Short-Term Plasticity at Inhibitory Synapses in Rat Striatum and Its Effects on Striatal Output

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Fitzpatrick, John S., Garnik Akopian, and John P. Walsh. Short-term plasticity at inhibitory synapses in rat striatum and its effects on striatal output. J Neurophysiol 85: 2088–2099, 2001. Two forms of short-term plasticity at inhibitory synapses were investigated in adult rat striatal brain slices using intracellular recordings. Intrastratal stimulation in the presence of the ionotropic glutamate receptor antagonists 6-cyano-7-nitroquinoxaline-2,3-dione (20 μM) and D,L-2-amino-5-phosphonovaleric acid (50 μM) produced an inhibitory postsynaptic potential (IPSP) that reversed polarity at $-76 \pm 1$ (SE) mV and was sensitive to bicuculline (30 μM). The IPSP rectified at hyperpolarized membrane potentials due in part to activation of K+ channels. The IPSP exhibited two forms of short-term plasticity, paired-pulse depression (PPD) and synaptic augmentation. PPD lasted for several seconds and was greatest at intersstimulus intervals (ISIs) of several hundred milliseconds, reducing the IPSP to 80 ± 2% of its control amplitude at an ISIs of 200 ms. Augmentation of the IPSP, elicited by a conditioning train of 15 stimuli applied at 20 Hz, was 119 ± 1% of control when sampled 2 s after the conditioning train. Augmentation decayed with a time constant of 10 s. We tested if PPD and augmentation modify the ability of the IPSP to prevent the generation of action potentials. A train of action potentials triggered by a depolarizing current injection of constant amplitude could be interrupted by stimulation of an IPSP. If this IPSP was the second in a pair of IPSPs, it was less effective in blocking spikes due to PPD. By contrast, augmented IPSPs were more effective in blocking spikes. The same results were achieved when action potentials were triggered by a depolarizing current injection of varying amplitude, a manipulation that produces nearly identical spike times from trial to trial and approximates the in vivo behavior of these neurons. These results demonstrate that short-term plasticity of inhibition can modify the output of the striatum and thus may be an important component of information processing during behaviors that involve the striatum.

INTRODUCTION

The strength of a synapse can vary with frequency of activation. This variation, known as short-term plasticity, can be an increase or decrease and can last just tens of milliseconds or as long as several minutes. Synapses in the behaving animal operate within this time frame (Alexander and Crutcher 1990; Chevalier and Deniau 1990; Crutcher and DeLong 1984), so short-term synaptic plasticity is likely an important mechanism in the functioning of biological neural networks.

Short-term plasticity can be due to a number of mechanisms. One is the intrinsic properties of neurotransmitter release. Several types of short-term increases in synaptic strength such as facilitation, augmentation, and posttetanic potentiation are due to the accumulation of residual calcium in the presynaptic terminal as a result of one or more preceding action potentials (Delaney and Tank 1994; Fisher et al. 1997; Kameya and Zucker 1994; Swandulla et al. 1991). The effects of previous calcium influxes combine nonlinearly with the effects of later calcium influxes, increasing the probability of neurotransmitter release. Sometimes, however, repetitive action potentials deplete the supply of neurotransmitter, thereby negating the effects of residual calcium and causing a short-term decrease in synaptic strength (Dittman and Regehr 1998; Swandulla et al. 1991).

Another mechanism responsible for short-term changes in synaptic strength is activation of neurotransmitter receptors on the presynaptic terminal. For example, activation of GABA_B receptors (Calabresi et al. 1991; Radnikow et al. 1997; Seabrook et al. 1991), muscarinic acetylcholine receptors (Marchi et al. 1990; Sugita et al. 1991), metabotropic glutamate receptors (Stefani et al. 1994), or adenosine receptors (Mori et al. 1996) can decrease synaptic strength at inhibitory synapses in striatum by decreasing the amount of GABA released. Activation of these receptors decreases release either by decreasing the calcium influx into the presynaptic terminal or by interfering with mechanisms of transmitter release (Wu and Saggau 1997). On the other hand, activation of nicotinic acetylcholine receptors can increase the amount of neurotransmitter released (Gray et al. 1996; Lena and Changeux 1997). Finally, synaptic strength can be decreased by the postsynaptic mechanism of receptor desensitization (Jones and Westbrook 1995; Otis et al. 1996).

GABAergic inhibition serves many important functions in the nervous system; it can prevent action potential generation (Bazemore et al. 1957), limit N-methyl-D-aspartate (NMDA) receptor activation (Dingledine et al. 1986; Kanter et al. 1996; Luhmann and Prince 1990; Staley and Mody 1992), hamper the backpropagation of action potentials into the dendritic tree (Kim et al. 1995; Larkum et al. 1999; Tsukamoto and Ross 1996), and synchronize membrane potential oscillations (Cobb et al. 1995). Short-term modulation of the strength of GABAergic synapses will affect all these processes and is thus impor-
tant for neuronal integration. GABAergic inhibition is particularly important in the striatum as over 95% of striatal neurons are GABAergic (Kemp and Powell 1971). We have therefore studied two forms of short-term plasticity, paired-pulse depression (PPD) and synaptic augmentation, at GABAergic synapses onto medium spiny neurons in the striatum. We have investigated their time course, parametric requirements, pharmacology, and impact on the ability of GABAergic synapses to prevent action potential generation in a situation approximating the in vivo behavior of these cells.

METHODS

Slice preparation

Adult Fisher 344 rats (2–3 mo) were anesthetized with pentobarbital sodium (60 mg/kg ip) and decapitated. Brains were quickly removed and submerged in cold (3–5°C), aerated (95% O2-5% CO2) sucrose artificial cerebral spinal fluid (ACSF). Sucrose ACSF consisted of (in mM) 248 sucrose, 5 KCl, 1.3 MgSO4, 28 NaHCO3, 1.25 NaH2PO4, and 10 glucose. Hemi-coriolar slices (400 μm) were cut on a Vibratome in sucrose ACSF, initially transferred to a mixture of half sucrose ACSF and half normal ACSF, then to normal ACSF, and allowed to warm to room temperature and recover for at least 1 h. Normal ACSF consisted of (in mM) 124 NaCl, 3 KCl, 2.4 CaCl2, 1.3 MgSO4, 26 NaHCO3, 1.25 NaH2PO4, and 10 glucose. Slices were transferred individually to a Haas-type interface chamber and perfused with aerated (95% O2-5% CO2) normal ACSF at 28–30°C.

In some experiments, we altered the normal ACSF. For Ba2+ ACSF, we substituted 0.5–1 mM BaCl2 for the corresponding concentration of CaCl2 and substituted MgCl2 for MgSO4 to prevent the precipitation of BaSO4. For Cs+ ACSF, we substituted CsCl for KCl.

Intracellular recordings

Intracellular recording electrodes (90–185 MΩ) were pulled with a Flaming/Brown P-87 puller (Sutter Instruments). Medium spiny neurons in the dorsal medial region of the striatum were impaled, and their responses to current injection and synaptic stimulation were recorded. Synaptic responses were evoked using a bipolar electrode (twisted, formvar-coated nichrome wire, 65 μm OD, A-M Systems) placed within the striatum about 100 μm from the recording electrode. Responses were filtered (3 kHz), amplified (Axoclamp 2A, Axon Instruments), digitized (10–20 kHz; Labmaster TL-1 or Digidata 1200B), and stored on computer (pClamp, Axon Instruments). All data were collected with ionotropic glutamate receptors blocked by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 20 μM), D,L-2-amino-5-phosphonovaleric acid (APV, 50 μM), and bicuculline (30 μM) partially blocked the nonglutamatergic response. Bicuculline (30 μM) partially blocked the non-NMDA response. Traces are averages of 5 consecutive responses. Sample IPSPs (b and c) are depolarizing because the resting membrane potential (~90 mV) was hyperpolarized relative to the IPSP reversal potential (~65 mV). Electrode was filled with K2HPO4. Stimulus artifacts are clipped. B, left: application of CNQX and APV reduced the amplitude of the response to intrastriatal stimulation by 85 ± 2% (n = 9). Right: application of bicuculline (in addition to CNQX, APV, and atropine) reduced the response by 61 ± 3% (n = 8). C: the IPSP reversed polarity at ~76 ± 1 mV and rectified at hyperpolarized membrane potentials (n = 29). These data were collected using electrodes filled with K2HPO4. Traces are single responses. In this example, the reversal potential was ~72 mV. Electrode was filled with K2HPO4, initially incubated in a 1% solution of Triton X-100 for 1 h to permeabilize the cell membranes. Injected cells were labeled by incubating the tissue with an avidin-horseradish peroxidase complex (ABC solution) at a dilution of 1:1000 in phosphate-buffered saline (Vector Labs, Burlingame, CA). After several rinses in phosphate-buffered saline over 1 h, the sections were reacted with diaminobenzidine (Sigma; 0.06%) and H2O2 (0.003%) in 0.15 M tris(hydroxymethyl)aminomethane buffer for 10 min. The sections were rinsed and mounted on gelatin-coated slides, which were then air dried, defatted, and coverslipped in Permound. Of the 64 neurons included in this study, 44 were successfully labeled with biocytin and directly identified as medium spiny neurons. The electrophysiological properties of

FIG. 1. Pharmacological isolation and voltage dependence of the inhibitory postsynaptic potential (IPSP). A: a 4-bar period line in the absence of drugs, 6-cyano-7-nitroquinolinic acid-2,3-dione (CNQX, 20 μM), D,L-2-amino-5-phosphonovaleric acid (APV, 50 μM), and atropine (1 μM) greatly reduced the synaptic response to intrastratal stimulation. Increasing stimulation intensity from 90 to 500 μA (↓) increased the amplitude of the nonglutamatergic response. Bicuculline (30 μM) partially blocked the nonglutamatergic response. Traces are averages of 5 consecutive responses. Sample IPSPs (b and c) are depolarizing because the resting membrane potential (~90 mV) was hyperpolarized relative to the IPSP reversal potential (~65 mV). Electrode was filled with K2HPO4. Stimulus artifacts are clipped. B, left: application of CNQX and APV reduced the amplitude of the response to intrastriatal stimulation by 85 ± 2% (n = 9). Right: application of bicuculline (in addition to CNQX, APV, and atropine) reduced the response by 61 ± 3% (n = 8). C: the IPSP reversed polarity at ~76 ± 1 mV and rectified at hyperpolarized membrane potentials (n = 29). These data were collected using electrodes filled with K2HPO4. Traces are single responses. In this example, the reversal potential was ~72 mV. Electrode was filled with K2HPO4, initially incubated in a 1% solution of Triton X-100 for 1 h to permeabilize the cell membranes. Injected cells were labeled by incubating the tissue with an avidin-horseradish peroxidase complex (ABC solution) at a dilution of 1:1000 in phosphate-buffered saline (Vector Labs, Burlingame, CA). After several rinses in phosphate-buffered saline over 1 h, the sections were reacted with diaminobenzidine (Sigma; 0.06%) and H2O2 (0.003%) in 0.15 M tris(hydroxymethyl)aminomethane buffer for 10 min. The sections were rinsed and mounted on gelatin-coated slides, which were then air dried, defatted, and coverslipped in Permound. Of the 64 neurons included in this study, 44 were successfully labeled with biocytin and directly identified as medium spiny neurons. The electrophysiological properties of
unidentified neurons were similar to those of identified medium spiny neurons, and thus we assume the unidentified neurons were also medium spiny neurons. In some experiments, we bathed the slice in Ba$^{2+}$ or Cs$^{+}$ to block K$^+$ channels. Under these conditions, cell type cannot be determined electrophysiologically because blockade of K$^+$ channels alters electrophysiological properties. Of 10 cells recorded in the presence of Ba$^{2+}$ or Cs$^{+}$, 9 were identified as medium spiny neurons through biocytin staining.

**Stimulation and analysis**

Stimulus current duration was 100 ms. During drug application, pairs of stimuli with 50-ms interstimulus intervals (ISIs) were applied every 20 s. In tests for paired-pulse modulation, pairs with ISIs from 50 ms to 10 s were applied. The interval between the second impulse of a given pair and the first impulse of the next pair was always 20 s. Each ISI was tested five times consecutively per cell, and pairs were presented in order of increasing ISIs. Paired-pulse modulation was quantified by dividing the maximum amplitude of the second response by the maximum amplitude of the first response, and the result was expressed as a percentage. In tests for augmentation, we applied a conditioning train of 15 stimuli at 20 Hz. This is the same type of stimulation we used to measure augmentation at excitatory synapses onto medium spiny neurons (Ou et al. 1997) and thus allows comparison of the magnitude and time course of augmentation at excitatory and inhibitory synapses. Also, this type of stimulation is physiologically relevant, as it is within the operating range of these synapses in awake animals (Wilson 1993; Wilson and Groves 1981). Test stimuli were applied 2, 5, 10, 20, and 30 s after the end of the conditioning train. This was repeated five times per cell with 2.5 min between conditioning trains. Augmentation was quantified by dividing the maximum amplitude of the response to a test stimulus by the maximum amplitude of the response to a control stimulus applied 15 s before the beginning of the train, and the result was expressed as a percentage. In experiments on the effects of different train lengths, conditioning trains of 1, 3, 5, 7, 9, 11, 13, and 15 stimuli were applied in either ascending or descending order. Recovery time between trains was 1.5 min, and the sequence of trains of different lengths was repeated three times per cell.

Membrane potential was corrected for any offset observed following electrode withdrawal but was not corrected for liquid junction potentials. Input resistance was determined with a −100-pA injection from a membrane potential of −90 mV. Results are expressed as mean ± SE. In the rectification experiments, overall statistical significance was determined with an omnibus F-test ANOVA, and post hoc Scheffe tests were used to identify the significant comparisons underlying the overall significance. Other tests for significance are identified when the results are stated.

The resting membrane potential of medium spiny neurons (87 ± 1 mV, n = 45) was negative with respect to the IPSP reversal potential (Fig. 1C), and thus IPSPs elicited at rest were depolarizing. We did not quantify PD and augmentation at membrane potentials depolarized to the IPSP reversal potential because we did not want to introduce problems associated with current injection into our analysis. The resistance of high-impedance electrodes changes with current injection, and imposing large depolarizations activates voltage-dependent currents. These problems create recording instability that could limit quantitative comparisons. In addition, there is much evidence that PD and augmentation are due to presynaptic mechanisms, and thus it is unlikely that sampling cells at their resting potential would affect the plasticity. We did perform a small qualitative sampling of the effects of PD and augmentation on IPSPs evoked at depolarized membrane potentials when we examined spike probability in Figs. 6 and 9.

**Drugs**

The following compounds were obtained from Sigma (St. Louis, MO): APV, atropine (sulfate salt), biocytin, cesium methanesulfonate, potassium acetate, dimethyl sulfoxide (DMSO), SCH 23390, sulpiride, and all components of the ACSF. Methyl potassium sulfate was obtained from Pfaltz and Bauer (Waterbury, CT). 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX) and (−)-bicuculline methiodide were obtained from Research Biochemicals (Natick, MA). CNQX was initially dissolved in DMSO, kept refrigerated as a stock solution for no more than 1 mo, and added to ACSF the day of the experiment. The final concentration of DMSO was 0.02%. CGP 35348 was a gift from Novartis Pharma AG.

**RESULTS**

**Properties of the IPSP**

Intrastriatal stimulation in control ACSF resulted in a depolarizing postsynaptic potential (Fig. 1A) which could trigger an action potential at higher stimulation intensities. Application of the ionotropic glutamate receptor antagonists CNQX (20 μM) and APV (50 μM) reduced the amplitude of the response by 85 ± 2% (n = 9; Fig. 1B) and revealed an IPSP with a reversal potential of −76 ± 1 mV (n = 29; Fig. 1C). The amplitude of the IPSP varied with stimulation intensity and was sensitive to the GABA$\lambda$ receptor antagonists bicuculline (30 μM) and picrotoxin (50–100 μM). Sometimes (8 of 32 neurons) the IPSP was multiphasic as previously reported (Seabrook et al. 1991) but only at depolarized membrane potentials.

The amplitude of the IPSP rectified at membrane potentials more hyperpolarized than −85 mV (Fig. 1C). This has also been observed in unitary IPSPs between striatal interneurons and medium spiny neurons (Koos and Tepper 1999). There are at least two possible mechanisms for this. The first is shunting by K$^+$ channels. The input resistance of medium spiny neurons decreases at hyperpolarized membrane potentials due to the activation of an inwardly rectifying K$^+$ current (Nisenbaum and Wilson 1995). This current has been shown to shunt excitatory responses to intrastriatal stimulation (Calabresi et al. 1990), so it may be responsible for shunting inhibitory responses as well. A second possible mechanism is the intrinsic properties of the GABA$\lambda$ receptor channel. Rectification of GABAergic responses has been observed in other preparations (Barker and Harrison 1988; Bormann et al. 1987; Collingridge et al. 1984; Weiss et al. 1988) and may be due to the difference between [Cl$^-$]$_i$ and [Cl$^-$]$_e$ (Barker and Harrison 1988) and to a voltage dependence of the kinetics of the GABA$\lambda$ receptor channel (Bormann et al. 1987; Dudel et al. 1980; Segal and Barker 1984; Weiss 1988).

To test if the rectification of the IPSP was due to shunting, we blocked K$^+$ currents by using either Ba$^{2+}$ ACSF or Cs$^+$ ACSF (see METHODS). In four of five experiments with Cs$^+$ ACSF, we also applied Cs$^+$ internally by filling the intracellular electrode with CsCl$_2$SO$_3$ (2 M). The average input resistance of cells bathed in Cs$^+$ ACSF (59 ± 3 MΩ; n = 5; P < 0.05) or Ba$^{2+}$ ACSF (97.4 ± 13 MΩ; n = 5; P < 0.001) was higher than the input resistance of cells bathed in normal ACSF (39 ± 2 MΩ; n = 29), indicating that these manipulations did in fact block K$^+$ channels (Fig. 2A). On the other hand, the mean IPSP reversal potential of cells bathed in Cs$^+$ ACSF (−79 ± 4 mV) or Ba$^{2+}$ ACSF (−76 ± 1 mV) was similar to that of cells bathed in normal ACSF (−76 ± 1 mV), indicating...
that these manipulations did not affect the transmembrane Cl\textsuperscript{−} gradient (Fig. 2C).

Bathing cells in Ba\textsuperscript{2+} ACSF or Cs\textsuperscript{+} ACSF reduced the rectification of the IPSP (Fig. 2, B and C). To quantify this effect, we calculated a rectification ratio for the IPSP from each cell. The rectification ratio was determined by performing a linear regression on the reversal data for the IPSP in a hyperpolarized region of membrane potentials (\(V_m \leq -90\) mV) and

**FIG. 2.** Rectification of the IPSP is reduced by Ba\textsuperscript{2+} or Cs\textsuperscript{+}. A: effect of K\textsuperscript{+} channel blockade on responses to current injection. Left: normal artificial cerebrospinal fluid (ACSF). Membrane potential was set to \(-89\) mV by constant injection of \(-40\) pA. Lowermost trace is the response to injection of \(-500\) pA for 50 ms. In successive traces, the current injection is incremented by 100 pA. Middle: Ba\textsuperscript{2+} ACSF. Membrane potential was set to \(-87\) mV by constant injection of \(-660\) pA. Lowermost trace is the response to injection of \(-250\) pA for 300 ms. In successive traces, the current injection is incremented by 100 pA. Right: Cs\textsuperscript{+} ACSF and CsCH\textsubscript{3}SO\textsubscript{4} (2 M) in electrode. Membrane potential is set to \(-87\) mV by constant injection of \(-510\) pA. Lowermost trace is the response to injection of \(-250\) pA for 300 ms. In successive traces, the current injection is incremented by 100 pA. Longer current injections were used in Ba\textsuperscript{2+} ACSF and Cs\textsuperscript{+} ACSF due to the longer membrane time constant and wider action potential. All traces are single responses. B: example of the effect of Cs\textsuperscript{+} on rectification. Rectification is present shortly after impaling the cell in Cs\textsuperscript{+} ACSF with an electrode filled with CsCH\textsubscript{3}SO\textsubscript{4} (○) but not 20-min later (●). C: voltage dependence of the IPSP in normal ACSF (○; \(n=29\)), Ba\textsuperscript{2+} ACSF (●; \(n=5\)), and Cs\textsuperscript{+} ACSF (▲; \(n=5\)). Rectification was reduced in Ba\textsuperscript{2+} ACSF or Cs\textsuperscript{+} ACSF. Note that the reversal potential was approximately the same in all 3 conditions. Control data are the same as in Fig. 1C. D: rectification was quantified for each cell by fitting the data points at hyperpolarized membrane potentials (B, left shading) and at depolarized membrane potentials (B, right shading) with separate regression lines and then dividing the slope of the regression line in the hyperpolarized region by the slope of the regression line in the depolarized region. The result is called the rectification ratio. The average rectification ratio was greater for cells bathed in Ba\textsuperscript{2+} ACSF or Cs\textsuperscript{+} ACSF than for cells bathed in normal ACSF. E: in the hyperpolarized region, the average slope of the regression line was greater for cells bathed in Ba\textsuperscript{2+} ACSF or Cs\textsuperscript{+} ACSF than for cells bathed in normal ACSF. F: in the depolarized region, the average slope of the regression line for cells bathed in Ba\textsuperscript{2+} ACSF or Cs\textsuperscript{+} ACSF was not significantly different from for cells bathed in normal ACSF. *\(P<0.001\).
for a depolarized region of membrane potentials \((V_m \approx -72\) mV). The rectification ratio is the slope of the regression line from the hyperpolarized region divided by the slope of the regression line from the depolarized region. If there is no rectification, the slopes in the two ranges are equal, and the rectification ratio is unity. If there is complete rectification, the slope in the hyperpolarized range is zero, and the rectification ratio is zero. The average IPSP rectification ratio for cells bathed in normal ACSF \((0.45 \pm 0.07; n = 5; P < 0.001)\) or Cs\(^+\) ACSF \((0.82 \pm 0.13; n = 5; P < 0.001)\) was greater than the average IPSP rectification ratio for cells bathed in normal ACSF \((0.09 \pm 0.02; n = 29; \text{Fig. 2}D)\). This was due to steeper slopes in the hyperpolarized region (Fig. 2E) and not to a difference between slopes in the depolarized region (Fig. 2F).

**PPD of the IPSP**

The first form of short-term plasticity we investigated was paired-pulse modulation. Stimulation using pairs of impulses with a wide range of ISIs revealed that the IPSP exhibits PPD (Fig. 3, A and B). PPD was negligible at the shortest ISI tested \((50\) ms; \(94 \pm 4\% ; n = 10)\) but was present at ISIs from 100 ms to 2 s and was greatest when the ISI was 500 ms \((80 \pm 2\%)\). (Six of these experiments were conducted with KC\(_2\)H\(_3\)O\(_2\) in the recording electrode and 4 with KCH\(_3\)SO\(_4\). PPD was similar for the 2 groups, so they were combined.) The delayed onset and long duration of PPD suggest that it may be due to the activation of metabotropic receptors on the presynaptic terminals of inhibitory synapses. Several types of metabotropic receptors have been shown to decrease GABA release in striatum and thus may mediate PPD. One type is muscarinic acetylcholine receptors (Marchi et al. 1990; Sugita et al. 1991). We tested whether these receptors are involved in PPD by stimulating with paired pulses in the presence of the muscarinic antagonist atropine \((1\) \(\mu\)M). Atropine did not block PPD (Fig. 3C; \(83 \pm 3\%\) at 500 ms ISI; \(n = 6; P > 0.35, 2\text{-tailed } t\text{-test})\), indicating that PPD is not mediated by muscarinic acetylcholine receptors. Activation of GABA\(_A\) receptors has also been shown to reduce IPSPs in striatum (Calabresi et al. 1991; Radnikow et al. 1997; Seabrook et al. 1991). We tested whether GABA\(_A\) receptors mediate PPD by measuring PPD in the presence of the GABA\(_A\) receptor antagonist CGP 35348 \((100\) \(\mu\)M). We found that PPD in the presence of CGP 35348 (Fig. 3C, \(79 \pm 3\%\) at 500 ms ISI; \(n = 5)\) is not significantly different from PPD in normal ACSF (Fig. 3C; \(P > 0.75, 2\text{-tailed } t\text{-test})\). Finally, we tested if activation of D1 or D2 dopamine receptors is responsible for PPD as dopamine receptor agonists have been observed to modulate GABA release in striatum (Harsing and Zigmond 1997; Wang and Johnson 1995) and GABA\(_A\) receptor currents in medium spiny neurons (Flores-Hernandez et al. 2000). A combination of the D1 antagonist SCH 23390 \((5\) \(\mu\)M) and the D2 antagonist sulpiride \((20\) \(\mu\)M) had no significant effect on PPD \((82 \pm 5\%\) at 500 ms ISI; \(n = 3; P > 0.65, 2\text{-tailed } t\text{-test})\).

**Augmentation of the IPSP**

We next investigated another form of short-term plasticity known as augmentation. Originally described at the frog neuromuscular junction (Magleby and Zengel 1976), augmentation results from the accumulation of calcium in the presynaptic terminal during repetitive activity (Delaney and Tank 1994; Kamiya and Zucker 1994; Swandulla et al. 1991; Tank et al. 1995). It decays with a time constant of several seconds and is often accompanied by facilitation and posttetanic potentiation, two other types of short-term plasticity (Fisher et al. 1997). We have previously shown that augmentation occurs at excitatory synapses onto medium spiny neurons (Ou et al. 1997) but is rather brief, decaying completely after 6 s. To determine if augmentation occurs at inhibitory synapses onto medium spiny neurons as well, we tested the effect of a brief conditioning train \((15\) stimuli at 20 Hz; Fig. 4, A and B) on the amplitude of the IPSP. We found that 2 s after the end of the train the amplitude of the IPSP was increased to 119 \(\pm 1\%\) of control (Fig. 4, C and D; \(n = 6; P < 0.005, \text{paired, 2\text{-tailed } t\text{-test})\). The augmentation decayed with a time constant of 10 s. We did not give test pulses at delays shorter than 2 s and thus do not know if the conditioning train also produced facilitation.
Stimulus artifacts are clipped.

From the end of the conditioning train. Each trace is the average of 5 trials.

is the control response elicited 15 s before the beginning of the conditioning train.

We tested whether this was true for IPSPs in medium spiny neurons by stimulating with brief conditioning trains when the slice was bathed in Ba\textsuperscript{2+} ACSF. The presence of Ba\textsuperscript{2+} did not affect augmentation at a delay of 2 s (116 ± 7%; \( n = 5 \); \( P > 0.5 \); 2-tailed t-test), but augmentation was decreased at longer delays of 5, 10, and 15 s. At the longest delay tested, 30 s, augmentation in control ACSF had completely decayed (100 ± 1%), but in Ba\textsuperscript{2+} ACSF the response was depressed (89 ± 4%; \( P < 0.05 \), 2-tailed t-test).

**Requirements for generating PPD versus augmentation**

With the preceding experiments we have established that inhibitory synapses onto medium spiny neurons exhibit two forms of short-term plasticity. One of these (PPD) weakens inhibition, while the other (augmentation) strengthens it. The requirements for generating the two forms of short-term plasticity differ only in the number of conditioning stimuli; PPD is generated by a single conditioning stimulus, while augmentation is generated by a train of 15 stimuli. To further investigate the relationship between PPD and augmentation, we varied the length of the conditioning train. We used conditioning trains of 1, 3, 5, 7, 9, 11, 13, and 15 stimuli and measured plasticity with a test pulse 2 s after the end of the train (Fig. 5A). We found, as expected, that shorter trains depressed the IPSP and longer trains augmented the IPSP (Fig. 5, B and C). There was a roughly linear transition between the two forms of short-term plasticity, with conditioning trains of 9 and 11 impulses producing no plasticity at all on average.

In these experiments, the longest conditioning trains were the same length as those in our initial augmentation experiments, yet they resulted in less augmentation (105 ± 3 vs. 119 ± 1% with test pulse after 2-s delay). This may be due to methodological differences between the two sets of experiments. In the initial augmentation experiments, we applied a total of five conditioning trains, all of them 15 stimuli in length, and allowed 2.5 min between trains for recovery. In the experiments in which we varied train length, we applied a total of 24 trains (3 trials each of 8 different lengths) and allowed 1.5 min between trains for recovery. To test if such methodological differences might affect the magnitude of augmentation, we separated the data in Fig. 5B by order of train presentation. Trains presented in descending order of length (\( n = 4 \)) tended to produce less depression and more augmentation than those presented in ascending order (\( n = 5 \)). Although this difference was not significant (\( P > 0.15 \); split plot factorial ANOVA), it indicates that the magnitude of augmentation may be sensitive to methodological parameters. This is further emphasized by results from pilot experiments in which we presented a single test stimulus 2 s after a 15-stimulus conditioning train and allowed 30 s between trains for recovery. This method resulted in less augmentation as well (111 ± 5%; \( n = 5 \); not shown).

**Effects of PPD and augmentation on action potential generation**

One of the purposes of inhibition is to prevent action potential generation. To test if PPD and augmentation change the ability of inhibition to block spikes, we elicited trains of action potentials by constant current injection and compared spike inhibition between control and depressed IPSPs and between control and augmented IPSPs. In four of four cells, a depressed IPSP was less effective in stopping the generation of action potentials, while in three of four cells an augmented IPSP was more effective (Fig. 6). This demonstrates that short-term plasticity of the IPSP can change the output of medium spiny neurons.

There are two drawbacks to eliciting action potentials with current injections of constant amplitude: the pattern of action potentials varies from trial to trial (Fig. 6) and constant current...
injection is not physiologically realistic. To avoid these drawbacks, we injected currents of varying amplitude. Such current injections were specified by random sequences of 2,440 or 4,960 values. Each value specified the level of current injection for 100 ms. To generate a sequence, values uniformly distributed between 2 and 1 were convolved with a rectangular window 40 – 80 values wide. During experiments, sequences were shifted in the positive direction and scaled until the neuron generated the desired number of action potentials. The amount of scaling and shifting was then fixed for the duration of the experiment.

We observed that a given varying current injection produced nearly the same pattern of action potentials in every trial (Fig. 7), replicating the results of a previous study in neocortical pyramidal cells (Mainen and Sejnowski 1995) and demonstrating that medium spiny neurons in vitro are capable of producing a reliable output. Also the response to a varying current better approximates the in vivo behavior of a medium spiny neuron than the response to a constant current (Wilson 1993; Wilson and Groves 1981).

Figure 8 demonstrates that an IPSP can change the response to a varying current. The top trace in Fig. 8 is the response to a varying current. In this case, we injected less current so that the cell would not spike. The middle trace in Fig. 8 is the response to the same varying current with an IPSP elicited 75 ms after current onset. The IPSP decreased the depolarization produced by the varying current, as shown by the difference between the two responses (Fig. 8, bottom trace).

Finally, we tested whether short-term plasticity affects the ability of an IPSP to block action potentials generated by varying current injection, and we found that it does (n = 4 for PPD, n = 2 for augmentation). This is illustrated in Fig. 9. In the top traces (Fig. 9, A1 and B1), the neuron generated six action potentials in response to varying current injection. In the middle traces (Fig. 9, A2 and B2), we elicited an IPSP during the varying current injection, and the IPSP blocked two action potentials. In the bottom left trace (Fig. 9 A3), the IPSP elicited during current injection was the second in a pair of IPSPs separated by 350 ms. As a result of PPD, the IPSP blocked the generation of one action potential instead of two. In the bottom right trace (Fig. 9 B3), we elicited the IPSP 2 s after a conditioning train. As a result of augmentation, this IPSP blocked the generation of three action potentials instead of two. This demonstrates that PPD and augmentation can modify the output of medium spiny neurons by modifying the ability of inhibitory inputs to block action potentials.

DISCUSSION

We have investigated the properties and short-term plasticity of an IPSP in medium spiny neurons of the striatum. Our main findings are that the IPSP rectifies at hyperpolarized membrane potentials due in part to shunting by K* channels, the IPSP exhibits augmentation and long-lasting PPD, and augmentation and PPD can modify the output of striatal neurons.
The most likely source of the GABAergic synapses studied here are the medium spiny neurons, as they constitute the vast majority of striatal neurons (Kemp and Powell 1971). The postsynaptic neurons in this study are also medium spiny neurons, and while there is anatomical evidence that medium spiny neurons synapse on each other (Somogyi et al. 1981; Wilson and Groves 1980), attempts to demonstrate this physiologically have not been successful (Jaeger et al. 1994). Two other possible sources are GABAergic striatal interneurons (Kawaguchi et al. 1995; Koos and Tepper 1999) and GABAergic afferents from substantia nigra pars reticulata (Rodriguez and Gonzalez-Hernandez 1999). Given the type of stimulating electrode used and the graded relationship between stimulation intensity and response amplitude, the IPSP described here probably results from the activation of many synapses, and properties of this IPSP are probably average properties of synapses from several different sources.

Rectification of the IPSP

The IPSP rectified at hyperpolarized membrane potentials. This rectification was reduced when the cell was exposed to Ba\(^{2+}\) or Cs\(^{+}\), demonstrating that K\(^{+}\) channels were partially responsible for the rectification. The fraction of rectification insensitive to Ba\(^{2+}\) and Cs\(^{+}\) may have been due to the voltage dependence of the GABA\(_{A}\) conductance (Bormann et al. 1987; Dudel et al. 1980; Segal and Barker 1984; Weiss 1988) or to the difference between \([\text{Cl}^{-}]_o\) and \([\text{Cl}^{-}]_i\) (Barker and Harrison 1988).

The finding that K\(^{+}\) channels shunt the IPSP is not surprising as K\(^{+}\) channels have previously been shown to shunt EPSPs in medium spiny neurons (Calabresi et al. 1990). The activity of medium spiny neurons is determined in part by the...
K⁺ channels that shunt the IPSP. In vivo intracellular recordings in both anesthetized and awake rats have shown that medium spiny neurons have two membrane potential states—a quiet, hyperpolarized state (the “down state”) and a noisy, depolarized state (the “up state”). A transition from the down state to the up state can be triggered by a barrage of excitatory input (Wilson 1993; Wilson and Groves 1981). The K⁺ channels are believed to maintain the neuron in the down state by shunting excitatory inputs (Nisenbaum and Wilson 1995).

When the neuron is in the down state, the purpose of inhibition may be to prevent transitions to the up state. Inhibitory inputs produce a transient shunt, which supplements the persistent shunt by K⁺ channels. In the presence of this larger shunt, some excitatory inputs capable of overcoming the shunt by K⁺ channels may not trigger a transition if the neuron receives an inhibitory input at around the same time. Furthermore, the shunt provided by inhibitory inputs has a different voltage dependence than the shunt provided by K⁺ channels. The K⁺ channels inactivate with depolarization (Nisenbaum and Wilson 1995), while the conductance of GABAᵦ channels increases with depolarization (Bormann et al. 1987; Dudel et al. 1980; Segal and Barker 1984; Weiss 1988). This suggests that as the neuron becomes depolarized an increase in shunting by inhibitory inputs could compensate for a decrease in shunting by K⁺ channels. In these ways, inhibitory inputs may act to prevent medium spiny neurons from reaching the up state.

When in the up state, the neuron often generates action potentials (Wilson 1993; Wilson and Groves 1981), and the purpose of inhibition in the up state may be to prevent action potentials. The up state is more depolarized than the IPSP reversal potential, so IPSPs are hyperpolarizing in the up state. Also, in the up state, the amplitude of the IPSP is not restrained by shunting. Therefore an inhibitory input that occurs during the up state will produce a significant hyperpolarization capable of blocking action potential generation.

**PPD**

The IPSP exhibited PPD over a wide range of ISIs, as previously reported (Radnikow et al. 1997). The time course of PPD, with its slow onset and even slower recovery, suggests it is due to activation of presynaptic metabotropic receptors. Several types of presynaptic metabotropic receptors have been shown to decrease GABA release in striatum. These include GABAᵦ receptors (Calabresi et al. 1991; Radnikow et al. 1997; Seabrook et al. 1991), muscarinic acetylcholine receptors (Marchi et al. 1990; Sugita et al. 1991), adenosine A₂A receptors (Mori et al. 1996), and metabotropic glutamate receptors (Stefani et al. 1994). We found that neither CGP 35348 nor atropine blocked PPD, indicating that PPD is not mediated by GABAᵦ receptors (Radnikow et al. 1997) or muscarinic receptors. Activation of dopamine receptors has been reported to have both presynaptic (Harsing and Zigmond 1997; Wang and Johnson 1995) and postsynaptic (Flores-Hernandez et al. 2000) effects on GABAergic synapses in striatum, but D₁ and D₂ dopamine receptors do not seem to be involved in PPD, as combined application of SCH 23390 and sulpiride had no effect on PPD. This is in accord with the finding that dopamine...
application does not affect the IPSP (Nicola and Malenka 1998).

If PPD is not due to the activation of presynaptic metabotropic receptors, there are at least two other mechanisms that could be responsible. One is the depletion of neurotransmitter (Takeuchi 1958). Release of neurotransmitter in response to the first stimulus may reduce the number of synaptic vesicles available for release in response to the second stimulus, thereby decreasing the amplitude of the second IPSP. Another possible mechanism is receptor desensitization. Some GABA_A receptors may become desensitized during the first IPSP, rendering them unable to contribute to the second IPSP (Jones and Westbrook 1995; Mellor and Randall 1998; Tia et al. 1996). The time course of PPD does not support either of these mechanisms, however. PPD produced by depletion or desensitization would be greatest at the shortest ISIs and would monotonically recover to baseline, but we observed no PPD at the shortest ISI and increasing PPD until the ISI reached 200 ms. If depletion and desensitization do contribute to the PPD we observed, their effects must be obscured at shorter ISIs by some simultaneous facilitative process.

### Augmentation

Repetitive synaptic activity has been shown to temporarily enhance synaptic strength at many synapses across a variety of species (Fisher et al. 1997). After a strong conditioning train, the enhancement in synaptic strength typically decays with fourth-order kinetics. The four time constants of decay define four types of enhancement: Fast-decaying facilitation (F1) has a decay time constant in the range of tens of milliseconds, slow-decaying facilitation (F2) in the range of hundreds of milliseconds, augmentation in the range of seconds, and post-tetanic potentiation (PTP) in the range of tens of seconds to minutes. The enhancement of the IPSP that we observed decayed with a time constant of 10 ± 1 s and is therefore an example of augmentation.

The augmentation of the IPSP was unusual in two respects: it was rather small, and it was not accompanied by PTP. Both effects may be due to the relatively weak nature of the conditioning train. Studies that report greater augmentation and co-occurrence of PTP typically employ a conditioning train with 10–50 times as many stimuli, often at a higher frequency (e.g., Bittner and Baxter 1991; Magleby and Zengel 1976; Tanabe and Kijima 1992; Zengel et al. 1980). Moreover, studies employing conditioning trains of varying lengths have shown that longer trains produce greater augmentation and PTP (Magleby and Zengel 1976; Poage and Zengel 1993; Zengel and Magleby 1982).

There is strong evidence that augmentation is due to an increase in presynaptic [Ca^{2+}] after repetitive activity (Delaney and Tank 1994; Fischer et al. 1997; Kamiya and Zucker 1994; Swandulla et al. 1991). Studies showing that Ba^{2+} increases augmentation (Poage and Zengel 1993; Zengel and Magleby 1980; Zengel et al. 1980) also suggest that augmentation is dependent on Ca^{2+} as presynaptic Ca^{2+} channels are permeable to Ba^{2+}. We tested the effect of Ba^{2+} on augmentation of the IPSP and observed no change at shorter delays but a switch to depression at the longest delay. This suggests that the mechanism responsible for augmentation at inhibitory synapses onto medium spiny neurons may be different from the mechanism at other synapses.

We investigated the relationship between PPD and augmentation by varying the length of the conditioning train. We found that trains of 1 to 7 stimuli depressed the IPSP, trains of 9 or 11 stimuli did not affect the IPSP, and trains of 13 or 15 stimuli augmented the IPSP. This suggests that conditioning trains simultaneously activate both depressing mechanisms and augmenting mechanisms. With shorter trains the depressing mechanisms prevail, with longer trains the augmenting mechanisms prevail, and with trains of intermediate lengths the two mechanisms cancel.

### Effects of PPD and augmentation on action potential generation

We have shown that IPSPs can block action potentials generated in response to both constant and varying current injections, thus demonstrating that inhibitory inputs can shape the firing patterns of medium spiny neurons. We have also shown that the effectiveness of the IPSP in blocking action potentials is diminished by PPD and increased by augmentation, indicating that short-term synaptic plasticity at inhibitory synapses can modulate striatal output.

The type of stimulation we used probably activated many inhibitory synapses, and such synchronous inhibitory input may not be physiologically realistic. This raises the possibility that because we have activated the inhibitory inputs in an artificial manner, our results are not physiologically relevant. However, evidence from several other brain areas, including the striatum, indicates that the input of an individual inhibitory neuron is sufficient to change the behavior of its targets. For instance, single inhibitory neurons can alter action potential timing in striatal medium spiny neurons (Koos and Tepper 1999), hippocampal pyramidal cells (Cobb et al. 1995), and cerebellar Purkinje cells (Hauser and Clark 1997) and can alter backpropagating action potentials in neocortical pyramidal cells (Larkum et al. 1999). Furthermore striatal inhibitory interneurons may fire synchronously, as some are electrically coupled (Koos and Tepper 1999). Thus we believe that our findings represent inhibitory mechanisms operating in the behaving animal to modify the output of medium spiny neurons.

The changes in striatal output caused by short-term plasticity of inhibition may propagate through the circuitry of the basal ganglia to thalamus and cortex. This could influence the performance of tasks known to involve the striatum, such as movement (Hauber 1998) and memory (Knowlton et al. 1996). This is likely to be true not just for PPD and augmentation but for any other forms of plasticity, short- and long-term, that exist at these synapses.

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