Brief Communications

Loss of Muscarinic Autoreceptor Function Impairs Long-Term Depression But Not Long-Term Potentiation in the Striatum

Paola Bonsi,[1] **Giuseppina Martella,**[2] **Dario Cuomo,**[2] **Paola Platania,**[2] **Giuseppe Sciamanna,**[1,2] **Giorgio Bernardi,**[1,2] **Jürgen Wess,**[3] **and Antonio Pisani**[1,2]

[1]Fondazione Santa Lucia Instituto di Ricovero e Cura a Carattere Scientifico, Rome 00146, Italy, [2]Department of Neuroscience, University "Tor Vergata," Rome 00133, Italy, and [3]Molecular Signaling Section, Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892-0810

Muscarinic autoreceptors regulate cholinergic tone in the striatum. We investigated the functional consequences of genetic deletion of striatal muscarinic autoreceptors by means of electrophysiological recordings from either medium spiny neurons (MSNs) or cholinergic interneurons (ChIs) in slices from single M_4 or double M_2/M_4 muscarinic acetylcholine receptor (mAChR) knock-out ($-/-$) mice. In control ChIs, the muscarinic agonist oxotremorine (300 nM) produced a self-inhibitory outward current that was mostly reduced in $M_4^{-/-}$ and abolished in $M_2/M_4^{-/-}$ mice, suggesting an involvement of both M_2 and M_4 autoreceptors. In MSNs from both $M_4^{-/-}$ and $M_2/M_4^{-/-}$ mice, muscarine caused a membrane depolarization that was prevented by the M_1 receptor-preferring antagonist pirenzepine (100 nM), suggesting that M_1 receptor function was unaltered. Acetylcholine has been involved in striatal long-term potentiation (LTP) or long-term depression (LTD) induction. Loss of muscarinic autoreceptor function is predicted to affect synaptic plasticity by modifying striatal cholinergic tone. Indeed, high-frequency stimulation of glutamatergic afferents failed to induce LTD in MSNs from both $M_4^{-/-}$ and $M_2/M_4^{-/-}$ mice, as well as in wild-type mice pretreated with the M_2/M_4 antagonist AF-DX384 (11-[[2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5,1 1-dihydro-6H-pyrido[2,3b][1,4] benzodiazepin-6-one). Interestingly, LTD could be restored by either pirenzepine (100 nM) or hemicholinium-3 (10 μM), a depletor of endogenous ACh. Conversely, LTP induction did not show any difference among the three mouse strains and was prevented by pirenzepine. These results demonstrate that M_2/M_4 muscarinic autoreceptors regulate ACh release from striatal ChIs. As a consequence, endogenous ACh drives the polarity of bidirectional synaptic plasticity.

Key words: cholinergic interneuron; long-term depression; long-term potentiation; striatal slices; electrophysiology; muscarine

Introduction

Acetylcholine (ACh) plays an essential role in striatal synaptic plasticity and motor learning, as well as in the pathophysiology of movement disorders (Calabresi et al., 2000; Partridge et al., 2002; Pisani et al., 2007). Striatal ACh innervation is exclusively intrinsic, originating from large cholinergic interneurons (ChIs) (Bolam et al. 1984). Regardless of their paucity, cholinergic markers measured in this region are among the highest within the brain. ChIs exhibit autonomous pacemaker activity, firing action potentials at rest, providing a constant ACh tone in the striatum (Bennett and Wilson, 1999; Zhou et al., 2002). Maintenance of discrete ACh levels is ensured by either ACh degrading enzymes or by M_2/M_4 muscarinic autoreceptors, which exert a negative feedback control on ACh release, hyperpolarizing ChIs through Ca_v2 calcium current inhibition and the opening of potassium (K^+) currents (Yan and Surmeier, 1996; Calabresi et al., 1998).

Five distinct muscarinic acetylcholine receptors (mAChRs; M_1–M_5) have been cloned and divided into two main classes, according to their pharmacological properties and transduction mechanisms (Wess et al., 2007). Receptor localization studies have revealed a predominant expression of M_1 and M_4 receptors on striatal neuronal subpopulations, whereas M_2 and M_3 are clearly less abundant (Zhang et al., 2002; Zhou et al., 2002). ACh also exerts its modulatory effects through nicotinic receptors, expressed on striatal dopaminergic terminals whose activation stimulates dopamine release. The close functional interplay between dopamine and nicotinic receptors is proven by the observation that nicotinic receptor antagonists prevent the induction of striatal synaptic plasticity (Partridge et al., 2002).

High-frequency stimulation (HFS) of the glutamatergic pathway causes either a long-lasting increase, or an enduring decrease in synaptic efficacy, referred to as long-term potentiation (LTP) or long-term depression (LTD), respectively (Calabresi et al., 1992a,b; Lovinger et al., 1993). Compelling evidence suggests that

intrastriatal ACh levels dictate the polarity of plastic changes via M_1 receptor activation, that are expressed on dendrites and spines of medium spiny neurons (MSNs), and are therefore strategically positioned to influence synaptic plasticity (Hersch et al. 1994, Yan et al., 2001). Indeed, M_1 receptor activation increases MSN excitability by reducing KCNQ and Kir2 currents (Galarraga et al., 1999; Shen et al., 2005, 2007). Consequently, M_1 receptor activation favors MSN depolarization, playing a permissive role in LTP (Calabresi et al., 2000). However, M_1 receptor activation also inhibits Ca_v1 and Ca_v2 calcium currents (Howe and Surmeier, 1995) that are necessary for LTD induction. It has been proposed recently that reducing ambient M_1 receptor stimulation promotes LTD through disinhibition of Ca_v1 channels (Wang et al., 2006).

The lack of selective ligands for mAChRs has made it difficult to define the role of striatal mAChRs by pharmacological means (Wess et al., 2007). We analyzed the consequences of muscarinic autoreceptor loss on striatal synaptic plasticity by using a combined pharmacological/molecular genetic approach. Specifically, we performed electrophysiological recordings from MSNs and ChIs using slices from wild-type (WT), single $M_4^{-/-}$, or double $M_2/M_4^{-/-}$ mice (Gomeza et al., 1999a,b, 2001).

Materials and Methods

Slice preparation. All procedures were performed in accord with the European Commission guidelines (86/609/EEC) and approved by the University "Tor Vergata." Mice (4–6 weeks old) were generated as described (Gomeza et al., 1999a,b, 2001). WT littermates of the same mixed genetic background were used as controls. Coronal corticostriatal slices (180–250 μm) were cut in Krebs' solution [containing (in mM) 126 NaCl, 2.5 KCl, 1.3 MgCl$_2$, 1.2 NaH$_2$PO$_4$, 2.4 CaCl$_2$, 10 glucose, and 18 NaHCO$_3$], bubbled with 95% O$_2$/and 5% CO$_2$. Individual slices were transferred in a recording chamber (~0.5–1 ml volume), continuously superfused with oxygenated Krebs' medium at 2.5–3 ml/min (32–33°C).

Electrophysiology. Current-clamp recordings were performed using sharp microelectrodes filled with 2 M KCl (40–60 MΩ). Signal acquisition and off-line analysis were performed using an Axoclamp 2B amplifier and pClamp9 software (Molecular Devices). Glutamatergic EPSPs were evoked with a bipolar electrode placed in the white matter. Test stimuli were delivered at 0.1 Hz in bicuculline. For high-frequency stimulation (three trains, 3 s duration, 100 Hz frequency, 20 s intervals), stimulus intensity was raised to suprathreshold levels. The amplitude of EPSPs was averaged and plotted as % of the control amplitude for ~10 min before HFS. EPSPs did not reveal significant differences in the three groups of mice (data not shown) ($p > 0.05$) and were suppressed by the AMPA receptor antagonist CNQX (10 μM).

Whole-cell recordings were made of ChIs visualized by means of infrared-differential interference contrast videomicroscopy, as described (Bonsi et al.,
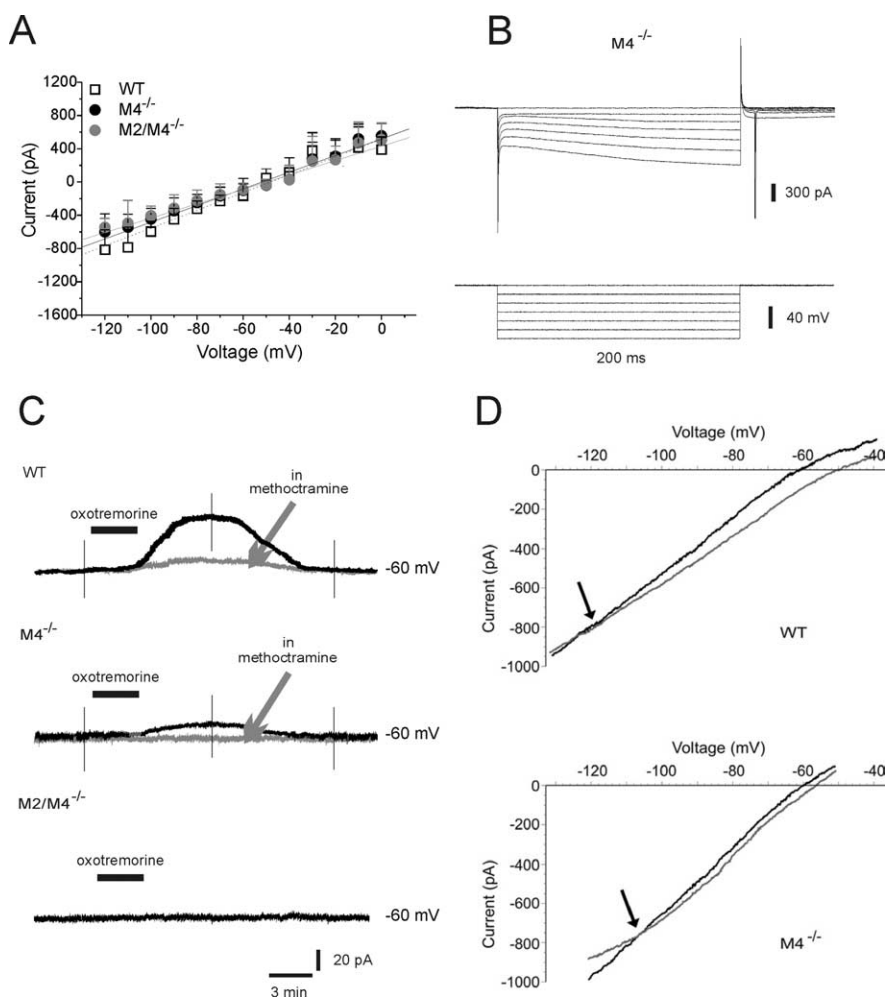

Figure 1. Muscarinic autoreceptor responses recorded from ChIs of knock-out mice. ***A***, Current–voltage relationships of ChIs recorded in slices obtained from WT, $M_4^{-/-}$, and $M_2/M_4^{-/-}$ mice are not significantly different from each other (ANOVA, $p > 0.05$). ***B***, Representative recording of a ChI from $M_4^{-/-}$ mice showing no change in the typical sag caused by I_h activation at hyperpolarized potentials. ***C***, Top, In a representative recording, bath application of oxotremorine (300 nM, 3 min) induced an outward current (black trace). This response was reduced by preincubation with the M_2/M_4 receptor antagonist methoctramine (gray trace; 300 nM). Middle, The response to oxotremorine (black trace) is mostly reduced compared with WT, and was completely blocked by methoctramine in $M_4^{-/-}$ ChIs (gray trace). Bottom, The recording shows the absence of any detectable response to bath-applied oxotremorine in $M_2/M_4^{-/-}$ mice. The deflections represent voltage ramps applied before, at the peak of the oxotremorine-induced current, and after drug washout. ***D***, Plots of a recording from a WT (top) and $M_4^{-/-}$ ChI (bottom) showing the estimated reversal potential for the oxotremorine-induced current. Gray line, Voltage ramp in control condition; black line, voltage ramp at the peak of oxotremorine effect. Holding potential, −60 mV. Error bars indicate SEM.

2007). Borosilicate glass pipettes (3–5 MΩ) contained (in mM) 125 K$^+$-gluconate, 10 NaCl, 1 CaCl$_2$, 2 MgCl$_2$, 0.5 BAPTA [1,2-bis (2-aminophenoxy) ethane-N,N,N,N-tetraacetic acid], 19 HEPES, 0.3 GTP, and 1 Mg-ATP, adjusted to pH 7.3 with KOH. Membrane currents were monitored (Axopatch 200B; Molecular Devices) and access resistances measured in voltage clamp were in the range of 5–30 MΩ before electronic compensation (60–80% was routinely used). To compare correctly current–voltage relationships before and after drug perfusion, series resistances (8–15 MΩ) were monitored by the peak amplitude of the capacitive transient induced by a −5 mV step applied before a voltage ramp (from −120 to −40 mV, 6 mV/s). Neurons in which series resistance changed by >10% were discarded from the statistics. Values given are mean ± SEM. Student's t test and nonparametric Mann–Whitney tests were used to compare means before and after HFS/drug. ANOVA test and *post hoc* Tukey's test were performed among groups ($p < 0.05$; $\alpha = 0.01$). Drugs were from Tocris Cookson, except for muscarine, methoctramine, hemicholinium-3, and bicuculline (Sigma), and were applied by switching the control perfusion to drug-containing solution.
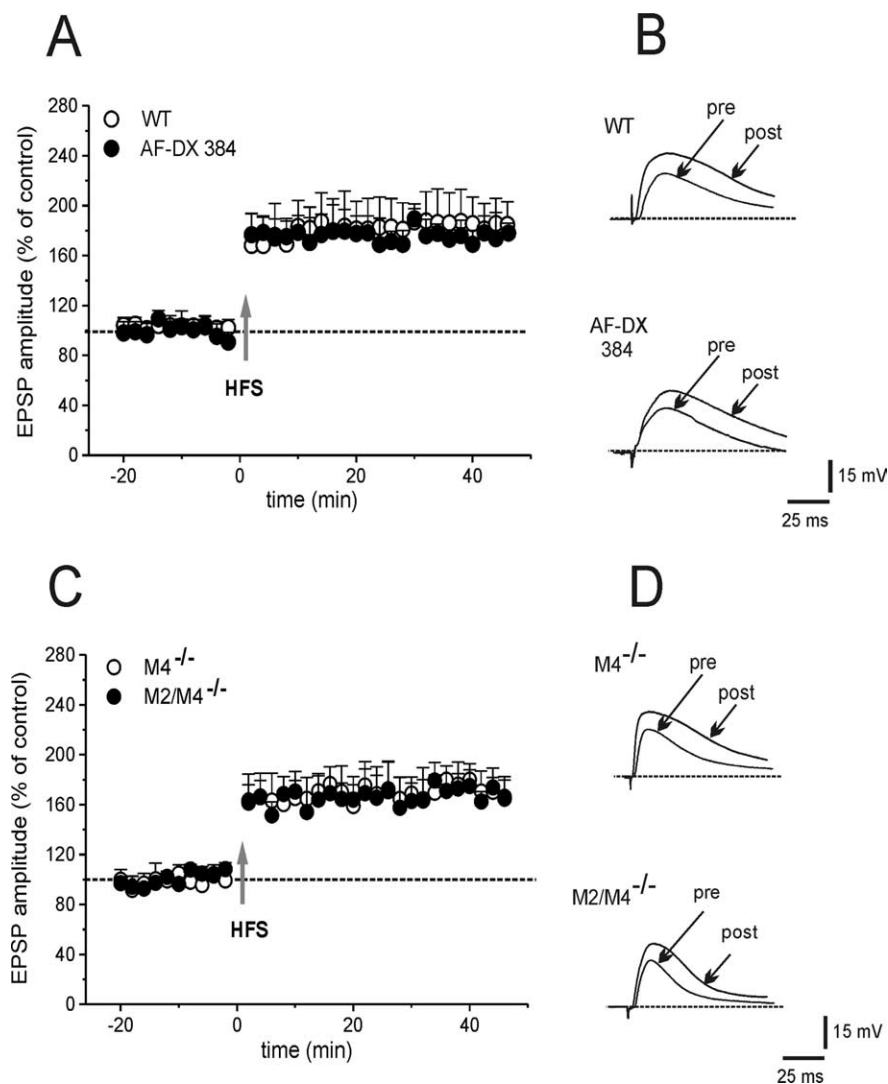
Figure 2. Normal LTP in mice lacking muscarinic autoreceptors. **A**, Time course of changes in EPSP amplitude recorded from WT MSNs. In the absence of external magnesium (LTP induction protocol), HFS induced a marked potentiation of cortically evoked EPSP (open circles), which persisted for >40 min of recording. LTP was unaffected by 300 nM AF-DX384 in WT mice (filled circles). **B**, Representative traces of the EPSPs recorded before (pre) and 25 min after (post) HFS in WT (top) and in WT pretreated with AF-DX 384 (bottom). **C**, HFS induced a stable LTP in $M_4^{-/-}$ (open circles) as well as in $M_2/M_4^{-/-}$ mice (filled circles). **D**, The superimposed traces show the EPSP before (pre) and after (post) HFS in slices from $M_4^{-/-}$ and $M_2/M_4^{-/-}$ mice. Each data point represents the mean ± SEM.

Results

Loss of autoreceptor response in cholinergic interneurons

Large ChIs ($n = 61$) were identified by means of their peculiar electrophysiological properties (Kawaguchi, 1993; Bonsi et al., 2007). Intrinsic membrane properties were not significantly different among ChIs from WT mice and $M_4^{-/-}$ or $M_2/M_4^{-/-}$ mice (ANOVA, $p > 0.05$): these interneurons showed depolarized resting membrane potential (RMP; WT, -56 ± 1.3 mV, $n = 10$; $M_4^{-/-}$, -57 ± 1.7 mV, $n = 9$; $M_2/M_4^{-/-}$, -53 ± 4.7 mV, $n = 6$) and high input resistance (WT, 150 ± 3.2 MΩ, $n = 10$; M4$^{-/-}$, 144 ± 3.9 MΩ, $n = 9$; $M_2/M_4^{-/-}$, 135 ± 4.4 MΩ, $n = 6$). More than half of the recorded cells exhibited a spontaneous firing activity. Hyperpolarizing current protocols activated a typical sag in the voltage response, indicative of an I_h current (Fig. 1B) whose amplitude (WT, 389.7 ± 93.4 pA, $n = 10$; $M_4^{-/-}$, 325.5 ± 78.0 pA, $n = 9$; $M_2/M_4^{-/-}$, 300.3 ± 41.3 pA, $n = 6$) and density (WT, 2.4 ± 0.3 pA/pF; $M_4^{-/-}$, 2.7 ± 0.2 pA/pF; $M_2/M_4^{-/-}$,

2.8 ± 0.4 pA/pF) did not differ among the three strains (ANOVA, $p > 0.05$). The current–voltage relationship did not show any significant change between ChIs from WT mice and $M_4^{-/-}$ or $M_2/M_4^{-/-}$ mice (Fig. 1A).

It has been shown that activation of M_2/M_4 autoreceptors in ChIs generates a membrane hyperpolarization or an outward current (Calabresi et al., 1998; Pisani et al., 1999). In the presence of tetrodotoxin (1 μM), oxotremorine (300 nM, 3 min) induced an outward current in the recorded cells from WT mice (Fig. 1C) (55 ± 2.6 pA; $n = 7$) coupled to a decreased conductance ($81 \pm 3.2\%$ of control; $n = 7$; $p < 0.05$), which was blocked by incubation with the M_2/M_4 receptor-preferring antagonist methoctramine (300 nM, 10 min). The reversal potential for this outward current was close to the calculated equilibrium potential for K$^+$ ions (Fig. 1D) (-120 ± 4.0 mV; $n = 6$). Surprisingly, oxotremorine was still able to induce an outward current in $M_4^{-/-}$ mice (Fig. 1C) (18 ± 2.6 pA; $n = 7$). This response was antagonized by methoctramine (Fig. 1C) and reversed at -105 ± 2.3 mV (Fig. 1D). Conversely, the response to oxotremorine (300 nM) was fully abolished in $M_2/M_4^{-/-}$ mice (Fig. 1C) ($n = 4$).

Membrane and synaptic responses of MSNs

MSNs from both WT and knock-out mice (WT, $n = 24$; $M_4^{-/-}$, $n = 36$; $M_2/M_4^{-/-}$, $n = 16$) did not exhibit differences both of RMP (WT, -89 ± 6.1 mV, $n = 19$; $M_4^{-/-}$, -86 ± 5.3 mV, $n = 21$; $M_2/M_4^{-/-}$, -91 ± 7.7 mV, $n = 13$; ANOVA, $p > 0.05$) and input resistance (WT, 62.4 ± 9.2 MΩ, $n = 19$; M4$^{-/-}$, 59.6 ± 10.6 MΩ, $n = 21$; $M_2/M_4^{-/-}$, 61 ± 8 MΩ, $n = 13$; ANOVA, $p > 0.05$), as measured by means of intracellular recordings. Depolarizing current pulses showed membrane rectification and tonic action potential discharge. The current–voltage relationship did not show any change among groups.

M_1 receptor activation has been shown to depolarize MSNs (Calabresi et al., 2000). Accordingly, perfusion of muscarine (30 μM, 1 min) caused a reversible membrane depolarization of MSNs from WT (5.6 ± 3 mV; $n = 8$) as well as from $M_4^{-/-}$ (5.8 ± 3.5 mV; $n = 6$) and $M_2/M_4^{-/-}$ (5.5 ± 4 mV; $n = 5$) mice that was prevented by the M_1 receptor-preferring antagonist pirenzepine (100 nM) (supplemental Fig. 1, available at www.jneurosci.org as supplemental material).

Knock-out mice lacking striatal mAChRs retain LTP

Removal of magnesium from the perfusing solution reveals an NMDA-mediated component of EPSPs and optimizes LTP induction (Calabresi et al., 1992b). Accordingly, HFS invariably induced LTP in MSNs from WT mice (Fig. 2A,B) ($180 \pm 28\%$ of

control, 25 min post-HFS; $n = 7$; Mann–Whitney, $p = 0.005$). Moreover, blocking M_1 receptors with 100 nM pirenzepine prevented LTP in WT MSNs (108 ± 8.8%; $n = 6$; t test, $p = 0.9$) (data not shown), confirming that M_1 receptors promote LTP. In $M_4^{-/-}$ or $M_2/M_4^{-/-}$ mice, HFS induced a LTP of a magnitude similar to that measured in WT littermates (Fig. 2C,D) ($M_4^{-/-}$, 172 ± 22%, $n = 7$; $M_2/M_4^{-/-}$, 164 ± 17%, $n = 5$; ANOVA, $p > 0.05$). In both knock-out strains, pretreatment with 100 nM pirenzepine did not cause significant changes of basal EPSP, but prevented LTP (data not shown) ($n = 3$). To verify these data pharmacologically, WT slices were incubated with the M_2/M_4 receptor antagonist 11-[[2-[(diethylamino)-methyl]-1-piperidinyl]acetyl]-5,1 1-dihydro-6H-pyrido[2,3b][1,4] benzodiazepin-6-one (AF-DX384; 300 nM, 20 min). Under this experimental condition, no significant effect on control EPSP was recorded. Yet, LTP induction was not different from control LTP (Fig. 2A,B) (172.8 ± 17.2%; $n = 6$; ANOVA, $p = 0.2$). Therefore, either genetic or pharmacological blockade of striatal muscarinic autoreceptors failed to alter LTP.

In principle, blockade of LTP by pirenzepine could be a consequence of a facilitatory action on LTD. Thus, in a final set of experiments in $M_4^{-/-}$ mice, slices were treated with sulpiride to block LTD (3 μM, 15 min) and then pirenzepine was added (100 nM, 15 min). In this experimental condition, HFS failed to induce LTP both in WT and $M_4^{-/-}$ mice (110.6 ± 13.7%; $n = 6$; t test, $p = 0.38$), confirming that M1 receptors are indeed necessary for LTP induction.

Lack of LTD at glutamatergic synapses
Ambient ACh concentrations exert a central role both in LTD or LTP. Loss of striatal muscarinic autoreceptor function is therefore expected to affect LTD by elevating cholinergic tone. HFS caused LTD in MSNs from WT mice (Fig. 3A,B) (49.3 ± 11% of control, measured 25 min post-HFS; $n = 6$; t test, $p < 0.01$). Conversely, in MSNs from $M_4^{-/-}$ or $M_2/M_4^{-/-}$ mice, HFS failed to induce LTD (Fig. 3A,B) ($M_4^{-/-}$, 100.3 ± 8%; $M_2/M_4^{-/-}$, 96.6 ± 8%; $n = 6$ for each strain; t test, $p = 0.3$). Moreover, in WT slices, pretreatment with the M_2/M_4 receptor antagonist, AF-DX384 (300 nM, 20 min), fully prevented LTD (data not shown) (103 ± 17%; $n = 5$, $p = 0.62$), suggesting that either pharmacological blockade or genetic deletion of muscarinic autoreceptors impairs striatal LTD.

Assuming that this impairment was caused by an increase in striatal ACh release, lowering cholinergic tone is expected to restore LTD. Slices were bathed with hemicholinium-3 (10 μM, 20 min), which depletes endogenous ACh (Parikh and Sarter, 2006). Hemicholinium-3 did not change EPSP amplitude per se, but completely rescued LTD in both $M_4^{-/-}$ and $M_2/M_4^{-/-}$ mice (Fig. 3C,D) (58.1 ± 13.6% and 53.2 ± 9% of control; $n = 6$ for M_4 and M_2/M_4, respectively; t test, $p < 0.001$). Hemicholinium-3 did
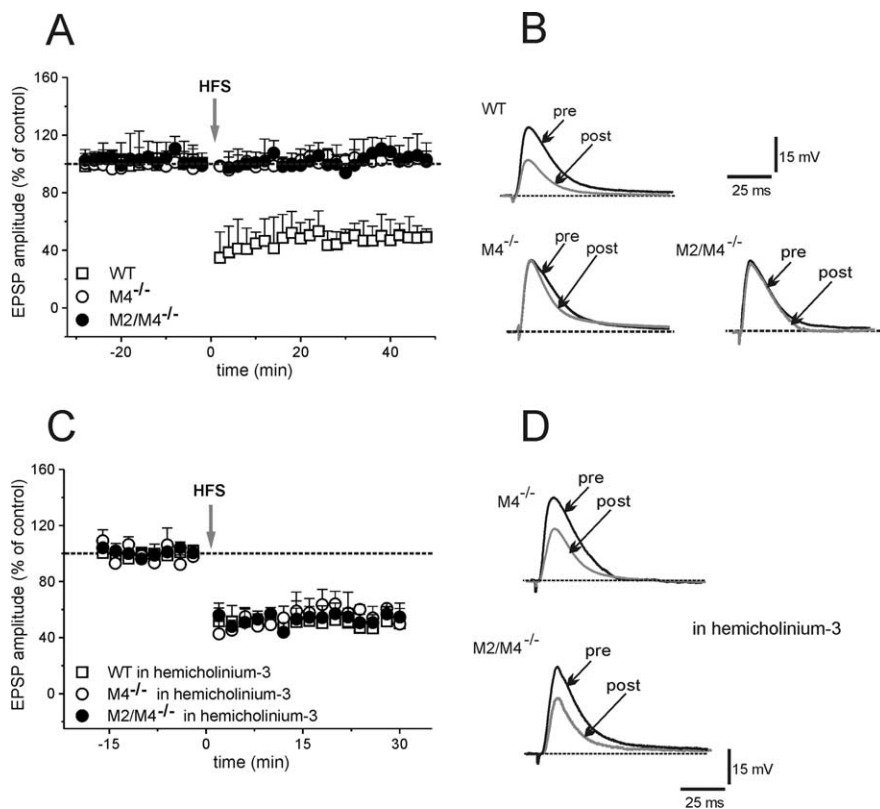
Figure 3. Impairment of striatal LTD. **A**, In MSNs from WT mice, HFS of glutamatergic fibers induced LTD of EPSPs (+/+, open square). The induction protocol failed to cause any synaptic change in MSNs from $M_4^{-/-}$ or $M_2/M_4^{-/-}$ mice (open and filled circles, respectively). **B**, The superimposed traces represent the EPSPs recorded before (pre) and 25 min after (post) HFS in WT and knock-out mice. **C**, In slices incubated with hemicholinium-3 (300 nM), HFS restored LTD in $M_4^{-/-}$ and $M_2/M_4^{-/-}$ mice (open and filled circles, respectively) without affecting LTD in WT mice (open square). **D**, Representative traces show the EPSP recorded before (pre) and after (post; 25 min) HFS in slices from both $M_4^{-/-}$ and $M_2/M_4^{-/-}$ mice in the presence of hemicholinium-3. Each data point represents the mean ± SEM.

not cause any change in LTD in MSNs from WT mice (Fig. 3C) (58 ± 9%; $n = 3$; $p = 0.13$).

To verify whether the impairment of LTD involved M_1 receptors, slices were treated with either the non-subtype-selective mAChRs antagonist scopolamine or with the M_1 receptor-preferring antagonist pirenzepine. Both 3 μM scopolamine (20 min) and pirenzepine (100 nM, 20 min) did not modify basal EPSP amplitude, but restored LTD (Fig. 4) (scopolamine: 67.3 ± 11.3%, $n = 5$, Mann–Whitney, $p < 0.0001$ for $M_4^{-/-}$ mice; 65 ± 14.4%, $n = 5$, $p < 0.0001$ for $M_2/M_4^{-/-}$ mice; pirenzepine: 53 ± 10.5%, $n = 6$, t test $p < 0.0001$ for $M_4^{-/-}$ mice; 51.4 ± 9.5%, $n = 6$, $p < 0.0001$ for $M_2/M_4^{-/-}$ mice; ANOVA among groups, $p = 0.7$).

"Competition" between LTD and LTP could hypothetically account for the rescue of LTD by pirenzepine. To address this hypothesis, the effects of pirenzepine on LTD were tested also in the presence of MK-801 [(+)-5-methyl-10,11-dihydro-5H-dibenzo [a,d] cyclohepten-5,10-imine maleate; 30 μM, 15 min], an NMDA receptor antagonist, to block LTP. In such experimental condition, HFS was still able to restore LTD in $M_4^{-/-}$ mice (48.7 ± 11.6%; $n = 5$; Mann–Whitney, $p < 0.0001$).

Discussion
Maintenance of ACh homeostasis in the striatum is of primary importance for correct motor control (Pisani et al., 2007). Loss of autoreceptors expressed on striatal ChIs is therefore expected to lead to a tonic increase in ACh levels and altered locomotion.
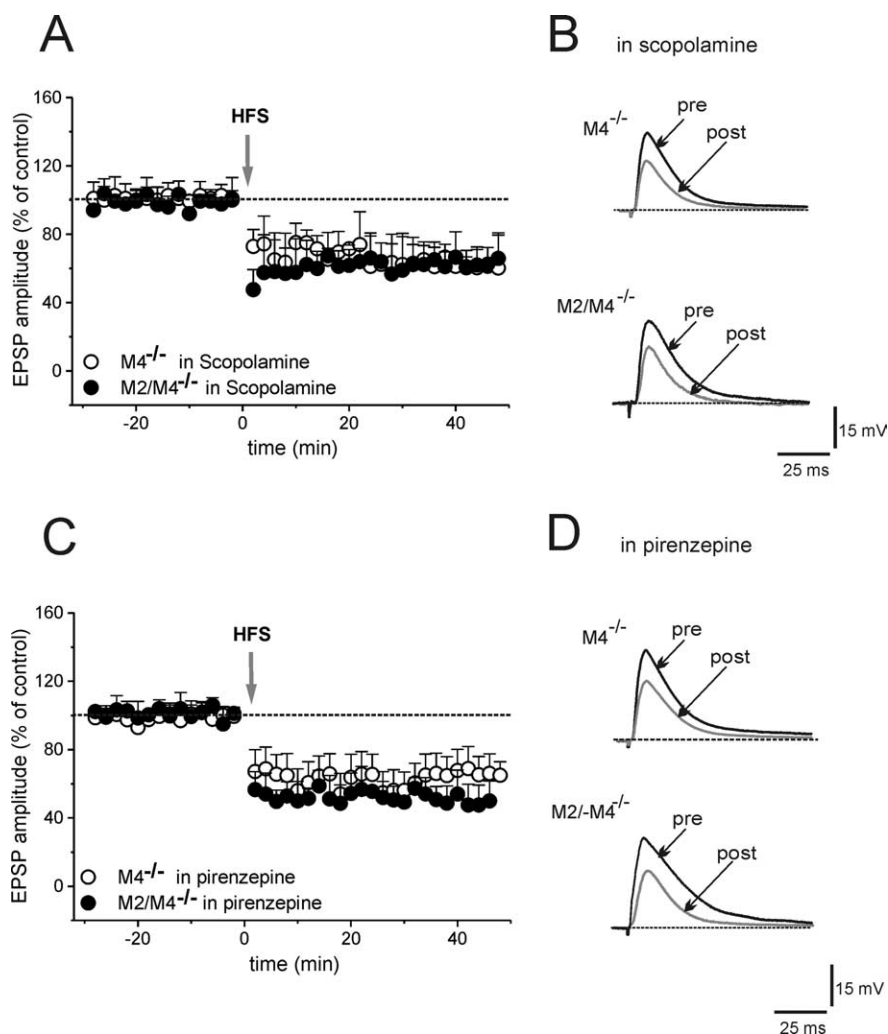
Figure 4. Blockade of M_1 muscarinic receptors restores LTD. ***A***, EPSP amplitudes in MSNs from $M_4{}^{-/-}$ (open circles) and $M_2/M_4{}^{-/-}$ (filled circles) are plotted in the presence of scopolamine (3 μM). ***B***, Representative traces of EPSP recorded before and 25 min after HFS in scopolamine from both groups of mice. ***C***, Time course of EPSP amplitudes in pirenzepine (100 nm) in $M_4{}^{-/-}$ (open circles) and $M_2/M_4{}^{-/-}$ (closed circles) mice. ***D***, Individual traces shown before (pre) and after (post) HFS. Data points are mean ± SEM.

Indeed, the oxotremorine-mediated inhibition of K^+-stimulated [^{3}ACh] efflux was completely lost in cortical and striatal tissues from $M_2/M_4{}^{-/-}$ mice (Zhang et al., 2002). Similarly, a large elevation in basal ACh content was found in the hippocampus of either M_2 or M_4 single or M_2/M_4 double knock-out mice (Tzavara et al., 2003). Moreover, in behavioral studies, $M_4{}^{-/-}$ mice displayed increased basal locomotor activity (Gomeza et al., 1999b).

Consistent with these findings, we found that the outward current recorded in ChIs was mostly reduced in single $M_4{}^{-/-}$ and abolished in $M_2/M_4{}^{-/-}$ mice, implicating both receptors in the cholinergic autoreceptor response. Accordingly, anatomical and pharmacological evidence identified both the M_2 and M_4 subtypes as striatal autoreceptors (Zhang et al., 2002).

Disruption of the M_2/M_4-mediated autoregulatory mechanism has been shown to alter synaptic plasticity in the hippocampus as well as in the visual cortex (Tzavara et al., 2003; Origlia et al., 2006). We tested the hypothesis that a presumed increase in cholinergic tone would influence striatal synaptic plasticity, providing the first evidence that striatal synaptic plasticity is indeed critically dependent on the levels of endogenous ACh, with

higher levels favoring LTP and lower levels promoting LTD. First, we found that MSNs from both $M_4{}^{-/-}$ or $M_2/M_4{}^{-/-}$ mice retain LTP. Second, pharmacological blockade of M_2/M_4 receptors with AF-DX384 prevented LTD, but did not alter LTP induction in WT mice. Because ACh exerts its permissive role in LTP induction through M_1 receptor activation, we examined whether this pathway was preserved in MSNs of knock-out mice. In both $M_4{}^{-/-}$ and $M_2/M_4{}^{-/-}$ mice, muscarine depolarized MSNs, confirming that M1 receptors were functional. In addition, LTP induction depended on M_1 receptor activation in both knock-out mice, as it was fully prevented by pirenzepine.

Conversely, no striatal LTD was observed in either $M_4{}^{-/-}$ or $M_2/M_4{}^{-/-}$ mice. In keeping with the assumption that elevated striatal ACh release affected LTD in mice lacking muscarinic autoreceptors, we were able to restore LTD by lowering striatal cholinergic tone with hemicholinium-3, which blocks the high-affinity choline transporters, preventing ACh resynthesis (Parikh and Sarter, 2006). Reducing M_1 receptor activity has been proposed to facilitate LTD induction (Wang et al., 2006). Accordingly, we found that blockade of M_1 receptors with pirenzepine was able to fully rescue LTD in both $M_4{}^{-/-}$ and $M_2/M_4{}^{-/-}$ mice.

Our data are consistent with the novel concept that bidirectional synaptic plasticity in the striatum is strictly regulated by ACh provided by ChIs. Under physiological conditions, striatal dopamine inhibits the activity of ChIs and ACh release (De-Boer et al., 1996; Pisani et al., 2000; Maurice et al., 2004). Moreover, different pathophysiological conditions characterized by striatal dopaminergic dysfunction, including Parkinson's disease and dystonia, are accompanied by abnormal cholinergic function (Ding et al., 2006; Pisani et al., 2007). Accordingly, mAChRs antagonists are used in the symptomatic treatment of these movement disorders (Lang and Lees, 2002; Horn and Comella, 2007). The findings reported here may contribute to a better understanding of how alterations in striatal circuitry are linked to motor dysfunction and eventually help identify novel therapeutic strategies to fine tune striatal cholinergic function in human disease.

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