Journal of Neurochemistry, 2007, **102**, 1115–1124 doi:10.1111/j.1471-4159.2007.04621.x

Paradoxical modulation of short-term facilitation of dopamine release by dopamine autoreceptors

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Abstract

Electrophysiological studies have demonstrated that dopaminergic neurons burst fire during certain aspects of reward-related behavior; however, the correlation between dopamine release and cell firing is unclear. When complex stimulation patterns that mimic intracranial self-stimulation were employed, dopamine release was shown to exhibit facilitated as well as depressive components (Montague *et al.* 2004). Understanding the biological mechanisms underlying these variations in dopamine release is necessary to unravel the correlation between unit activity and neurotransmitter release. The dopamine autoreceptor provides negative feedback to dopamine release, inhibiting release on the time scale of a few seconds. Therefore, we investigated this D_2 receptor to see whether it is one of the biological mechanisms responsible for the history-dependent modulation of dopamine release. Striatal dopamine release in anesthetized rats was evoked with stimulus trains that were designed to promote the variability of dopamine release. Consistent with the well established D_2-mediated autoinhibition, the short-term depressive component of dopamine release was blocked by raclopride, a D_2 antagonist, and enhanced by quinpirole, a D_2-receptor agonist. Surprisingly, these same drugs exerted a similar effect on the short-term facilitated component: a decrease with raclopride and an increase with quinpirole. These data demonstrate that the commanding control exerted by dopamine autoreceptors over short-term neuroadaptation of dopamine release involves both inhibitory and paradoxically, facilitatory components.

Keywords: carbon-fiber microelectrode, dopamine autoreceptor, dopamine, *in vivo* voltammetry, synaptic plasticity.
J. Neurochem. (2007) **102**, 1115–1124.

Measurements in both alert primates (Waelti *et al.* 2001; Tobler *et al.* 2005) and rats (Pan *et al.* 2005) have shown that dopaminergic neurons exhibit phasic activity during reward related tasks that follows the pattern of a prediction error, one of the basic tenets of learning theory (Schultz *et al.* 1997; Schultz and Dickinson 2000). Transient firing of dopaminergic neurons leads to release in terminal regions that has the form of a transient increase in extracellular dopamine that is subsequently returned to baseline as a consequence of clearance by the dopamine transporter (Chergui *et al.* 1994; Wightman and Robinson 2002). This release and uptake can be directly measured with rapid voltammetric techniques. Initial modeling of dopamine release evoked by burst firing suggested that the concentration released by each impulse was constant (Wightman *et al.* 1988), but subsequent studies showed that the concentration of released dopamine fluctuates, exhibiting both facilitation and depression based on stimulation history (Garris *et al.* 1999; Yavich and MacDonald 2000; Cragg 2003; Montague *et al.* 2004).

Early voltammetric measurements showed that a long-term (minutes) depression of dopamine release occurred with prolonged stimulation (Ewing *et al.* 1983; Michael *et al.* 1987) or rapidly repeated trains (Yavich 1996; Yavich and MacDonald 2000). This depression was shown to depend on the capacity of the newly synthesized, readily releasable pool and the ability of a reserve pool of dopamine to restore the releasable compartment. Recent

Received February 23, 2007; accepted March 10, 2007.

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Abbreviations used: D_1 and D_2, dopamine receptors; LTP, long term potentiation.

work suggests that the reserve pool is maintained by synapsin, a protein that interacts with the surface of synaptic vesicles (Venton *et al.* 2006). Another critical control point that influences the relationship between impulse flow and dopamine release is the D_2-like dopamine autoreceptor. Inhibition of the dopamine autoreceptor potentiates release of dopamine (Gonon and Buda 1985; Yavich 1996) because activation of the autoreceptor causes inhibition of dopamine synthesis and a lowering of the release probability (Sibley 1999). Activation of the autoreceptor inhibits dopamine release rapidly, operating on a second time scale (Phillips *et al.* 2002).

We have recently investigated dynamic changes in dopamine release using a mathematical model that incorporates both facilitatory and depressive components (Montague *et al.* 2004). Using stimulation conditions similar to those imposed during intracranial self-stimulation of dopamine cell bodies (Wise 2004), dopamine release was found to be modulated by a short-term facilitation component, a short-term depression component, and a long-term depression component (Montague *et al.* 2004). Thus, the model enabled us to quantify the previously described facilitation and depression of dopamine release. Here, we consider the neurobiological correlates of these components. The long-term depression has been attributed to the slow refilling of the releasable pool. Mechanisms underlying the short-term facilitation and depression terms have not been assigned, although an autoreceptor mechanism seems likely for the short-term depression component. In this work we directly probe the role of the dopamine autoreceptor by administering exogenous D_2 antagonist and agonist and examining electrically stimulated release. We find that activation of the D_2 receptor not only causes depression of dopamine release but that the same treatment also augments the short-term facilitation. In contrast, blockade of the D_2 receptor removes both short-term components leaving only a long-term depression factor that regulates dopamine release. These findings indicate that the plasticity of short-term changes in dopamine release is a direct consequence of D_2-dopamine autoreceptor activation.

Methods

Animals and surgery

Male Sprague–Dawley rats (225–350 g; Charles River, Wilmington, MA, USA) were anesthetized with urethane (1.5 g/kg, i.p.) and placed in a stereotaxic frame (Kopf, Tujunga, CA, USA). A heating pad (Harvard Apparatus, Holliston, MA, USA) maintained a constant body temperature of 37°C. Holes were drilled in the skull for the working, reference, and stimulation electrodes at coordinates selected from the atlas of Paxinos and Watson (Paxinos and Watson, 1986). The carbon-fiber microelectrode was placed in the striatum (AP +1.2, ML +2.0, and DV −4.5). The stimulating electrode was placed in the substantia nigra (AP −5.2, ML +1.0, and DV −7.5). Both the carbon-fiber and stimulating electrodes were adjusted in the dorsal–ventral coordinate while stimulating to achieve maximal dopamine release. An Ag/AgCl reference electrode was inserted in the contralateral side.

Electrical stimulation

An untwisted bipolar stimulating electrode (Plastics One, Roanoke, VA, USA) was used to stimulate dopaminergic neurons. The stimulus was provided by an analog stimulus isolator (A-M Systems, Sequim, WA, USA). The stimulation train consisted of biphasic pulses (±300 μA, 2 ms/phase unless otherwise noted). The frequency and number of pulses per train were varied as noted in the text. Stimulations used were designed to mimic those for which an animal will lever-press during intracranial self-stimulation. The pulses were generated by a computer and applied between the cyclic voltammograms to avoid electrical interference.

Electrochemistry

Cylindrical carbon fiber microelectrodes were prepared using T650 carbon fibers (3 μm radius, Amoco, Greenville, SC, USA) and encased in glass capillaries (A-M Systems) and pulled with a micropipette puller (Narashige, East Meadow, NY, USA). The protruding fiber was then cut to a length of 50–100 μm. On the day of use, the electrode was soaked for 10 min in isopropanol purified with activated carbon (Bath *et al.* 2000). To make contact with the carbon fiber, a wire coated with silver paint was inserted into the open end of the capillary and twisted to ensure solid contact with the fiber. The wire was then secured using epoxy. The reference electrode was chloridized by placing a silver wire in an HCl solution and applying 5 V.

Fast-scan cyclic voltammetry was used in all experiments (Bath *et al.* 2000). The instrumentation controlled the potential of the carbon-fiber electrode while the reference electrode was held at ground potential. The potential of the working electrode was held at −0.4 V versus Ag/AgCl between scans and was ramped to +1.0 V at 300 V/s and repeated at a frequency of 10 Hz. After the experiment the working electrode was calibrated *in vitro* using dopamine solutions of known concentration.

Data analysis

Data were analyzed in Graph Pad Prism (Graph Pad Software, San Diego, CA, USA) and are expressed as mean ± SEM. Statistical significance was determined using a two-way ANOVA, and *post hoc* comparisons were performed using the method of least squares with a Bonferroni correction.

Peak amplitude for each stimulation train was calculated by subtracting the difference between the concentration at the base and the apex of the response. This minimizes the effect produced by the inhibition of uptake which slows the return to baseline. For experiments involving five stimulation trains, three post-drug files were collected and averaged to fully capture the effect of the drug. Each post-drug train was then normalized to its corresponding pre-drug train to compare the overall facilitation and depression at each train in the pattern.

Simulations of the data were performed on the basis of the dynamic model described previously (Montague *et al.* 2004). The model was developed to predict dopamine release when the neuron is exposed to a

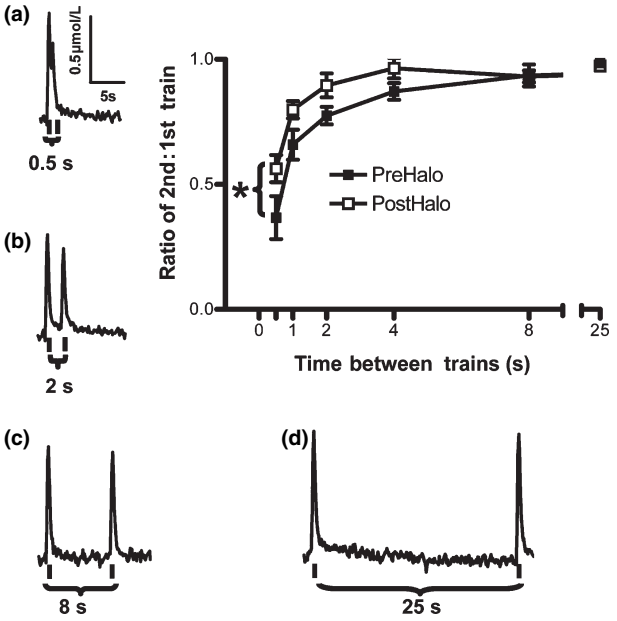

Fig. 1 Effect of stimulation train spacing on autoreceptor function. (a–d) Each panel shows the representative time course of dopamine evoked release in a single animal. Vertical bars indicate stimulus train (50 Hz, 12p, 300 μA) delivery. Time intervals between trains: (a) 0.5 s (b) 2 s (c) 8 s (d) 25 s. Inset: amplitude of the second peak normalized to the amplitude of the first peak in each train. This ratio was compared at different time intervals ($n = 7$), as well as before and after administration of a 0.5 mg/kg i.p. injection of haloperidol ($n = 7$). At spacing of 0.5 s, the pre- and post-drug values were significantly different [$F(1,91) = 9.202$, $p < 0.0031$, Bonferroni t-test]. At time intervals larger than 8 s, both before and after haloperidol the normalized amplitudes were identical. Error bars represent SEM.

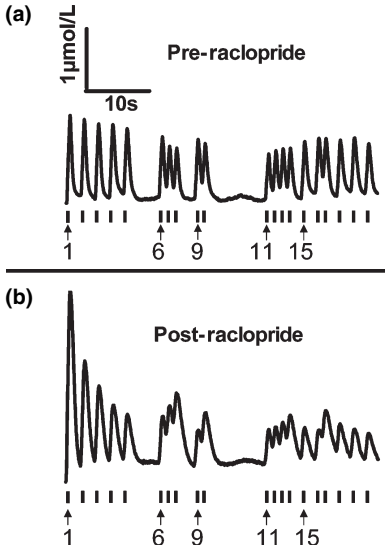

Fig. 2 Effect of raclopride on stimulated dopamine release during a complex stimulation pattern. Each panel is a representative concentration versus time trace of dopamine release in a single animal. Vertical bars indicate stimulus train (50 Hz, 24p, 300 μA) delivery. Data were recorded every 20 min. (a) Trace of dopamine release before drug administration. (b) The effect of a 1 mg/kg i.p. injection of raclopride on stimulated dopamine release 20 min after administration.

complex series of stimulations such as what occurs during intracranial self-stimulation. During a stimulation train, the amount of dopamine released per stimulus pulse $[DA]_p$ can be defined by the following equation:

$$[DA]_p = a_0 * f * d_1 * d_2 \quad (1)$$

where a_0 is the initial concentration of dopamine released per stimulus pulse at the beginning of a stimulation train, f is the short-term facilitation, d_1 is the short-term depression, and d_2 is the long-term depression. Each of these dynamic terms is multiplicative to give either an overall depression ($f * d_1 * d_2 < 1$) or an overall facilitation ($f * d_1 * d_2 > 1$) of release. Between stimulation events, each term decays exponentially with first order kinetics to its original value of 1, with a time constant τ_j^{-1}, with I_j representing any of the three variables in eqn 1:

$$\frac{dI_j}{dt} = \tau_j^{-1}(1 - I_j). \quad (2)$$

This model was used to create a simulation program written in LabVIEW (National Instruments, Austin, TX, USA). The program was then used to predict whether facilitation or depression would occur under specific stimulation conditions.

Materials

All chemicals and drugs were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used as received. Solutions were prepared using doubly distilled deionized water (Megapure system, Corning, NY, USA). The Tris buffer solution used for post-calibration was prepared using 12 mmol/L Tris, 140 mmol/L NaCl, 3.2 mmol/L KCl, 1.2 mmol/L $CaCl_2$, 1.25 mmol/L NaH_2PO_4, 1.2 mmol/L $MgCl_2$, 2.0 mmol/L Na_2SO_4 at pH 7.4. Drugs were dissolved in saline and injected intraperitoneally.

Results

Paired-train paradigm

To examine how rapid changes in extracellular dopamine concentration could affect the dynamics of subsequent dopamine release in the caudate-putamen of anesthetized rats, we used the paired-train paradigm, where the maximal release evoked by one stimulus train is compared with a second stimulus train at some variable time in the future. This approach has been effective to study D_2 inhibition of release in both brain slices, where local electrical stimulation has been used to evoke dopamine release (Kennedy *et al.* 1992; Phillips *et al.* 2002; Rice and Cragg 2004), as well as *in vivo* (Benoit-Marand *et al.* 2001). Trains with 12 pulses delivered at 50 Hz were used and the second train was initiated 0.5–25 s after the beginning of the first train (Fig. 1). When the second train was delivered 0.5 s after the

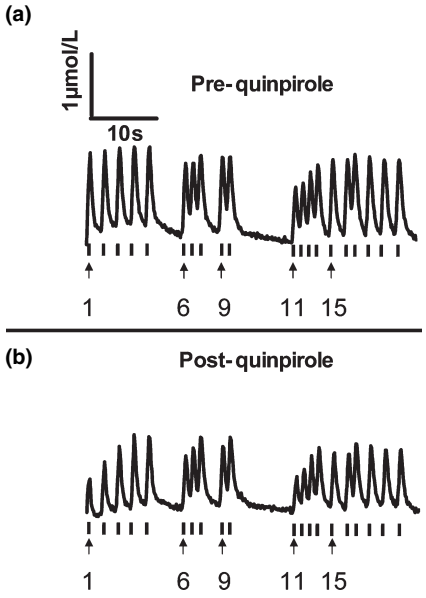

Fig. 3 Effect of quinpirole on stimulated dopamine release during a complex stimulation pattern. Each panel is a representative concentration versus time trace of dopamine release in a single animal. Other conditions as in Fig. 2. (a) Trace of dopamine release before quinpirole administration (b) The effect of a 1 mg/kg i.p. injection of quinpirole on stimulated dopamine release 20 min after administration.

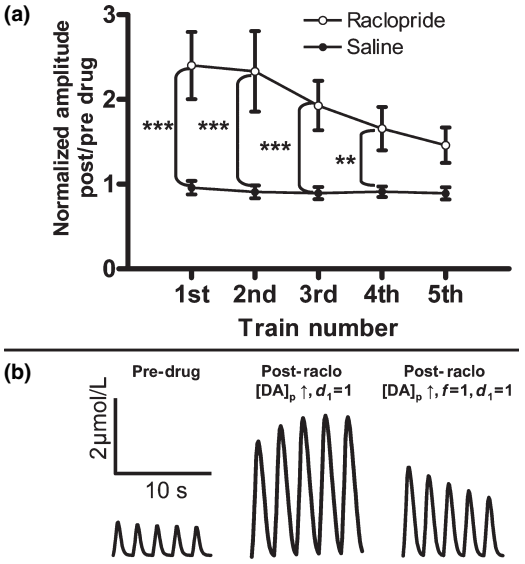

Fig. 4 Effect of raclopride on maximal stimulated dopamine release in consecutive trains. Release during five trains, 2 s apart, acquired as in Fig. 2. (a) Results following 1 mg/kg injection of raclopride (n = 6) or saline (n = 9) were normalized by the pre-injection maximal release amplitudes. Raclopride was given i.p. Twenty minutes later, a second file was collected with the same stimulation parameters. There was significance between raclopride and saline in the first four trains (p < 0.001, 0.001, 0.001, and 0.01 respectively, Bonferroni t-test). (b) Simulations of responses expected for the five stimulus trains. Left panel: simulation of pre-drug response. Parameters used were a_o = 100 nmol/L, uptake terms [K_m = 200 nmol/L, V_{max} = 4 (μmol/L)/s], kickback factors were: short-term facilitation = 1.01 with a time constant of 4.41 s, short-term depression = 0.989 with a time constant of 3.23 s, and long-term depression = 0.997 with a time constant of 840 s. Middle panel: simulation of post-raclopride response with short-term depression removed; a_o was doubled, short-term kick back factor was set to one, and the other terms were left at their pre-drug value. Right panel: identical to middle panel except short-term facilitation kick back factor was set to one. Error bars represent SEM.

first its amplitude was depressed (Fig. 1a). However, as the time between trains was increased, the depression diminished until at 8 s both trains produced similar magnitudes of dopamine release (Fig. 1c). Following a 0.5 mg/kg i.p. injection of haloperidol, the amplitude of the first train doubled (n = 7 animals), clearly indicating that dopamine release is normally under D_2 receptor control (Wiedemann *et al.* 1992). Consistent with prior experiments conducted *in vivo* and in brain slices, the relative amplitude of the second train was not attenuated as greatly at short intertrain intervals with the antagonist present. These experiments demonstrate that the D_2 receptor can inhibit subsequent dopamine release on a time scale similar to the short-term depression factor captured by the model, 3.24 s (Montague *et al.* 2004).

Examination of facilitation and depression of dopamine release

To investigate the plasticity of dopamine release during stimulations, an extended series of trains (all 24 pulse, 50 Hz, 300 μA) separated by different times was used [representative examples are shown in the panels (a) of Figs 2 and 3]. Prior to drug administration, stimulation sequences were repeated every 5 min until the release was stable between sequences. Within this stimulation pattern, the first five trains probed the short-term dynamics of dopamine release, while the later trains probed the dynamics of the longer-term component. There is some biological variability between animals in the pattern as demonstrated by the amplitude of dopamine release: some animals exhibit slight depression in the first five trains (Fig. 2a) while others exhibit slight facilitation (Fig. 3a). However, facilitation occurs in later trains in both examples. For example, the responses to train 15 are approximately 25% larger than seen during train 11.

Effect of raclopride on dopamine release

The responses were compared before (Fig. 2a) and 20 min after (Fig. 2b) a 1 mg/kg i.p. injection of the D_2 antagonist raclopride. [Responses 20 min after saline were virtually unchanged (vide infra)]. Like haloperidol, raclopride caused approximately a two-fold increase in dopamine release on the first train of each pattern (Fig. 2b). However, the effect is less

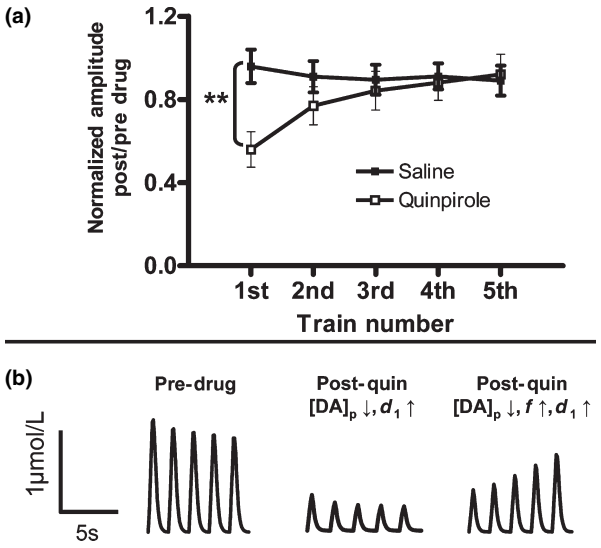

Fig. 5 Effect of quinpirole on maximal stimulated dopamine release in consecutive trains. Release during five trains, 2 s apart, acquired as in Fig. 3. (a) Results following a 1 mg/kg injection of quinpirole ($n = 7$) or saline ($n = 9$) were normalized by the pre-administration values. There was significance between quinpirole and saline in the first train ($p < 0.01$, Bonferroni *t*-test). (b) Simulations of responses expected for the five stimulus trains. Left panel: simulation of pre-drug response. Parameters used were $a_o = 100$ nmol/L, uptake terms [$K_m = 200$ nmol/L, $V_{max} = 4$ (μmoL/L)/s], kickback factors were: short-term facilitation = 1.01 with a time constant of 4.41 s, short-term depression = 0.989 with a time constant of 3.23 s, and long-term depression = 0.997 with a time constant of 840 s. Middle panel: simulation of release following quinpirole with only the short-term depression increased; a_o was decreased in half to account for the reduced release and short-term depression was enhanced (kickback factor = 0.985). Right panel: same as middle panel except the short-term facilitation was also enhanced (kickback factor = 1.02). Error bars represent SEM.

prominent during the next four trains as the amplitude of release diminishes. The within train facilitation observed between trains 11 and 15 is virtually abolished by raclopride administration (Fig. 2b).

The relative amplitude of the first five trains was pooled from several animals (Fig. 4, $n = 6$ for raclopride, $n = 9$ for saline). The maximum dopamine amplitude for each train was normalized by its pre-saline or pre-raclopride counterpart. For saline, the responses were essentially unity indicating no change in dopamine release in the 20 min interval between train applications. In contrast, dopamine release in the first train was enhanced by a factor of 2.5 following raclopride (Fig. 4a). With each successive train, however, the enhancement of release diminished with the values decreasing toward unity [$F(1,65) = 66.05$, $p < 0.0001$, two-way ANOVA]. Trains 1–4 exhibited significant difference from saline values ($p < 0.001$ for trains 1, 2, and 3, $p < 0.01$ for train 4, Bonferroni *t*-test). Train 5 was not significantly different from pre-drug values ($p > 0.05$, Bonferroni *t*-test). Thus, administration of the D_2 antagonist changes the release pattern to one of long-term depression.

We attempted to simulate these results with the Montague model (Fig. 4b). The pre-drug response, using kinetic values from Montague *et al.* (2004), predicts little change in maximal dopamine during the first five trains as is found in the experimental data (compare the left panel, Fig. 4b with the first five trains in Figs 2a and 3a). Because D_2 antagonists can enhance release (Fig. 1), we simulated the results after raclopride administration with an increased concentration of release (a_o) to account for this. Furthermore, we eliminated the short-term depression, d_1, as suggested by the data in Fig. 1. The simulation result (middle panel, Fig. 4b) predicts that dopamine release will be facilitated with each train, behavior unlike the experimental data. However, when both the short-term depression and facilitation, d_1 and f, were eliminated, stimulated release was predicted to decrease with each train as a consequence of the long-term depression, d_2. This trend closely resembles with that obtained experimentally after raclopride (compare right panel, Fig. 4b, with Fig. 2b or the raclopride mean values Fig. 4a).

Effect of quinpirole on dopamine release

The effects of a 1 mg/kg i.p. dose of the D_2 agonist quinpirole were examined. Release measurements were made 20 min after quinpirole was administered. The first release train was decreased by 50% consistent with previous reports (Joseph *et al.* 2002), but by the fourth train dopamine release had returned to its pre-drug value (Fig. 3b). At the beginning of each subset of closely spaced stimulation trains (trains 1, 6, 9, and 11), release was inhibited but recovered by the end of the subset.

As with raclopride, maximal dopamine release from the first five trains from multiple animals ($n = 7$) following 1 mg/kg quinpirole were normalized to their corresponding pre-drug values and compared with release data collected in a similar way following saline ($n = 9$). The maximum dopamine concentration for each post-drug train was normalized to its corresponding response in the pre-drug train, with values <1 indicating decreased release relative to pre-drug values. Quinpirole caused dopamine release on the initial train to diminish by a factor of two (Fig. 5), consistent with previous experiments involving i.p. injection of quinpirole (Stamford *et al.* 1991). However, with each consecutive train, the amount of release incremented [$F(1,70) = 5.331$, $p < 0.0239$, two-way ANOVA]. Other than for the first train, there was no significant difference between the values following saline and the quinpirole values ($p < 0.01$ for train 1, $p > 0.05$ for trains 2–5, Bonferroni *t*-test).

The results following quinpirole were also simulated with the Montague model (Fig. 5b). Because D_2 agonists can enhance the short-term depression, we simulated the

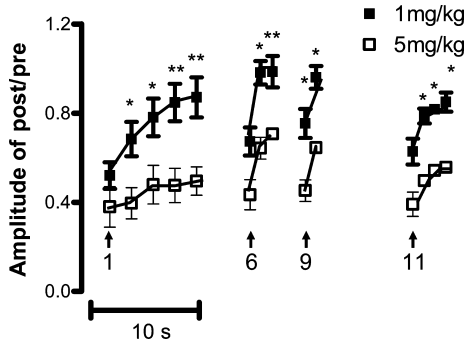

Fig. 6 Effect of quinpirole concentration on maximal stimulated dopamine release. Each data point is the maximal dopamine release induced by a 50 Hz, 24p, 300 μA train normalized by release observed in the pre-drug train. Post-quinpirole files were collected 20 min after administration. Values <1 correspond to a depressed train, while values >1 correspond to a facilitated train. Two different doses of quinpirole were used, 1 mg/kg ($n = 6$) and 5 mg/kg ($n = 5$). Error bars represent SEM. There was significance in trains 2, 3, 4, 5, 7, 8, 9, 10, 12, 13, 14, 15 ($p < 0.05$ for all, except trains 4, 5, and 7 with $p < 0.01$).

results after quinpirole administration with a decreased concentration of release, a_o, and with the short-term depression, d_1, enhanced. This simulation shows an overall decrease in the amount of dopamine release, while also increasing the amount of observed depression (middle panel, Fig. 5b), behavior unlike the experimental data. However, when both short-term depression and facilitation, d_1 and f, are enhanced, the observed trend closely resembles that obtained experimentally after quinpirole (compare right panel, Fig. 5b, with Fig. 3b or the quinpirole mean values Fig. 5a).

The effects of quinpirole on depression and facilitation of the later trains were also examined (Fig. 6). The inhibition is rapidly restored when there is a pause between stimulation trains. Between trains 5 and 6, there is approximately 5 s between stimulations, and, during this time, release reverts to a more inhibited state. During trains 6–8, the release increases, but during the pause between trains 8 and 9, there is again a reversion to more inhibited release. The time between trains 10 and 11 is relatively long (10 s), and dopamine release returns to an inhibited level similar to that caused by quinpirole on the first train. However, release facilitates quickly as is apparent in the trains immediately following trains 6, 9, and 11. Increasing the dose of quinpirole to 5 mg/kg caused increased inhibition on the first train, but the rate of facilitation is significantly lower (Fig. 6). The first train after each pause (trains 1, 6, 9, and 11) all have approximately the same value, but the rate of facilitation is slowed in the higher drug response files.

The D_1 receptor was also investigated to ascertain its effect on facilitation and depression of release. A 1 mg/kg i.p. injection of the D_1-agonist SCH-23390 was given (data not shown). There was no significant difference between release following SCH-23390 and saline [$F(1,60) = 1.931$, $p < 0.168$, Bonferroni t-test] indicating that the D_1 receptor is uninvolved.

Stimulation intensity

We wished to know how increased concentrations of the endogenous ligand, dopamine, could affect the dynamics of subsequent dopamine release. As above, the paired-train paradigm was used, where the effect of one stimulus train is compared with a second stimulus train at some time in the future. In this case, the number of stimulus pulses in the train was varied, which affects the amount of dopamine released (Wightman *et al.* 1988). 50 Hz trains spaced by 2 s containing at least six pulses were used to allow the effects of facilitation and depression to accumulate. At six pulses per train, the amplitude of the second train was smaller than that of the first train (Fig. 7a). Thus, as in Fig. 1, a small amount of depression of release occurs under these conditions. At 60 pulses for each train, the results were dramatically different with the second train facilitated relative to the first train (Fig. 7a). Indeed, a trend of facilitation was seen with pulse numbers greater than six [$F(6,72) = 4.302$, $p < 0.0001$, two-way ANOVA]. A 0.5 mg/kg i.p. dose of haloperidol blocked this apparent facilitation at 60 pulses (Fig. 7c).

Discussion

The experiments in this paper address mechanisms that govern dynamic fluctuations of dopamine release. They investigate whether the short-term depression identified by Montague *et al.* 2004 is in fact D_2-mediated autoinhibition, while additionally revealing the paradoxical role of the dopamine autoreceptor in facilitated dopamine release. Previous electrochemical measurements using the paired-pulse paradigm have shown that activation of D_2 autoreceptors inhibits dopamine release on the second pulse (Limberger *et al.* 1991; Kennedy *et al.* 1992; Benoit-Marand *et al.* 2001; Phillips *et al.* 2002) and that this inhibition decays on a time scale of a few seconds. Our results with paired trains that directly evoke somatodendritic action potentials in dopamine neurons (Kuhr *et al.* 1987) produce the same results, and show that the time scale of the recovery during paired-trains (Fig. 1) is consistent with the short-term depression ($\tau = 3.2$ s) described by Montague *et al.* (2004). We also demonstrate that release is depressed in the presence of a D_2 agonist, quinpirole, for the first train. Repeated trains reveal more complex regulation. As previously shown (Yavich and MacDonald 2000), both facilitation and depression are apparent in the absence of drug (Figs 2 and 3). Our work establishes that these processes are linked to the D_2-receptor. First, an antagonist removes both short-term depression and facilitation of dopamine release (Fig. 4). Second, facilitation becomes more apparent with repeated trains in the presence of a D_2

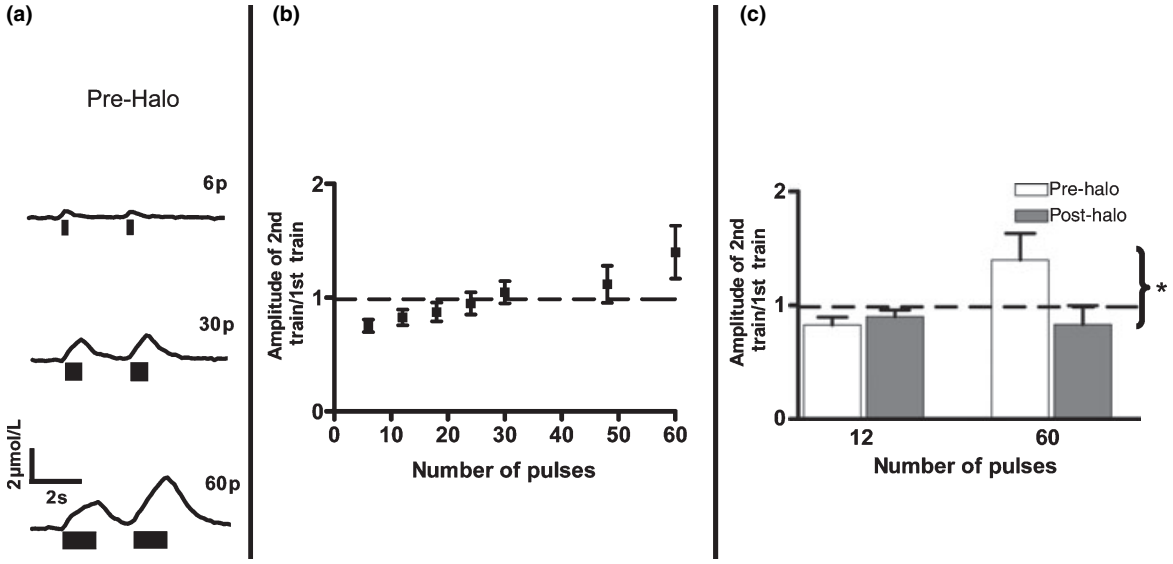

Fig. 7 Effect of the number of stimulus pulses on dopamine release. Each train was at 50 Hz and 300 μA. (a) Representative traces of the paired train paradigm using 6p, 30p, and 60p before administration of haloperidol. (b) Panel showing the effect of 6p, 12p, 18p, 24p, 30p, 48p, and 60p on the paired-train paradigm. The amplitude of the second train was normalized to the amplitude of the first train. Depression occurred at low numbers of pulses, while facilitation occurred at higher numbers of pulses. This trend was continuous from 6–60p [$F(6,72) = 2.89$, $p < 0.014$, two-way ANOVA]. (c) Administration of haloperidol abolished facilitation at 60 pulses. Error bars represent SEM.

agonist, quinpirole (Figs 5 and 6). Thus, the short-term dynamics of dopamine release, both facilitation and depression, are due to regulation by the D_2-autoreceptor. As will be discussed below, the facilitation component may in fact be release from inhibition.

The D_2-like population of dopamine receptors is comprised of the D_2-dopamine receptor that serves as the striatal dopamine autoreceptor (Benoit-Marand *et al.* 2001) and the D_3-dopamine receptor that plays only a minor role (Joseph *et al.* 2002). Consistent with those prior findings, quinpirole, an agonist at both of these receptor types, inhibited maximal dopamine release evoked by the first train relative to its pre-drug value. Similarly, the increased dopamine release observed following raclopride or haloperidol, D_2-receptor antagonists, is consistent with prior work (Gonon and Buda 1985; Stamford *et al.* 1988; Yavich 1996). D_2 antagonists have also been shown to lower dopamine uptake rates (Wu *et al.* 2002), a factor clearly apparent in the prolonged responses of the dopamine signals after raclopride (Fig. 2, lower panel). The Montague model does not account for such changes in uptake. Therefore, we have not modeled the entire set of responses, but rather have used the predictions of the model to guide our experimental design and interpretation of the observed responses.

Although raclopride potentiated maximal dopamine release evoked by the first train, each subsequent train in a closely spaced series of stimulations exhibited less release (Fig. 4a), i.e., there was a predominant depression of release throughout the stimulation sequence. Similar results have been obtained in mouse striatum following repeated trains following haloperidol administration (Yavich 1996). This effect could be modeled by eliminating both the short-term depression (d_1) and facilitation (f) factors in the Montague model (Fig. 4b, right panel). Thus, the data are consistent with the concept that long-term depression of dopamine release is the sole adaptive factor when dopamine D_2 receptors are blocked. This long-term depression is consistent with the slow time course of replenishing the readily releasable pool (Yavich 1996; Yavich and MacDonald 2000) from the recycling and reserve pools (Rizzoli and Betz 2005). With intense stimulations such as used here, sustained release requires mobilization of the reserve pool, which comprises the majority of vesicles (Richards *et al.* 2003). Alternatively, released dopamine may compete with raclopride at the dopamine autoreceptor so that the dopamine released by each train may further reinstate a depression of release. While this could occur by simple binding competition (Ross and David 1989; Seeman *et al.* 1990), it seems unlikely because the time scale of the depression responses are adequately described by the long-term depression described in the Montague model.

Although activation of the autoreceptor with quinpirole caused depression of the dopamine release evoked on the first train, dopamine release incremented for subsequent stimulations (Figs 3 and 5). Similar behavior has also been observed for dopamine release under the control of nicotinic

acetylcholine receptors. In that case, receptor activation leads initially to short-term depression, but the nicotinic receptor desensitizes resulting in an apparent facilitation (Rice and Cragg 2004). Similarly, in our case, the data following quinpirole could not be modeled by simply increasing the kick-back factor for short-term depression (d_1) but also required increasing it for the short-term facilitation (f) (Fig. 5b, right panel), suggesting a link between the two processes. Thus, while the data indicate that the short-term depression in the Montague model is mediated by the dopamine autoreceptor, the short-term facilitation is consistent with desensitization of this pathway. When the full stimulation train was employed, it became apparent that at later trains (11–14), the likelihood of facilitation was much larger, presumably because of the prolonged activation and subsequent desensitization of the autoreceptor by dopamine (Figs 2 and 3). The importance of the duration of activation of the autoreceptor was directly probed by increasing train duration; as the number of pulses in the paired-train paradigm was increased, the second train became increasingly larger than the first (Fig. 7a), again indicating desensitization via a dopamine dependent process. A major contributor to this effect was the depression of release on the first train. The greatest difference was found between the six pulse and 60 pulse trains: the maximal release was only 4.0 times greater with 60 pulses, far less than the 10-fold difference based upon the number of pulses. So, again, the apparent facilitation on the second train is actually due to depressed release on the first train. This facilitatory effect is D_2-receptor mediated because it is abolished following haloperidol (Fig. 7c). Careful inspection shows that all of the cases of apparent facilitation are actually a removal of depression (see, for example, Fig. 2a, trains 11–14, Fig. 3a and b and Figs 5–7).

Desensitization is a common feature of G-protein coupled receptors when they experience prolonged exposure to agonist (Leaney *et al.* 2004; Xu-Friedman and Regehr 2004). Our experiments provide 20 min between injection of the D_2 agonist quinpirole and the measurements (Fig. 5). During this time, the D_2 receptor could be primed for desensitization, for example by phosphorylation of the G-protein (Krasel *et al.* 2004). This prepares the protein for arrestin binding and subsequent desensitization upon agonist binding (Vilardaga *et al.* 2003; Sinclair *et al.* 2006), processes that occur on the 10-s time scale of our observations. An increased dose of quinpirole did not lead to a significantly greater inhibition on the first train. This ceiling effect is the asymptote of the intrinsic activity of quinpirole and has been demonstrated in brain slices where the maximum effect of quinpirole at D_2 receptors is only a partial reduction of dopamine release (Joseph *et al.* 2002). At the same time, the apparent desensitization decreased (Fig. 6). The high dose of quinpirole may saturate the receptors, preventing subsequent release of dopamine from significantly affecting receptor dynamics. Indeed, throughout these studies, the competition between endogenous dopamine and exogenous drugs complicates quantitative interpretation of the data.

The pre-synaptic modulation of dopamine release, and its regulation by D_2 receptors, will contribute to the complex modulation of the strength of cortico–striatal interactions. The plasticity in dopamine release seen here and in other *in vivo* work (Yavich and MacDonald 2000), as well as in brain slices (Cragg 2003; Rice and Cragg 2004), provides clear evidence that pre-synaptic plasticity does occur, a feature demonstrated in other preparations (Lu and Hawkins 2006). However, pre-synaptic facilitation of neurotransmitter release is typically thought to involve intracellular calcium dynamics (Atluri and Regehr 1996; Zucker and Regehr 2002). Intracellular calcium influx after a conditioning stimulus does not immediately return to resting levels, and, upon subsequent stimulations, this ''residual'' calcium is summated with the subsequent influx to increase release probability. The D_2 receptor can modulate intracellular calcium via its G-protein $\beta\gamma$ subunits by activation of phospholipase C and by inhibition of calcium channels on the plasma membrane (Neve *et al.* 2004), providing another possible D_2-controlled facilitation pathway.

Dopamine synapses are strategically located to modulate striatal neurons. Many are found on the neck of spines of medium spiny neurons, and the cortical inputs to these neurons, which use glutamate as their neurotransmitter, synapse on the head of the spines (Sesack *et al.* 1994). These cortico-striatal synapses can exhibit both long term depression (LTD) and long term potentiation (LTP) following tetanic stimulation (Charpier and Deniau 1997; Charpier *et al.* 1999; Nishioku *et al.* 1999). Furthermore, cortico-striatal LTP can be induced by bursts of dopamine release, either introduced by iontophoresis (Arbuthnott *et al.* 2000), pressure injection (Wickens *et al.* 1996), or released from endogenous stores using stimuli similar to those used in this work (Reynolds *et al.* 2001; Reynolds and Wickens 2002). Both D_1 (Reynolds *et al.* 2001; Centonze *et al.* 2003) and D_2 (Calabresi *et al.* 1997) receptors participate in the dopamine mediated synaptic plasticity. Our findings have direct pertinence to intracranial self-stimulation results, an experiment that evokes LTP (Reynolds *et al.* 2001), as our stimulation trains are the same as those used in intracranial self-stimulation experiments. The data demonstrate that D_2 receptors provide a pre-synaptic component that not only can modulate autoinhibition, but also short-term facilitation of dopamine release, and therefore impacts both D_1- and D_2-mediated post-synaptic plasticity. As phasic dopamine release occurs in response to cues predicting reward, the pre-synaptic plasticity observed here likely plays an important role in the modulation of these types of responses as well (Schultz 1998; Carelli and Wightman 2004).

These findings have important implications for the downstream actions of D_2-receptor ligands that are used clinically.

For example, D_2 receptor antagonists are used therapeutically for the management of schizophrenia (Seeman *et al.* 2006). These ''typical'' neuroleptics commonly have low affinity for dopamine D_1-like receptors, and thus permit dopamine neurotransmission via this route. Our data predicts that this D_1-mediated transmission will also be perturbed as there is gross loss of dynamic modulation, and therefore putative information content in the dopamine that is released to act upon these receptors. Thus, when reconciling the mechanisms involved in the unwanted side effects of neuroleptics, these important actions on pre-synaptic plasticity need to be considered.

Acknowledgements

This work was supported by NIH grants (NS 15841 and DA 10900 to RMW; DA 17155 to PEMP; NS 36298 to PAG). JMK is a NSF GRFP fellow.

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