the swim CPG output to be identified, although alterations in dopamine signaling are known to modify glutamate receptor expression and synaptic connectivity in the mammalian CNS in a region-specific manner (Lidow et al. 2001; Tarazi et al. 2002) and recent data have suggested that D1-like but not D2-like receptor pathways can enhance AMPA channel-mediated glutamatergic transmission onto neonatal mouse lumbar motoneurons (Han and Whelan 2009). Moreover, both D1- and D2-like receptor subtypes are present in the ventral horn of the mammalian spinal cord (Zhu et al. 2007), the area where locomotory circuitry is located.

Changes in dopamine receptor activity can also modify expression of G proteins and cause specific upregulation of the G protein in that cascade (Hervé and Girault 2005). In addition, abnormalities in dopamine systems can result in altered expression of proteins (Chen and Gurlung 1999; Lidow et al. 2001) that interact with dopamine receptors in multiprotein complexes (Koh et al. 2003). Thus a number of important substrates are known to exist that potentially could enable different dopamine levels in the spinal cord, via a concentration-dependent recruitment of D1-like or D2-like receptor pathways, to exert divergent modulatory actions on the same target CPG network.

It is interesting that a bimodal action of dopamine signaling similar to that found here in Xenopus larvae has been reported in C. elegans (Chase et al. 2004; Jorgensen 2004), in which the amine’s presence can have antagonistic effects on locomotion, instructing the worm to terminate or initiate swimming, depending on the type (D2- and D1-like, respectively) of receptor activated. However, although the two receptor subtypes are coexpressed in nematode locomotory motoneurons, it is unclear how dopamine’s opposing actions are exerted at the central network level, if dopamine’s respective receptor subtypes are also targeted within the swim circuit, or indeed whether the same or different networks are involved (Jorgensen 2004). Here, we present evidence that in a vertebrate rhythmic motor system a single neuromodulator has the potential to act differently on the same central neural circuitry according solely to the magnitude of its concentration present, and that differences in levels of release could enable a dynamic switch in dopaminergic modulatory actions within the spinal cord.

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No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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