Dopamine D-1/D-5 Receptor Activation Is Required for Long-Term Potentiation in the Rat Neostriatum In Vitro

J.N.D. KERR AND J. R. WICKENS
Department of Anatomy and Structural Biology, University of Otago, Dunedin, New Zealand

Received 8 June 2000; accepted in final form 12 September 2000

Kerr, J.N.D. and J. R. Wickens. Dopamine D-1/D-5 receptor activation is required for long-term potentiation in the rat neostriatum in vitro. *J Neurophysiol* 85: 117–124, 2001. Dopamine and glutamate are key neurotransmitters involved in learning and memory mechanisms of the brain. These two neurotransmitter systems converge on nerve cells in the neostriatum. Dopamine modulation of activity-dependent plasticity at glutamatergic corticostriatal synapses has been proposed as a cellular mechanism for learning in the neostriatum. The present research investigated the role of specific subtypes of dopamine receptors in long-term potentiation (LTP) in the corticostriatal pathway, using intracellular recording from striatal neurons in a corticostriatal slice preparation. In agreement with previous reports, LTP could be induced reliably under Mg^{2+}-free conditions. This Mg^{2+}-free LTP was blocked by dopamine depletion and by the dopamine D-1/D-5 receptor antagonist SCH 23390 but was not blocked by the dopamine D-2 receptor antagonist remoxipride or the $GABA_A$ antagonist picrotoxin. In dopamine-depleted slices, the ability to induce LTP could be restored by bath application of the dopamine D-1/D-5 receptor agonist, SKF 38393. These results show that activation of dopamine D-1/D-5 receptors by either endogenous dopamine or exogenous dopamine agonists is a requirement for the induction of LTP in the corticostriatal pathway. These findings have significance for current understanding of learning and memory mechanisms of the neostriatum and for theoretical understanding of the mechanism of action of drugs used in the treatment of psychotic illnesses and Parkinson's disease.

INTRODUCTION

Activity-dependent synaptic plasticity is a widely used model for learning and memory mechanisms of the brain (Bliss and Collingridge 1993). Although most extensively studied in the hippocampus, activity-dependent synaptic plasticity has also been described in several other brain areas including the neostriatum. The neostriatum is a brain region involved in certain types of learning including reward-related (Beninger 1983) and motor (Graybiel 1995) learning. It receives inputs from all regions of the cerebral cortex (McGeorge and Faull 1989) via an extensive glutamatergic projection (McGeer et al. 1977). The neostriatum also receives a major dopaminergic projection from the substantia nigra, which terminates in close proximity to the corticostriatal inputs (Smith et al. 1994). A number of models have proposed activity-dependent synaptic plasticity in the corticostriatal pathway as a mechanism for learning-related functions of the neostriatum (Beninger 1983; Groves 1983; Miller 1981; Wickens 1990). These models assume that synaptic plasticity in the corticostriatal pathway is regulated by the dopamine inputs from the substantia nigra.

The corticostriatal pathway is of critical importance for the function of the neostriatum. Corticostriatal inputs to the neostriatum synapse directly on the spiny projection neurons (Somogyi et al. 1981), which are the output neurons of the neostriatum (Preston et al. 1980). Thus the corticostriatal synapses are the direct connection between the input and output of the neostriatum. Furthermore the spiny projection neurons are relatively quiescent, and their firing activity occurs in response to excitation by cortical inputs (Wilson and Groves 1981; Wilson et al. 1983). Thus the efficacy of the corticostriatal synapse is a major determinant of the action potential activity of the spiny projection neurons, and plasticity in these synapses is a candidate mechanism for the learning functions of the neostriatum.

Both long-term potentiation (LTP) and long-term depression (LTD) have been described in the corticostriatal pathway. LTD can be induced by high-frequency stimulation (HFS) of the cortical afferents to the neostriatum (Calabresi et al. 1992b; Lovinger et al. 1993; Wickens et al. 1996, 1998), and the requirements for its induction have been extensively characterized (Calabresi et al. 1992a,b, 1994, 1995). In contrast to LTD, neostriatal LTP cannot be induced reliably by HFS in standard solutions. It was first reported after HFS in slices bathed in Mg^{2+}-free fluid (Walsh 1991). This form of LTP was subsequently shown to be blocked by *N*-methyl-D-aspartate (NMDA) receptor antagonists (Calabresi et al. 1992c), suggesting that the unmasking of LTP in Mg^{2+}-free fluid was due to removal of the voltage-dependent Mg^{2+} block of the NMDA channels (Nowak et al. 1984).

In addition to occurring in Mg^{2+}-free conditions, striatal LTP can be induced in normal bathing solutions if dopamine is applied in pulses timed to coincide with cortical HFS (Wickens et al. 1996). A similar phenomenon occurs in response to substantia nigra stimulation in the intact animal (Reynolds and Wickens 2000) but not in dopamine-depleted animals, suggesting that LTP can be induced under more physiological conditions and that it is a dopamine-dependent phenomenon. There are also indications that Mg^{2+}-free LTP is blocked by chronic dopamine depletion by 6-hydroxydopamine (Centonze et al. 1999). These findings suggest a possible link between the induction of neostriatal LTP under Mg^{2+}-free conditions and

Address for reprint requests: J. R. Wickens, Dept. of Anatomy and Structural Biology, School of Medical Sciences, University of Otago, PO Box 913, Dunedin, New Zealand (E-mail: jeff.wickens@stonebow.otago.ac.nz).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

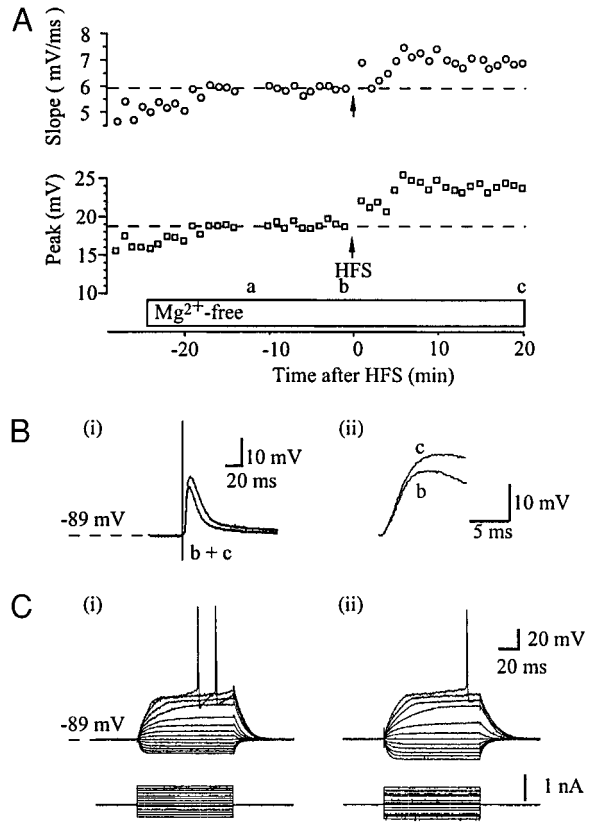

FIG. 1. Striatal long-term potentiation (LTP) in Mg^{2+}-free solution. ↑, time of high-frequency stimulation (HFS). □, perfusion with Mg^{2+}-free solution. - - -, baseline taken as the average of last 5 min responses before HFS. *A*: measurements of the slope (○) and peak amplitude (□) of postsynaptic responses, showing at each time point the average of 6 consecutive responses evoked at 0.1 Hz. *B*: response to cortical test stimulus just prior to HFS and 20 min after HFS. *i*: traces at time points b and c shown overlaid; *ii*: initial part of traces on expanded timebase so that differences in slope can be seen. Each trace is an average of 6 consecutive responses. *C*: superimposed responses to a series of current pulses 10 min before HFS (*i*, time a) and 20 min after HFS (*ii*, time c). All data from same cell.

LTP associated with endogenous dopamine release or direct application of dopamine.

The present experiments used intracellular recording techniques to investigate the role of specific dopamine receptor subtypes in corticostriatal LTP. We report a stimulation protocol that reliably induces LTP of the corticostriatal pathway in Mg^{2+}-free conditions. We also show that this form of LTP is blocked reliably by a dopamine D-1/D-5 receptor antagonist but not by a dopamine D-2 receptor antagonist or $GABA_A$ receptor antagonist. Finally, we show that LTP is prevented by dopamine depletion but can be restored by a dopamine D-1 receptor agonist.

METHODS

Male Wistar rats (190–240 g) were deeply anesthetized with ether and decapitated. The brain was quickly removed and chilled in ice-cold artificial cerebrospinal fluid (ACSF, see following text). After cooling for 3 min, the brain was removed from solution, the hemispheres were separated, and a block of brain tissue was prepared by sectioning one hemisphere in a horizontal plane 45° to the base of the brain. The block containing the neostriatum and overlying cortex was fixed to the stage of a Campden vibroslice, and 400-μm slices were cut in which the cortex, neostriatum, and corticostriatal connecting fibers were preserved (Arbuthnott et al. 1985; Kawaguchi et al. 1989). Slices were maintained at room temperature before being transferred to a recording chamber in which they were superfused with ACSF containing (in mM) 124 NaCl, 2.5 KCl, 2.0 $MgSO_4$, 2.5 $CaCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, and 11 glucose that was gassed with 95% O_2-5% CO_2 mixture and maintained at a temperature of 35 ± 0.1°C (mean ± SD). During slice preparation, an ACSF solution in which sucrose (248 mM) was substituted for NaCl was used to maximize the yield of good impalements (Aghajanian and Rasmussen 1989). Slices were allowed to equilibrate in the recording chamber for at least 1 h before use.

Intracellular records were obtained from neostriatal cells using glass microelectrodes filled with 2 M potassium acetate solution (95–120 MΩ). For inclusion in the study, cells were required to meet the following criteria: resting membrane potential more negative than −80 mV and stable throughout the recording period (at least 50 min) without use of holding current; action potential overshoot greater than 10 mV; and, action potential onset delayed at least 50 ms from the onset of a just-suprathreshold current pulse. Cells were rejected from the study at the start of the experiment if they did not meet these criteria. These criteria were adopted after extensive preliminary work had shown that less stringent criteria resulted in greater variability in both cellular properties and LTP. A total of 29 cells meeting these inclusion criteria were used in the present study.

Electrophysiological traces were recorded using an Axoprobe 1A intracellular amplifier (Axon Instruments). Traces were digitized at a sampling rate of 10 kHz per channel using pClamp software (Axon Instruments) and saved to hard disk for analysis off-line. Postsynaptic potentials (PSPs) were evoked by stimulation of the deeper layers of the cortex and adjacent white matter (bipolar electrodes, monophasic constant current pulses, 0.1 ms, 150 μA max). Stimulus intensity was adjusted to give initial postsynaptic potential (PSP) amplitudes of

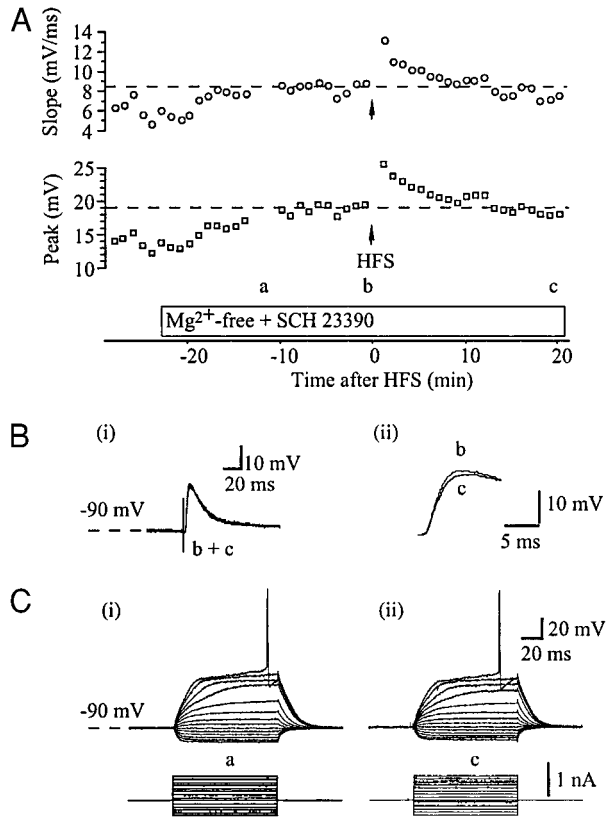

FIG. 2. Effects of SCH 23390 on striatal LTP in Mg^{2+}-free solution. Same layout as in Fig. 1.

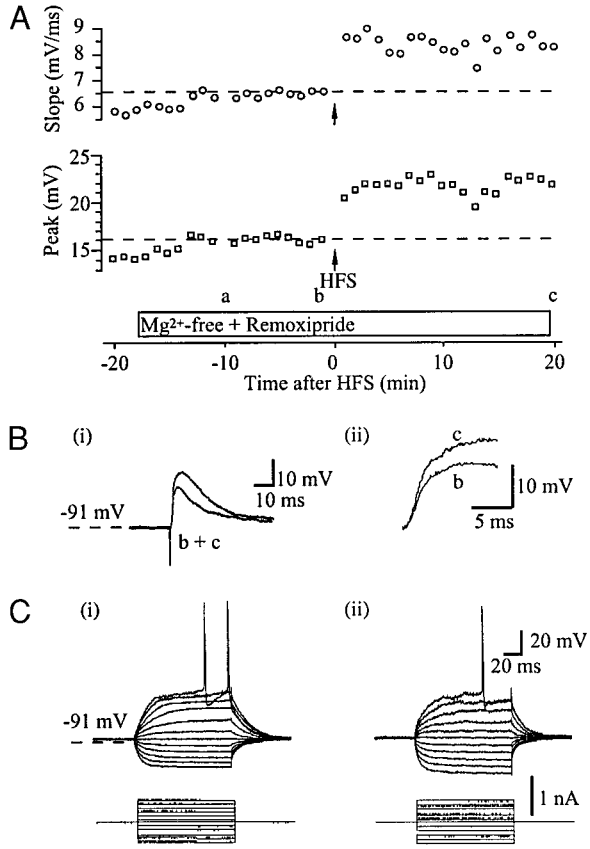

FIG. 3. Effects of remoxipride on striatal LTP in Mg^{2+}-free solution. Same layout as in Fig. 1.

10–15 mV. After a 10-min baseline period in normal solution, perfusion was switched to a Mg^{2+}-free solution. During exchange of Mg^{2+}-free for normal solution, PSPs were recorded for 20 min by which time the PSP amplitude and waveform had stabilized. Thus recordings were made for at least 30 min before HFS.

The cortical stimulus current used during HFS was the same as that used for test pulses and thus was not a suprathreshold stimulus. To ensure a conjunction of both presynaptic activity and action potential firing of the postsynaptic cell, HFS of the cortex (trains of 50 pulses at 100 Hz repeated 6 times at 10-s intervals) was paired with depolarization of the postsynaptic neuron using an intracellular current pulse. Prior to HFS, the depolarizing current pulse for each cell was adjusted to an intensity that ensured action potential firing in response to cortical stimulation (520-ms pulse, 0.2–1.2 nA). Test responses were recorded for at least 20 min following HFS. No intracellular holding current was used to maintain the membrane potential, and cells were discarded if the membrane potential changed during the recording period. Only one cell per slice was used to avoid effects of prior HFS on synaptic plasticity.

Measurements of PSP peak amplitude and slope were made using in-house programs based on Axograph 2.0 software (Axon Instruments). Peak PSP values were measured from the resting membrane potential to the maximum depolarization during the PSP. The PSP slope was the maximum rate of rise, obtained from the maximum value of slope of the moving regression line fitted to eight consecutive sample points (corresponding to 0.8 ms) between onset and peak of the PSP.

Measurements of cellular properties (input resistance and action potential characteristics) were made shortly after impalement and repeated 10 min before and 20 min after HFS. Input resistance was determined from the slope of a regression line fitted to the membrane potentials produced by a series of subthreshold depolarizing current pulses. Threshold for action potential firing was defined as the point on the voltage trajectory at which the rate of depolarization exceeded 8 mV/ms. Action potential amplitude was defined as the difference between threshold and the peak of the action potential waveform. Action potential duration was measured at the voltage midway between threshold and peak potentials. The amplitude of the afterhyperpolarization potential (AHP) was defined as the difference between threshold and the minimum of the hyperpolarization that followed each action potential. Threshold was used as the baseline for AHP measurements rather than resting membrane potential because the equilibrium potential for AHPs is more depolarized than the hyperpolarized resting membrane potential of spiny projection neurons.

Remoxipride (10 μM, Sigma), SCH 23390 (10 μM, RBI), SKF 38393 (5 μM, RBI), and picrotoxin (50 μM, Sigma) were dissolved to their desired final concentration in the Mg^{2+}-free superfusing fluid. Dopamine-depleted slices were prepared from animals injected with alpha-methyl para tyrosine (AMPT, 300 mg/kg ip, RBI) 2.5 h before slice preparation. The administration of AMPT depletes up to 86% of the releasable stores of dopamine (White et al. 1993) by inhibiting tyrosine hydroxylase, an enzyme catalyzing the rate limiting step in the production of dopamine (Cumming et al. 1994). Previous work has shown that the protocol used in the present experiments abolishes dopamine release within 45 min (Williams and Millar 1990). All animals in the AMPT group showed reduced motor activity (consistent with dopamine depletion) prior to slice preparation, and recording was completed within 5 h of the AMPT injection.

Statistical analysis of synaptic plasticity was based on the percentage change in response from baseline values (average of 5 min of test responses prior to HFS). Between-group differences were tested for statistical significance using a one-way ANOVA followed by Student-Newman multiple comparison procedure. Statistical analysis of cellular properties used a two-tailed *t*-test for independent samples (between group comparison) and a paired *t*-test (within group comparison of cellular properties 10 min before and 20 min after HFS). The probability level for statistical significance was set at $P = 0.05$.

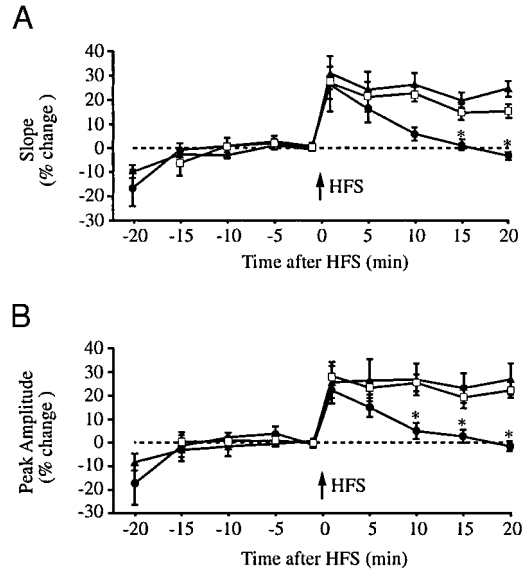

FIG. 4. Dopamine D-1/D-5 receptor antagonists, but not D-2 antagonists, block Mg^{2+}-free LTP in the neostriatum. Percentage change from baseline, taken as the average of last 5 min responses before HFS, group averages across all cells in each treatment group. *A*: change in postsynaptic potential (PSP) slope. *B*: change in PSP peak amplitude. □, Mg^{2+}-free controls ($n = 6$); ▲, Mg^{2+}-free plus remoxipride ($n = 4$); ●, Mg^{2+}-free plus SCH 23390 ($n = 5$). *, significant difference from controls ($P < 0.05$).

RESULTS

Switching of the ACSF to a Mg^{2+}-free solution was associated with an increase in the slope and peak of the test responses. These changes reached a steady state and stabilized within 20 min of changeover from normal to Mg^{2+}-free ACSF. In these slices, cortical HFS paired with depolarization of the postsynaptic cell reliably induced a long-term increase in both the slope and peak of the cortically evoked test responses, which we refer to as Mg^{2+}-free LTP. Averaging across all cells tested ($n = 6$), there was an increase in the PSP peak amplitude (22.3 ± 3.4%) and slope (15.3 ± 2.9%) measured 20 min after HFS. These findings are illustrated in Fig. 1.

To test whether the Mg^{2+}-free LTP was due to potentiation of a reversed inhibitory postsynaptic potential (IPSP), LTP was measured in the presence of picrotoxin, a $GABA_A$ receptor antagonist. The group average ($n = 3$) showed an increase in the PSP peak amplitude (40.8 ± 5.1%) and slope (28.4 ± 5.4%) measured 20 min after HFS that was not significantly different from the LTP seen in the Mg^{2+}-free control group (data not shown).

Subtype-specific dopamine receptor antagonists were used to test whether dopamine receptors play a role in Mg^{2+}-free LTP. The D-1/D-5 antagonist SCH 23390 (10 μM) abolished Mg^{2+}-free LTP (Fig. 2). In slices treated with SCH 23390, there was no change in the group average ($n = 5$) measures of PSP peak amplitude (−1.6 ± 2.2%) or slope (−3.3 ± 1.6%) measured 20 min after HFS.

To determine whether dopamine D-2 receptors play a role in Mg^{2+}-free LTP, the D-2 receptor antagonist remoxipride was also tested (Fig. 3). Averaging across all slices tested ($n = 4$), remoxipride (10 μM) did not prevent LTP of the PSP peak amplitude (26.9 ± 6.7%) or slope (24.7 ± 3.4%) measured 20 min after HFS.

Group average data for the control (Mg^{2+}-free), dopamine D-1/D-5 receptor antagonist (SCH 23390) and dopamine D-2 receptor antagonist (remoxipride) are shown in Fig. 4. The difference between Mg^{2+}-free control and SCH 23390 groups was significant ($P < 0.05$). There was no significant difference between Mg^{2+}-free control and remoxipride groups. Although the magnitude of Mg^{2+}-free LTP appeared to be greater in the remoxipride-treated slices, this difference was not significant. Thus dopamine D-1/D-5 receptor activation, but not D-2 receptor activation, was found to be a requirement for neostriatal LTP in Mg^{2+}-free conditions.

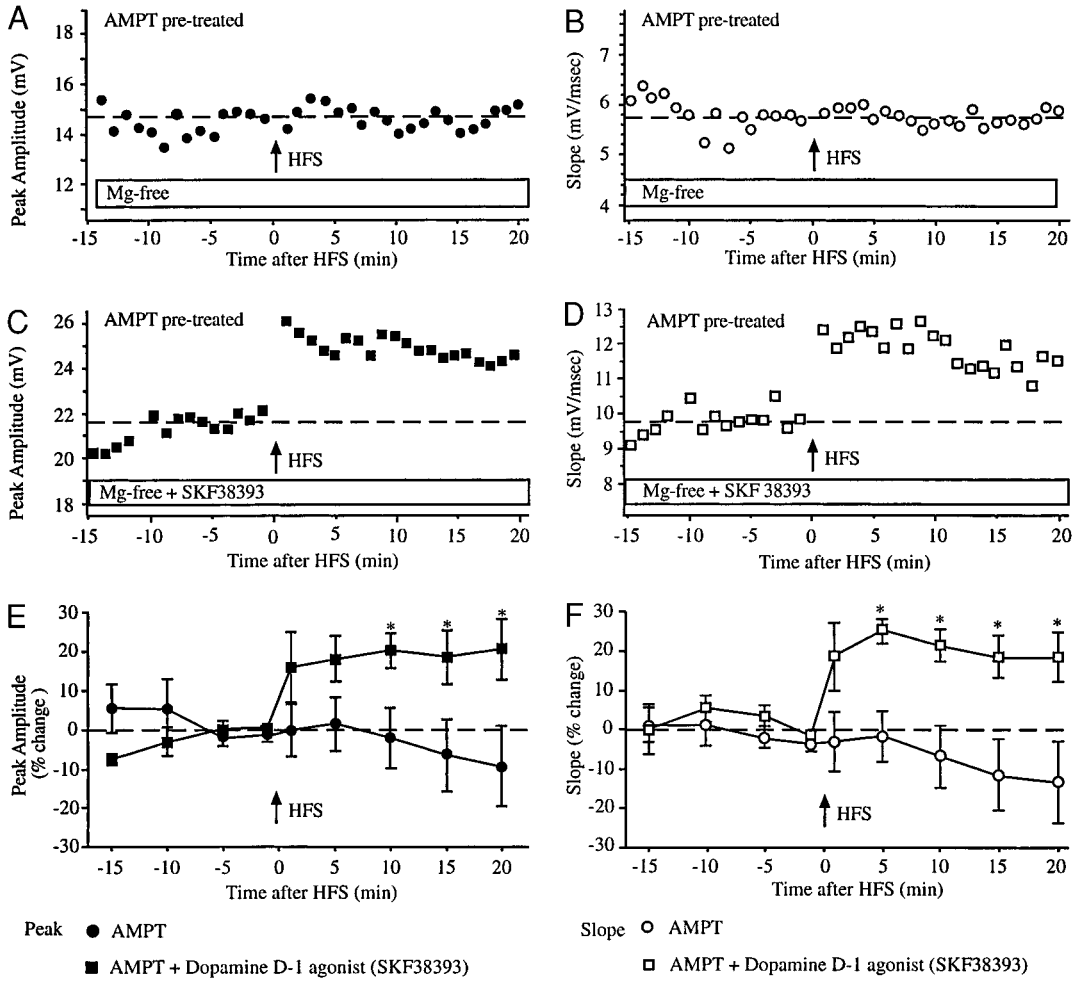

FIG. 5. Mg^{2+}-free LTP is blocked by dopamine depletion and restored by dopamine D-1 receptor agonist. *A*: alpha-methyl para tyrosine (AMPT) pretreatment blocks Mg^{2+}-free LTP of PSP peak amplitude. *B*: AMPT pretreatment blocks Mg^{2+}-free LTP of PSP slope. *C*: after AMPT pretreatment, addition of the D-1 agonist SKF 38393 restores Mg^{2+}-free LTP of PSP peak amplitude. *D*: SKF 38393 restores Mg^{2+}-free LTP of PSP slope. *E*: group average of peak amplitude data from AMPT-treated controls and slices treated with SKF 38393. *F*: slope data for same slices as in *E*. *, significant difference between groups ($P < 0.05$).

TABLE 1. *Cellular properties before and after HFS*

	Control	SCH 23390	Remoxipride	AMPT	AMPT + SKF 38393
n	6	5	4	6	5
Membrane potential, mV					
Pre	−95.3 ± 4.6	−95.4 ± 2.2	−96.5 ± 1.2	−94.5 ± 1.0	−94.2 ± 1.5
Post	−97.0 ± 3.1	−93.6 ± 3.3	−98.5 ± 1.3	−93.0 ± 1.5	−96.2 ± 1.9
Input resistance, MΩ					
Pre	36.5 ± 8.8	44.7 ± 4.6	56.5 ± 17.6	50.4 ± 9.9	44.5 ± 3.1
Post	33.6 ± 6.0	41.7 ± 0.9	59.9 ± 16.8	42.5 ± 15.4	37.1 ± 2.4
Firing threshold, mV					
Pre	−53.7 ± 0.2	−53.6 ± 2.8	−53.2 ± 5.1	−58.2 ± 1.1	−49.9 ± 3.1
Post	−50.5 ± 2.0	−52.5 ± 2.5	−53.7 ± 3.5	−57.0 ± 1.5	−48.3 ± 6.1
Action potential amplitude, mV					
Pre	78.0 ± 6.5	77.4 ± 2.1	75.9 ± 1.4	76.9 ± 3.2	76.5 ± 2.5
Post	68.6 ± 9.3	76.4 ± 4.3	73.5 ± 1.8	78.7 ± 2.0	79.7 ± 2.4
Action potential duration, ms					
Pre	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.9 ± 0.0	0.7 ± 0.0
Post	0.7 ± 0.2	0.7 ± 0.1	0.8 ± 0.0	0.8 ± 0.0	0.7 ± 0.0
AHP amplitude, mV					
Pre	14.1 ± 1.7	14.5 ± 1.7	14.0 ± 0.3	12.0 ± 0.5	13.5 ± 0.6
Post	15.4 ± 1.5	13.6 ± 1.3	11.2 ± 1.0	13.9 ± 0.5	16.1 ± 0.9
EPSP slope, mV/ms					
Pre	6.8 ± 1.0	5.3 ± 1.2	7.0 ± 0.4	5.9 ± 0.7	7.2 ± 1.2
Post	7.7 ± 1.0	5.0 ± 1.0	8.7 ± 0.4	5.2 ± 0.4	10.7 ± 1.3
EPSP peak, mV					
Pre	16.0 ± 2.0	16.7 ± 3.3	19.2 ± 1.6	17.7 ± 1.5	18.0 ± 2.0
Post	19.4 ± 2.2	16.5 ± 3.5	23.1 ± 1.2	16.0 ± 1.6	22.2 ± 2.8

Cellular properties were measured routinely 10 min before high-frequency stimulation (HFS Pre) and 20 min after HFS (Post). There were no significant differences in cellular properties between treatment groups (2-tailed *t*-test) or within groups before and after HFS (paired *t*-test). Values are means ± SE. AMPT, alpha-methyl paratyrosine; AHP, afterhyperpolarization; EPSP, excitatory postsynaptic potential.

The blockade of Mg^{2+}-free LTP by a dopamine D-1/D-5 receptor antagonist suggests that endogenous dopamine may be involved in this form of LTP. The requirement for endogenous dopamine was tested in slices depleted of releasable dopamine by pretreatment with AMPT. Pretreatment of slices with AMPT blocked Mg^{2+}-free LTP (Fig. 5, *A* and *C*). Averaging across all slices tested ($n = 6$), there was no change in the PSP peak amplitude (−9.4 ± 10.2%) or slope (−13.2 ± 10.2%) measured 20 min after HFS. The difference between the Mg^{2+}-free control group and the AMPT-treated group was significant ($P < 0.05$).

Application of the dopamine D-1 receptor agonist SKF 38393 restored the ability of AMPT-treated slices to show Mg^{2+}-free LTP (Fig. 5, *B* and *C*). There was LTP of PSP peak amplitude (20.5 ± 7.8%) and slope (18.4 ± 6.6%) measured 20 min after HFS ($n = 5$). The difference between the control (dopamine-depleted) group and the SKF 38393 (dopamine-depleted) group was significant ($P < 0.05$).

An apparent short-term facilitation of both PSP slope and peak was observed in slices exposed to SCH23390 (Fig. 2). This short-term facilitation was not significantly different in magnitude from the initial potentiation seen in control slices, slices exposed to Remoxipride, or AMPT-treated slices exposed to SKF 38393 (Fig. 4). Short-term facilitation was, however, completely abolished in slices from AMPT-treated animals (Fig. 5).

Changes (or the absence of changes) in postsynaptic response measures (slope and peak of the PSP) could be secondary to changes in cellular properties other than synaptic efficacy. This possibility was tested by measuring selected cellular properties 10 min before and 20 min after HFS. No significant changes were observed in resting membrane potential, input resistance, action potential threshold, action potential amplitude and duration, or AHPs (Figs. 1*C*, 2*C*, and 3*C* and Table 1).

Between-group differences in the induction of Mg^{2+}-free LTP could also in theory be secondary to differences in cellular properties affecting neuronal excitability. However, there were no significant between group differences in cellular properties as a result of treatment with SCH 23390, remoxipride, AMPT, or AMPT plus SKF 38393 (Table 1). To exclude possible differences in the level of depolarization during HFS, the intensity of the applied current and the responses of the postsynaptic cell to HFS plus depolarization were also compared between groups. There were no significant differences between groups in the intensity of the current pulse used during HFS, the number of action potentials fired, or the level of depolarization produced in the postsynaptic neuron during HFS (data not shown).

DISCUSSION

The main finding of the present study was that dopamine D-1/D-5 receptor activation is a necessary requirement for Mg^{2+}-free LTP in the neostriatum. The present result is in agreement with previous studies showing that LTP could reliably be induced by HFS of the corticostriatal pathway in slices bathed in Mg^{2+}-free solution (Calabresi et al. 1992c; Walsh 1991). In addition, we found that Mg^{2+}-free LTP was blocked by the dopamine D-1/D-5 receptor antagonist SCH 23390 and not blocked by the dopamine D-2-selective antagonist remoxipride. To our knowledge, this is the first study showing that dopamine D-1/D-5 receptor activation is necessary for Mg^{2+}-free LTP in the neostriatum.

The slices treated with SCH 23390 showed an apparent short-term facilitation that was not evident in other groups.

This may be an early phase of Mg^{2+}-free LTP that is unmasked by the prevention of the later phase of LTP in the presence of SCH 23390. Previously a role of dopamine D-1/D-5 receptors in the persistence of LTP has been described in the hippocampal CA1 area, where Frey et al. (1991) showed that the presence of SCH 23390 resulted in prevention of late LTP stages (more than 1–2 h). This effect in the hippocampus occurs over a longer time course than that described in the present experiments (hours rather than minutes) but may involve similar cellular mechanisms.

At the doses of SCH 23390 used in the present study (10 μM), it is not possible to rule out an effect on other monoamine receptors such as those for serotonin. However, Mg^{2+}-free LTP was also blocked in slices which were dopamine depleted by pretreatment with AMPT, which does not lead to depletion of serotonin. The only difference between these treatments was the complete abolition of short-term facilitation in the AMPT group, which indirectly suggests a possible link between short-term facilitation and serotonergic effects of SCH 23390; this might warrant further experimental investigation.

It has been reported recently that Mg^{2+}-free LTP was blocked after chronic dopamine depletion with 6-hydroxydopamine (Centonze et al. 1999). Treatment with 6-hydroxydopamine has also been shown to cause loss of synapses in the striatum (Ingham et al. 1998), and this may affect the synapses that are normally potentiated in Mg^{2+}-free LTP. The present results show, however, that in slices acutely depleted of endogenous dopamine by pretreatment with AMPT, HFS in Mg^{2+}-free solutions similarly failed to induce LTP. Together with the previous result, these findings suggest that endogenous dopamine release at the time of HFS is a necessary requirement for Mg^{2+}-free LTP.

Another important finding of the present study is that after acute dopamine depletion with AMPT, Mg^{2+}-free LTP is restored to control levels by bath application of the dopamine D-1 receptor agonist SKF 38393. We are not aware of any previous reports showing that dopamine D-1 receptor activation restores LTP in dopamine-depleted slices. Together with the effects of dopamine depletion and dopamine D-1/D-5 receptor antagonists, the effects of the D-1 receptor agonist strongly implicate dopamine D-1/D-5 receptors in Mg^{2+}-free LTP. The neostriatal dopamine D-1/D-5 receptors are G-protein-coupled receptors that activate adenylyl cyclase leading to intracellular accumulation of cyclic AMP (cAMP). Thus Mg^{2+}-free LTP probably involves elevation of cAMP and triggering of related biochemical cascades present in neostriatal neurons. Elevation of cAMP by direct activation of adenylyl cyclase (Colwell and Levine 1995) or dopamine D-1/D-5 receptors (Price et al. 1999) causes enhancement of responses to glutamatergic agonists. In contrast, the dopamine D-2 receptor is negatively coupled to adenylyl cyclase. We found that the dopamine D-2 receptor antagonist, remoxipride, did not block Mg^{2+}-free LTP. These results are compatible with dopamine D-1/D-5 receptor mediated activation of adenylyl cyclase being necessary for the enhancement of synaptic transmission in Mg^{2+}-free LTP.

The magnitude of Mg^{2+}-free LTP was not reduced in the presence of the $GABA_A$ antagonist, picrotoxin. This finding is important because previous studies have not ruled out the possibility that Mg^{2+}-free LTP included a component of LTP of feed-forward inhibitory IPSPs (Calabresi et al. 1992c; Walsh 1991). At the hyperpolarized resting membrane potentials typical of spiny neostriatal neurons, IPSPs may be depolarizing because the equilibrium potential of $GABA_A$-activated conductances is more depolarized (Misgeld et al. 1982). This made it necessary to test if the changes in cortically evoked test responses were due to changes in feedforward IPSPs. The finding that Mg^{2+}-free LTP was not blocked or reduced in magnitude by the $GABA_A$ antagonist picrotoxin shows that Mg^{2+}-free LTP is not due to changes in $GABA_A$ mediated IPSPs. It implies that the LTP is due to changes in excitatory postsynaptic potentials.

In the present study, LTP could reliably be induced by HFS in Mg^{2+}-free solution in all cells tested. The reliability of induction of this form of LTP is consistent with a previous study in which longer HFS trains were applied. Calabresi et al. (1992c) described LTP in Mg^{2+}-free solution after 900 suprathreshold stimulus pulses (applied as 3 trains of 300 pulses at 100 Hz). In contrast, Walsh (1993) found that a somewhat milder induction protocol involving 400 subthreshold stimulus pulses (applied as 4 trains of 100 pulses at 100 Hz) did not reliably induce LTP but induced short-term potentiation lasting from 5 to 45 min. In the present study, LTP lasting for as long as recordings were continued (at least 20 min and up to 40 min) was elicited by HFS consisting of a total of 300 subthreshold stimulus pulses (applied as 6 trains of 50 pulses at 100 Hz) in conjunction with suprathreshold depolarization of the postsynaptic neuron. These results show that Mg^{2+}-free LTP is a robust phenomenon that is readily reproduced in different laboratories despite marked differences in HFS protocols.

Previous work has shown that the induction of Mg^{2+}-free LTP in the neostriatum can be blocked by NMDA receptor antagonists (Calabresi et al. 1992c). In normal bathing solutions, NMDA receptor-operated channels play a minor role in corticostriatal synaptic transmission (Kita 1996). This is because at hyperpolarized membrane potentials such channels are closed by a magnesium block (Nowak et al. 1984) and, as in the present and previous studies (Jiang and North 1991), the resting membrane potential of the neostriatal neurons is strongly hyperpolarized. Thus it is necessary to consider whether the facilitation of LTP in Mg^{2+}-free is due to the greater influx of calcium postsynaptically in Mg^{2+}-free solution. In Mg^{2+}-free conditions, HFS of cortical afferents paired with suprathreshold current injection as used in the present study can be expected to produce influx of calcium via NMDA channels. This failed to produce LTP in slices that had been dopamine depleted by pretreatment with AMPT or slices in which dopamine D-1/D-5 receptors were blocked by a selective antagonist. Thus the present results show that activation of NMDA channels per se is not sufficient for induction of Mg^{2+}-free LTP.

Calabresi et al. (1997) have shown abnormal induction of LTP in slices made from mice lacking dopamine D-2 receptors. In slices made from dopamine D-2 receptor knockout mice, HFS of the corticostriatal pathway under conditions that would normally induce LTD produced LTP. In contrast to the present results showing blockade of Mg^{2+}-free LTP in wild-type rats, the abnormal form of LTP seen in the D-2 receptor-deficient mice was not blocked by SCH 23390. A potentially important factor in these differences is that D-2 receptor-deficient mice have abnormal dopamine function throughout life. Dopaminergic receptor mechanisms can be disrupted by abnormal dopa-

mine receptor stimulation during development. For example, rats given 6-OHDA lesions as neonates show a substantial sub-sensitivity to both D-2 and D-1 antagonists as adults (Duncan et al. 1987). Thus it is plausible that in the D-2 receptor-deficient mice, the dose of SCH 23390 used by Calabresi et al. (1997) may not produce effective D-1 blockage in dopamine receptor knockout animals.

Another possible cause of the difference in response to SCH 23390 in the present experiments and in the D-2 receptor-deficient mice (Calabresi et al. 1997) is that the neurons in the present study were relatively polarized (−95 mV in comparison to −85 mV in the D-2 receptor-deficient mice). No holding current was used in the present experiments, and the resting membrane potential reflects the selection criteria used, the quality of the impalement, the ionic composition of the extracellular fluid, and the effects of the ionic composition of the intracellular electrode solution (Nisenbaum and Wilson 1995). Furthermore during the plasticity-inducing HFS, depolarizing current was applied to ensure suprathreshold depolarization of the recorded cells. This minimizes any potential interaction between resting membrane potential and the production of LTP. In addition, because there was no significant difference in resting membrane potential between the groups in the present experiments (Table 1), the polarized resting membrane potential is unlikely to have influenced the results.

While showing that activation of NMDA channels is not sufficient for LTP induction, the present results do not exclude a complex modulatory effect of dopamine D-1/D-5 receptor activation on NMDA receptor-mediated channels, which may also be necessary for LTP induction. Previous work has shown that dopamine application leads to an enhancement of NMDA receptor-mediated responses that is apparently mediated by dopamine D-1/D-5 receptors (Cepeda et al. 1992, 1993; Levine et al. 1996). Such enhancement of NMDA receptor-mediated channels may be necessary for LTP to occur in Mg-free solutions, suggesting that both dopamine D-1/D-5 receptor activation and NMDA receptor-mediated channel activation may be necessary. Futher experiments are needed to investigate this possibility.

An alternative explanation for the facilitation of striatal LTP in Mg^{2+}-free conditions is that these conditions favor dopamine release; an effect that itself is mediated by activation of presynaptic NMDA receptors (Desce et al. 1992; Krebs et al. 1991a,b; Roberts and Sharif 1978) presumably located on dopaminergic nerve terminals. The dopaminergic terminals on spiny projection neurons synapse in close proximity to the glutamatergic corticostriatal terminals (Freund et al. 1984; Hersch et al. 1995; Smith et al. 1994; Yung et al. 1995). Thus glutamate released from corticostriatal terminals during cortical HFS might act directly on adjacent dopaminergic terminals to cause dopamine release. Spillover of glutamate under Mg^{2+}-free conditions favors NMDA-mediated release of endogenous dopamine. A model in which Mg^{2+}-free conditions favor LTP by facilitating endogenous dopamine release is compatible with the present findings as well as previous results showing that in the absence of exogenous dopamine NMDA receptor activation is necessary for Mg^{2+}-free LTP (Calabresi et al. 1992c) and that pulsatile application of exogenous dopamine facilitates LTP under physiological conditions (Wickens et al. 1996). To confirm this possibility, further experiments are needed to measure endogenous dopamine release in response to HFS in Mg^{2+}-free and control conditions.

In summary, the present study has confirmed previous reports that Mg^{2+}-free LTP is a reliable and robust phenomenon in the neostriatum. It has extended understanding of this phenomenon by showing that Mg^{2+}-free LTP is dopamine dependent and requires activation of dopamine D-1/D-5 receptors but not dopamine D-2 receptors. Furthermore it has shown that LTP can be restored in dopamine-depleted slices by the application of a dopamine D-1/D-5 agonist. These results are significant for current understanding of learning and memory mechanisms of the neostriatum and are compatible with behavioral evidence that D-1/D-5 receptors are important in reward-related learning (Sutton and Beninger 1999; Wickens and Kotter 1995). The results are also significant for theoretical understanding of the mechanism of the therapeutic effect of dopamine receptor antagonist drugs used in the treatment of psychotic illnesses (Miller et al. 1990) and dopamine receptor agonists in the treatment of Parkinson's disease (Rascol et al. 1999).

We thank Prof. W. C. Abraham and Dr. D. Plenz for helpful comments on the manuscript.

This work was supported by the New Zealand Neurological Foundation, the New Zealand Health Research Council, the New Zealand Schizophrenia Fellowship, the HS and JC Anderson Trust, and the New Zealand Lottery Grants Board.

Present address of J.N.D. Kerr: Unit of Neural Networks Physiology, LSN/NIMH, National Institutes of Health, Bethesda, MD 20892-4075.

REFERENCES

AGHAJANIAN G AND RASMUSSEN K. Intracellular studies in the facial nucleus illustrating a simple new method for obtaining viable motoneurons in adult brain slices. *Synapse* 3: 331–338, 1989.

ARBUTHNOTT GW, MACLEOD N, AND RUTHERFORD A. The rat cortico-striatal pathway in vitro (Abstract). *J Physiol (Lond)* 367: 102P, 1985.

BENINGER RJ. The role of dopamine in locomotor activity and learning. *Brain Res* 287: 173–196, 1983.

BLISS TVP AND COLLINGRIDGE GL. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 361: 31–39, 1993.

CALABRESI P, FEDELE E, PISANI A, FONTANA G, MERCURI NB, BERNARDI G, AND RAITERI M. Transmitter release associated with long-term synaptic depression in rat corticostriatal slices. *Eur J Neurosci* 7: 1889–1894, 1995.

CALABRESI P, MAJ R, MERCURI NB, AND BERNARDI G. Coactivation of D1 and D2 dopamine receptors is required for long-term synaptic depression in the striatum. *Neurosci Lett* 142: 95–99, 1992a.

CALABRESI P, MAJ R, PISANI A, MERCURI NB, AND BERNARDI G. Long-term synaptic depression in the striatum: physiological and pharmacological characterization. *J Neurosci* 12: 4224–4233, 1992b.

CALABRESI P, PISANI A, MERCURI NB, AND BERNARDI G. Long-term potentiation in the striatum is unmasked by removing the voltage-dependent magnesium block of NMDA receptor channels. *Eur J Neurosci* 4: 929–935, 1992c.

CALABRESI P, PISANI A, MERCURI NB, AND BERNARDI G. Post-receptor mechanisms underlying striatal long-term depression. *J Neurosci* 14: 4871–4881, 1994.

CALABRESI P, SAIARDI A, PISANI A, BAIL J-H, CENTONZE D, MERCURI NB, BERNARDI G, AND BORRELLI E. Abnormal synaptic plasticity in the striatum of mice lacking dopamine D2 receptors. *J Neurosci* 17: 4536–4544, 1997.

CENTONZE D, GUBELLINI P, PICCONI B, CALABRESI P, GIACOMINI P, AND BERNARDI G. Unilateral dopamine denervation blocks corticostriatal LTP. *J Neurophysiol* 82: 3575–3579, 1999.

CEPEDA C, BUCHWALD NA, AND LEVINE MS. Neuromodulatory actions of dopamine in the neostriatum are dependent upon the excitatory amino acid receptor subtypes activated. *Proc Natl Acad Sci USA* 90: 9576–9580, 1993.

CEPEDA C, RADISAVLJEVIC Z, PEACOCK W, LEVINE MS, AND BUCHWALD NA. Differential modulation by dopamine of responses evoked by excitatory amino acids in human cortex. *Synapse* 11: 330–341, 1992.

COLWELL CS AND LEVINE MS. Excitatory synaptic transmission in neostriatal neurons: regulation by cyclic AMP-dependent mechanisms. *J Neurosci* 15: 1704–1713, 1995.

CUMMING P, VENKATACHALAM TK, RAJAGOPAL S, DIKSIC M, AND GJEDDE A. Brain uptake of alpha-[14C] methyl-para-tyrosine in the rat. *Synapse* 17: 125–128, 1994.

DESCE JM, GODEHEU G, GALLI T, ARTAUD F, CHERAMY A, AND GLOWINSKY J. L-glutamate-evoked release of dopamine from synaptosomes of the rat striatum: involvement of AMPA and N-methyl-D-aspartate receptors. *Neuroscience* 47: 333–339, 1992.

DUNCAN GE, CRISWELL HE, MCCOWN TJ, PAUL IA, MUELLER RA, AND BREESE GR. Behavioral and neurochemical responses to haloperidol and SCH-23390 in rats treated neonatally or as adults with 6-hydroxydopamine. *J Pharmacol Exp Ther* 243: 1027–1034, 1987.

FREUND TF, POWELL JF, AND SMITH AD. Tyrosine hydroxylase-immunoreactive boutons in synaptic contact with identified striatonigral neurons, with particular reference to dendritic spines. *Neuroscience* 13: 1189–1215, 1984.

FREY U, MATTHIES H, REYMANN KG, AND MATTHIES H. The effect of dopaminergic D1 blockade during tetanization on the expression of long-term potentiation in the rat CA1 region in vitro. *Neurosci Lett* 129: 111–114, 1991.

GRAYBIEL AM. Building action repertoires: memory and learning functions of the basal ganglia. *Curr Opin Neurobiol* 5: 733–741, 1995.

GROVES PM. A theory of the functional organisation of the neostriatum and the neostriatal control of voluntary movement. *Brain Res Rev* 5: 109–132, 1983.

HERSCH SM, CILIAX BJ, GUTEKUNST CA, REES HD, HEILMAN CJ, YUNG KKL, BOLAM JP, INCE E, YI H, AND LEVEY AI. Electron microscopic analysis of D1 and D2 dopamine receptor proteins in the dorsal striatum and their synaptic relationships with motor corticostriatal afferents. *J Neurosci* 15: 5222–5237, 1995.

INGHAM CA, HOOD SH, TAGGART P, AND ARBUTHNOTT GW. Plasticity of synapses in the rat neostriatum after unilateral lesion of the nigrostriatal dopaminergic pathway. *J Neurosci* 18: 4732–4743, 1998.

JIANG Z-G AND NORTH RA. Membrane properties and synaptic responses of rat striatal neurones in vitro. *J Neurophysiol* 443: 533–553, 1991.

KAWAGUCHI Y, WILSON CJ, AND EMSON PC. Intracellular recording of identified neostriatal patch and matrix spiny cells in a slice preparation preserving cortical inputs. *J Neurophysiol* 62: 1052–1068, 1989.

KITA H. Glutamatergic and GABAergic postsynaptic responses of striatal spiny neurons to intrastriatal and cortical stimulation recorded in slice preparations. *Neuroscience* 70: 925–940, 1996.

KREBS MO, DESCE JM, KEMEL ML, GAUCHY C, GODEHEU G, CHERAMY A, AND GLOWINSKY J. Glutamatergic control of dopamine release in the rat striatum: evidence for presynaptic *N*-methyl-D-aspartate receptors on dopaminergic nerve terminals. *J Neurochem* 56: 81–85, 1991a.

KREBS MO, TROVERO F, DESBAN M, GAUCHY C, GLOWINSKY J, AND KEMEL M. Distinct presynaptic regulation of dopamine release through NMDA receptors in striosome and matrix-enriched areas of the rat striatum. *J Neurosci* 11: 1256–1262, 1991b.

LEVINE MS, LI ZW, CEPEDA C, CROMWELL HC, AND ALTEMUS KL. Neuromodulatory actions of dopamine on synaptically-evoked neostriatal responses in slices. *Synapse* 24: 65–78, 1996.

LOVINGER DM, TYLER EC, AND MARRITT A. Short- and long-term depression in the rat neostriatum. *J Neurophysiol* 70: 1937–1949, 1993.

MCGEER PL, MCGEER EG, SCHERER U, AND SINGH K. A glutamatergic corticostriatal path? *Brain Res* 128: 369–373, 1977.

MCGEORGE AJ AND FAULL RL. The organisation of the projections from the cerebral cortex to the striatum in the rat. *Neuroscience* 29: 503–537, 1989.

MILLER R. *Meaning and Purpose in the Intact Brain.* Oxford: Clarendon Press, 1981.

MILLER R, WICKENS JR, AND BENINGER R. Dopamine D-1 and D-2 receptors in relation to reward and performance: a case for the D-1 receptor as a primary site of therapeutic action of neuroleptic drugs. *Prog Neurobiol* 34: 143–183, 1990.

MISGELD U, WAGNER A, AND OHNO T. Depolarizing IPSPs and depolarization by GABA of rat neostriatum cells in vitro. *Exp Brain Res* 45: 108–114, 1982.

NISENBAUM ES AND WILSON CJ. Potassium currents responsible for inward and outward rectification in rat neostriatal spiny projection neurons. *J Neurosci* 15: 4449–4463, 1995.

NOWAK L, BREGESTOVSKI P, ASCHER P, HERBET A, AND PROCHIANTZ A. Magnesium gates glutamate-activated channels in mouse central neurones. *Nature* 307: 462–465, 1984.

PRESTON RJ, BISHOP GA, AND KITAI ST. Medium spiny neuron projection from the rat neostriatum: an intracellular horseradish peroxidase study. *Brain Res* 183: 253–263, 1980.

PRICE CJ, KIM P, AND RAYMOND LA. D1 dopamine receptor-induced cyclic AMP-dependent protein kinase phosphorylation and potentiation of striatal glutamate receptors. *J Neurochem* 73: 2441–2446, 1999.

RASCOL O, BLIN O, THALAMAS C, DESCOMBES A, SOUBROUILLARD C, AZULAY P, FABRE N, VIALLET F, LAFNITZEGGER K, WRIGHT S, CARTER JH, AND NUTT JG. ABT-431, a D1 receptor agonist prodrug, has efficacy in Parkinson's disease. *Ann Neurol* 45: 736–741, 1999.

REYNOLDS JNJ AND WICKENS JR. Substantia nigra dopamine regulates synaptic plasticity and membrane potential fluctuations in the rat neostriatum, in vivo. *Neuroscience* 99: 199–203, 2000.

ROBERTS PJ AND SHARIF NA. Effects of L-glutamate and related amino acids upon the release of [3H] dopamine from rat striatal slices. *Brain Res* 157: 391–395, 1978.

SMITH Y, BENNETT BD, BOLAM JP, PARENT A, AND SADIKOT AF. Synaptic relationships between dopaminergic afferents and cortical or thalamic input in the sensorimotor territory of the striatum in monkey. *J Comp Neurol* 344: 1–19, 1994.

SOMOGYI JP, BOLAM JP, AND SMITH AD. Monosynaptic cortical input and local axon collaterals of identified striatonigral neurons. A light and electron microscope study using the Golgi-peroxidase transport degeneration procedure. *J Comp Neurol* 195: 567–584, 1981.

SUTTON MA AND BENINGER RJ. Psychopharmacology of conditioned reward: evidence for a rewarding signal at D1-like dopamine receptors. *Psychopharmacology* 144: 95–110, 1999.

WALSH JP. Long-term potentiation (LTP) of the excitatory synaptic input to medium spiny neurons of the rat striatum. *Soc Neurosci Abstr* 17: 852, 1991.

WALSH JP AND DUNIA R. Synaptic activation of *N*-methyl-D-aspartate receptors induces short-term potentiation at excitatory synapses in the striatum of the rat. *Neuroscience* 57: 241–248, 1993.

WHITE FJ, HU XT, AND HENRY DJ. Electrophysiological effects of cocaine in the rat nucleus accumbens: microiontophoretic studies. *J Pharmacol Exp Ther* 266: 1075–1084, 1993.

WICKENS JR. Striatal dopamine in motor activation and reward-mediated learning. Steps towards a unifying model. *J Neural Transm* 80: 9–31, 1990.

WICKENS JR, BEGG AJ, AND ARBUTHNOTT GW. Dopamine reverses the depression of rat cortico-striatal synapses which normally follows high frequency stimulation of cortex in vitro. *Neuroscience* 70: 1–5, 1996.

WICKENS JR AND KOTTER R. Cellular models of reinforcement. In: *Models of Information Processing in the Basal Ganglia*, edited by Houk JC, Davis JL, and Beiser DG. Cambridge, MA: MIT Press, 1995, p. 187–214.

WICKENS JR, MCKENZIE D, CONSTANZO E, AND ARBUTHNOTT GW. Site of action of potassium channel blockers in striatal long-term potentiation. *Neuropharmacology* 37: 523–533, 1998.

WILLIAMS GV AND MILLAR J. Differential actions of endogenous and iontophoretic dopamine in rat striatum. *Eur J Neurosci* 2: 658–661, 1990.

WILSON CJ, CHANG HT, AND KITAI ST. Disfacilitation and long-lasting inhibition of neostriatal neurons in the rat. *Exp Brain Res* 51: 227–235, 1983.

WILSON CJ AND GROVES PM. Spontaneous firing patterns of identified spiny neurons in the rat neostriatum. *Brain Res* 220: 67–80, 1981.

YUNG KK, BOLAM JP, SMITH AD, HERSCH SM, CILIAX BJ, AND LEVEY AI. Immunocytochemical localization of D1 and D2 dopamine receptors in the basal ganglia of the rat: light and electron microscopy. *Neuroscience* 65: 709–730, 1995.