A Comparative Voltage and Current-Clamp Analysis of Feedback and Feedforward Synaptic Transmission in the Striatal Microcircuit In Vitro

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Gustafson, Nicholas, Elakkat Gireesh-Dharmaraj, Uwe Czubayko, Kim T. Blackwell, and Dietmar Plenz. A comparative voltage and current-clamp analysis of feedback and feedforward synaptic transmission in the striatal microcircuit in vitro. J Neurophysiol 95: 737–752, 2006. First published October 19, 2005; doi:10.1152/jn.00802.2005. Striatal spiny projection (SP) neurons control movement initiation by integrating cortical inputs and inhibiting basal ganglia outputs. Central to this control lies a “microcircuit” that consists of a feedback pathway formed by axon collaterals between GABAergic SP neurons and a feedforward pathway from fast spiking (FS) GABAergic interneurons to SP neurons. Here, somatically evoked postsynaptic potentials (PSPs) and currents (PSCs) were compared for both pathways with dual whole cell patch recording in voltage- and current-clamp mode using cortex-striatum-substantia nigra organotypic cultures. On average, feedforward inputs were 1 ms earlier, more reliable, and about twice as large in amplitude compared with most feedback inputs. On the other hand, both pathways exhibited widely varying, partially overlapping amplitude distributions. This variability was already established for single FS neurons targeting many SP neurons. In response to precisely timed action potential bursts, feedforward and feedback inputs consistently showed short-term depression \( \leq 50–70\% \) in voltage-clamp, although feedback inputs also displayed strong augmentation in current-clamp in line with previous reports. The augmentation of feedback inputs was absent in gamicidin D perforated-patch recording, which also showed the natural reversal potential for both inputs to be near firing threshold. Preceding depolarizing feedback inputs during the down state did not consistently change subsequent postsynaptic action potentials. We conclude that feedback and feedforward inputs have their dominant effect during the up-state. The reversal potential close to the up-state potential, which supports shunting operation with millisecond precision and the strong synaptic depression, should enable both pathways to carry time-critical information.

INTRODUCTION

It is now well established that GABAergic spiny projection (SP) neurons of the striatum, besides inhibiting basal ganglia output nuclei, also form functional synapses through their local axon collaterals (Czubayko and Plenz 2002; Guzman et al. 2003; Koos et al. 2004; Taverna et al. 2004; Tunstall et al. 2002; Venance et al. 2004). This feedback loop allows cortical inputs that achieve control over basal ganglia outputs to govern local striatal dynamics (Kitano et al. 2001; Plenz 2003; Plenz and Kitai 2000; Wickens 2002; Wickens et al. 1991). In addition, the corticostriatal inputs through GABAergic fast spiking (FS) interneurons inhibit SP neurons directly, which establishes a feedforward pathway that is independent of striatal output (Plenz 2003; Tepper et al. 2004).

Together, the two pathways form the basis of a striatal “microcircuit” (Bolam et al. 2006; Tepper and Plenz 2006), where each pathway might serve distinct functions. Synaptic inhibition from FS inputs (FS→SP) is reliable (Koos and Tepper 1999; Koos et al. 2004; Plenz and Kitai 1998b; Tepper and Plenz 2006) and predominantly targets the soma of SP neurons (Bennett and Bolam 1994; Kubota and Kawaguchi 2000). This suggests that feedforward inputs predominantly affect somatic spiking (Koos and Tepper 1999). Postsynaptic potentials (PSPs) and currents (PSCs) from SP inputs (SP→SP) are more variable. While SP→SP connections are on average smaller in amplitude and less reliable compared with feedforward inputs, they nevertheless cover a wide range in amplitudes and failure rates as shown in mature organotypic slices in whole cell patch recordings (Czubayko and Plenz 2002) and acute 3- to 4-wk-old slices (Guzman et al. 2003; Koos et al. 2004; Taverna et al. 2004; Venance et al. 2004). This variability originates in part from the finding that \( \approx 90\% \) of SP inputs target more distant SP dendrites, which attenuates their somatically recorded amplitudes (Koos et al. 2004). The variability and attenuation suggests that feedback inputs might not primarily control somatic spiking; however, alternative functional roles for these inputs are poorly understood.

Short-term plasticity has also been consistently reported to differ between the two pathways. FS→SP connections reliably show short-term depression (Koos et al. 2004; Plenz and Kitai 1998b), which tends to preserve the temporal information in synaptic transmission (Abbott et al. 1997; Fuhrmann et al. 2002; Thomson 2000). SP inputs, on the other hand, reveal paired-pulse and short-term depression or facilitation in voltage-clamp or current-clamp mode (Czubayko and Plenz 2002; Guzman et al. 2003; Koos et al. 2004; Taverna et al. 2004; Venance et al. 2004). This suggests that SP inputs might be less reliable in retaining temporal input information.

This study provides a comparative analysis of both pathways in the cortex-striatum-substantia nigra culture in vitro model, which has been successfully used in a variety of studies on striatal processing of corticostriatal inputs (Blackwell et al. 2003; Kerr and Plenz 2004; Plenz and Kitai 1998b). In the first part, we study the efficacy of both pathways to affect somatic membrane potentials and currents using voltage- and current-clamp techniques. This also allows for a more detailed comparison of the organotypic culture system with findings from mature organotypic slices.
the acute slices. We specifically address differences in short-term plasticity recorded for both pathways. We study these differences in the whole cell patch configuration using precisely timed presynaptic action potential bursts, thereby reducing trial-to-trial variability. We extend this approach using the less invasive gramicidin-D perforated-patch approach, which also preserves the intracellular chloride concentration.

We found that in the cultures, FS inputs are on average stronger and more reliable than SP inputs, which is in accordance with findings in the acute slice. We also show that SP→SP connections reveal consistent and strong short-term depression in current clamp when using perforated-patch recordings only. Finally, perforated-patch recordings show that fast GABAergic inputs to SP neurons are depolarizing at rest.

**METHODS**

**Preparation of organotypic cultures**

For the preparation of cortex-striatum-substantia nigra organotypic cultures (Plenz and Kitai 1998b), coronal sections from rat brains (Sprague Dawley, Taconic Farms, MD) at postnatal day 0–2 were cut on a vibroslicer (VT 1000 S, Leica Microsystems, Allendale, NJ). All animal procedures were performed in accordance with the National Institutes of Health Guide to the Care and Use of Laboratory Animals and with approval of the NIMH Animal Care and Use Committee.

Slices containing striatum (500 μm thickness), and cortex (350 μm thickness) were used for dissection of dorsolateral cortex and striatum. For the substantia nigra (including pars compacta and pars reticulata), ventrolateral sections from mesencephalic slices, 500 μm thick, were taken; medial tissue regions were avoided. The tissue was submerged in 25 μl of chicken plasma (Sigma, St. Louis, MO) on a cove slip, and 25 μl of bovine thrombin was added (1,000 NIH units/0.75 ml; Sigma). After plasma coagulation, individual cultures were placed in tubes (Nunc, Naperville, IL) with 800 μl of culture medium consisting of 50% basal medium Eagle, 25% Hanks balanced salt solution, 25% horse serum, 0.5% glucose, and 0.5 mM L-glutamine (all Gibco, Grand Island, NY). Cultures were rotated in a “rollertube” incubator (0.6 rpm; Heraeus, Göttingen, Germany) at 35.5°C in normal atmosphere. After 3 and 27 days in vitro (DIV), 10 μl of mitosis inhibitor was added for 24 h (4 mM cytosine-5-b-arabino-furanosid, 4.4 mM uridine, and 4.4 mM 5-fluoroodeoxyuridine; calculated to final concentration; all Sigma). Medium was changed every 3–5 days.

For electrophysiological recordings, cultures were placed in a custom-made, heated recording chamber maintained at 35.5 ± 0.5°C (TC-20, NPI, Tamm, Germany). Cultures were submerged in artificial cerebrospinal fluid (ACSF) containing (in mM) 126 NaCl, 0.3 NaH2PO4, 2.5 KCl, 0.3 KH2PO4, 1.6 CaCl2, 1.0 MgCl2, 0.4 MgSO4, 26.2 NaHCO3, and 11 D-glucose saturated with 95% O2-5% CO2 at a flow rate of 1.8 ml/min. The osmolarity of the ACSF was 300 ± 5 mOsm. The recording chamber was mounted on an upright microscope equipped with ×4 and ×60 water-immersion objectives (BX-50, Olympus, Tokyo, Japan). The microscope was placed on a custom-made sliding table allowing a change in field of view during the experiment. Pipettes for somatic patch recordings were pulled to a resistance of 4–6 MΩ (1.5 mm OD, 0.75 ID; P-97, Sutter Instruments, Novato, CA).

**Whole cell patch recordings**

For whole cell recordings, the intracellular patch solution contained (in mM) 132 K-gluconate, 6 KCl, 8 NaCl, 10 HEPES, 2 Mg-ATP, and 0.39 Na-GTP and was supplemented with 0.25% neurobiotin (Vector Laboratories, Burlingame, CA). The pH was adjusted to 7.2–7.4 with KOH, and the final osmolarity of the pipette solution was 290 ± 10 mOsm. The composition of intra- and extracellular solution resulted in a theoretical reversal potential of $E_{Cl} = -59$ mV for chloride and $E_{K} = -102$ mV for potassium. To reduce degradation of ATP and GTP, the intracellular working solution was kept on ice throughout the experiment.

Somatic gigascale (>2–4 GΩ) were made to visually identified cells, after which we compensated for electrode capacitance. After breakthrough, intracellular membrane potentials were first recorded in current clamp for each neuron (Axopatch 200D, Axon Instruments) and preamplified (Cyberamp380, Axon Instruments). No corrections were made for series resistance or junction potential. In current clamp, data were digitized for each neuron at 10 kHz for voltage and 5 kHz for current; whereas in voltage clamp, data were digitized at 5 kHz for voltage and 25 kHz for current. Data were collected continuously using the CED 1401 (Cambridge Electronic Design, UK). Electrophysiological data analysis was performed in Spike2 (CED).

After obtaining a current-voltage relationship for each neuron in current clamp for neuronal identification, a series of single action potentials (10–50) were elicited at 1 Hz to test for the presence of synaptic connections. Once a synaptic connection was identified, characterization of postsynaptic potentials was performed in current clamp, or alternatively, postsynaptic currents were characterized after switching to voltage clamp for the postsynaptic neuron. In several cases, both postsynaptic currents (PSCs) and postsynaptic potentials (PSPs) were recorded from the same neuron. In these latter cases, when testing for short-term plasticity, the order of voltage- and current-clamp recordings was randomized to control for possible serial effects that might arise from prolonged synaptic activation at high frequencies. After each paired recording, a new pair of neurons was selected for analysis, except when testing for multiple connections between one FS interneuron and several SP neurons. In the latter case, connectivity was analyzed by recording from the FS interneuron while testing up to five different postsynaptic SP neurons.

**Perforated-patch recording using gramicidin D**

Perforated-patch recording was performed using gramicidin D (Sigma-Aldrich), which forms ion pores that permit passage of Na+, K+, and other small cations, but prevents passage of proteins and anions like Cl− (Akaie 1996). This allows intracellular measurement of chloride-mediated synaptic responses without disturbing the natural $E_{Cl}$ and preserving second messenger–mediated pathways. Stock solution of gramicidin D was made in methanol at 10 mg/ml and diluted to a final concentration of 0.2 mg/ml before recording in standard intracellular pipette solution. For visual control of the perforated patch, the fluorescent dye Dextran Alexa Fluor 488 (Molecular Probes) was dissolved in the pipette solution at a concentration of 0.4 mg/ml. An intact perforated recording was characterized by a fluorescent electrode tip and absence of fluorescence inside the cell. Breaking the perforated patch artificially with high-voltage pulses filled the neuron with the fluorescent dye. Stability of the perforated patch was checked briefly every 10–15 min using standard fluorescent illumination (Olympus) during recordings. Recordings during which the perforated patch spontaneously broke and became whole cell were discarded. After gigaseal formation, the SP neuron was first observed in voltage-clamp mode with the holding potential set to −80 mV. The establishment of voltage clamp developed gradually over 10–15 min after gigaseal formation and was marked by an increase in current transients to voltage pulses and a decrease in spontaneous firing as the clamp became more effective. After stable access was obtained, dual recordings were continued in current-clamp mode.

**Neuronal types**

We identified neuronal types based on soma size and physiological criteria as described below. Striatal spiny projection neurons were identified by their spherical soma with a diameter of 10–12 μm. SP neurons were accepted for recordings if 1) the resting membrane potential was more negative than −70 mV, 2) suprathreshold current
pulse injection resulted in a ramp-like depolarization that delayed action potential discharge by several hundreds of milliseconds, and 7) neurons could fire action potentials repetitively ≤50 Hz. The likelihood of recording from interneurons was increased by visually selecting large somas for patching. FS interneurons were identified by a linear current-voltage relationship and high firing rates in response to current injections (Blackwell et al. 2003; Kawaguchi 1993; Plenz and Aertsen 1996; Plenz and Kitai 1998b). FS interneurons were accepted for recordings if they revealed a stable resting membrane potential and fired repetitively at high action potential frequency to prolonged suprathreshold current injections. For further identification, some FS interneurons were processed for anatomical reconstruction. Cultures were fixed in 4% paraformaldehyde overnight, and after wash, neurons were incubated in Avidin-Texas Red (Vector) as described previously (Plenz and Kitai 1998b). Fluorescence was measured using a confocal microscope (Zeiss) and images were processed using Adobe Photoshop (Adobe).

Basic electrophysiological parameters

Input resistance was measured in response to small hyperpolarizing current pulses applied at resting membrane potential. Similarly, membrane time constants were extracted from membrane potential trajectories using linear regression analysis on semi-logarithmic plots, ignoring the short, initial time constant and only using the longer of the two time constants. For extraction of action potential waveform parameters, ≤20 action potentials elicited by suprathreshold current injection were averaged. Action potential threshold was defined as the membrane potential at which the positive change in membrane potential reached 5% of its maximal value. Action potential amplitude was measured from threshold to peak, and action potential duration was taken as the width at half-height. Similarly, action potential afterhyperpolarization (AHP) was quantified by the time to half-maximum. Similarly, action potential afterhyperpolarization (AHP) was measured by the time to half-height. Similarly, action potential afterhyperpolarization (AHP) was quantified by the time to half-maximum.

Analysis of synaptic responses to single presynaptic action potentials

To block the occurrence of spontaneous up-states (Plenz and Kitai 1998b) and fast glutamatergic transmission in the culture, all recordings were performed in the presence of the glutamate antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX, 10 μM; Sigma), if not stated otherwise. Suprathreshold, exponentially decaying currents (half-width: 0.7 ms) were injected into the presynaptic cell to elicit precisely timed action potentials. The action potential peak defined the time of the presynaptic action potential. To analyze synaptic connections in response to single action potentials, single presynaptic action potentials were elicited at 1 Hz. PSPs were analyzed at resting potential in current-clamp mode and PSCs were analyzed at zero holding current in voltage-clamp mode to avoid shifting the neuron from its natural resting potential and introducing a clamp at the soma without being certain if a proper clamp has been achieved in the dendrites. Postsynaptic responses to single spikes were classified into successes, failures, and outliers using custom routines written in Spike2. To perform this classification, first the average response and SE to all presynaptic spikes were calculated, which served as a template. Then, individual responses were plotted at high temporal resolution and compared with the average template. An outlier was defined as an individual response distorted by spontaneous activity. A failure was identified in two ways. First, either as the absence of a response, i.e., the response did not cross threshold (±3 SD of prestimulus noise) during peak time of the average response (±2 ms). Alternatively, a putative response was delayed by more than ~2 ms with respect to the average response onset, suggesting this response to be spontaneous synaptic activity. All other responses were classified as “successes.” Successes and failures were averaged separately and saved for subsequent analysis. Typically, 20–200 trials were used to determine the mean PSP or PSC. To calculate the CV for PSCs, successes and failures were combined when calculating the mean and SD. Peak amplitudes were extracted from individual responses within 10 ms after a presynaptic action potential. In addition, maximal slope values were calculated from a linear approximation of the PSP or PSC trajectory between 20 and 80% of peak amplitudes. The 10–90% rise time was measured from averaged successes for PSCs and PSPs, respectively. Synaptic response onset was defined as the time from presynaptic spike peak to the midpoint of this slope.

Maximal synaptic conductance $g_s$ was calculated from whole cell voltage-clamp recordings as

$$g_s = \frac{PSC}{(V_m - E_{Cl})}$$

in which PSC was the average peak synaptic current elicited in the absence of failures, $V_m$ was the membrane holding potential at zero holding current, and $E_{Cl}$ was set to ~59 mV.

Numerical fit of single PSP and PSC time-course

Preliminary results showed that a standard $\alpha$-function of the form $y(t) = A \cdot \alpha \cdot e^{-t/\tau}$ was insufficient to capture the fast rise of the synaptic response. We therefore used a sum of exponential functions to achieve appropriate fits of the form $y(t) = \sum A_n \cdot e^{t/\tau_n} + Y_0$.

First, we compared the results between a double exponential fit ($n = 2$) and a triple exponential fit ($n = 3$) and found that the quality of fit was not significantly different between the two functions ($n = 57$ neurons). Second, plots of synaptic time courses in semi-logarithmic coordinates showed that the long-lasting decay for PSCs and PSPs was accurately captured by a single exponential decay (data not shown). Finally, when fitting PSPs with a biexponential function, a strong correlation was found with $\tau_{rise} \equiv \tau_{decay}$. This allowed us to simplify the exponential fit to a formula described by a single amplitude parameter $A$ combined with the difference of two exponential functions that capture the rise ($\tau_{rise}$) and decay of the response ($\tau_{decay}$), respectively.

Biexponential functions of the form

$$H(t) = A_0 \cdot (e^{-t/\tau_{rise}} - e^{-t/\tau_{decay}}) + Y_0$$

were used to parameterize the average PSP and PSP time-course (success) in response to single presynaptic spikes. The two time constants, $\tau_{rise}$ and $\tau_{decay}$, of the postsynaptic response captured the fast rise and slow decay of the response, respectively. $A_0$ is the magnitude and direction of the postsynaptic response. Therefore a PSP has a positive $A_0$ and a PSC has a negative $A_0$. $Y_0$ represents residual baseline activity of the postsynaptic neuron and is close to zero for PSCs and to resting membrane potential for PSPs. Best fits were obtained using a Levenberg-Marquardt iterative procedure until $\chi^2$ was minimized (Origin v6.0, Microcal, Southampton, MA).

Short-term plasticity of synaptic responses to presynaptic action potential bursts

Short-term plasticity was tested for each synaptic connection with precisely timed action potential bursts by using exponentially decaying suprathreshold current injections into the presynaptic cell. Each burst contained 10 action potentials and was repeated five times with an interburst interval >2 s. Burst frequencies between 5 and 60 Hz were tested and averaged separately for each frequency. In voltage-clamp mode, total postsynaptic charge transfer was calculated by integrating over the burst response duration at zero holding potential. Total charge transfer at 5 Hz was taken to normalize responses at higher frequencies. The average time-course for PSCs was calculated for each neuron and each frequency and averaged over the population. In current-clamp mode, PSPs in response to action potential bursts

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were recorded at resting membrane potential. After correcting for average prestimulus membrane potential (mean taken 50 ms before response onset), calculation of total postsynaptic depolarization, normalization to total depolarization achieved at 5 Hz, and averaging of time-courses were done as described for voltage-clamp recordings.

**Reversal potential estimate in perforated patch-recordings**

Perforated patch recordings to estimate the $E_{Cl}$ were performed in the presence of spontaneous up and down state activity, i.e., in the absence of DNQX. For each connection, about 10 postsynaptic responses to presynaptic bursts at 20 Hz were averaged at different membrane potentials induced by depolarizing somatic current injections. Changes in postsynaptic depolarization to the first spike in a burst were used to calculate $E_{Cl}$. These experiments required relatively strong current injections, which can result in an overestimation of membrane potential values due to the pipette serial resistance. This was partially corrected for by comparing the threshold of action potentials from suprathreshold steady-state current injections with the threshold of spontaneous action potentials during up-states. The differences in action potential thresholds were used to estimate the error from serial resistance for a given current injection, which was subtracted from corresponding measured steady-state voltages. These corrections were in the range of a few millivolts.

**Effect of depolarizing GABAergic feedback inputs on action potential timing on depolarization**

The effect of preceding depolarizing GABAergic inputs onto action potential firing and timing on suprathreshold depolarization was studied in two different ways. First, a presynaptic action potential burst (10 action potentials at 60 Hz) was used to elicit depolarizing PSPs in the postsynaptic neuron during the down-state. Immediately after the burst, a cortical single shock stimulation (100 μs, ~50 μA; monopolar tungsten electrode, WPI) was used to elicit an up-state in striatal neurons. Paired stimuli were alternated with cortical stimulation only at an inter up-state interval of 5 s. Number of action potentials and unpaired up-state activity. Second, a presynaptic action potential timing on depolarization after the burst, a cortical single shock stimulation (100 μs) was used to elicit depolarizing PSPs in the postsynaptic neuron during the down-state. Immediately after the burst, a cortical single shock stimulation (100 μs, ~50 μA; monopolar tungsten electrode, WPI) was used to elicit an up-state in striatal neurons. Paired stimuli were alternated with cortical stimulation only at an inter up-state interval of 5 s. Number of action potentials and delay to first action potential were analyzed for paired and unpaired up-state activity. Second, a presynaptic action potential burst (10 action potentials at 60 Hz) was followed immediately by a suprathreshold somatic current pulse injection (1-s duration). The intensity of the suprathreshold current pulse was adjusted to achieve a delay in action potential onset of ~100–400 ms. About 10 responses were averaged for each neuron and the delay to first action potential in response to current injection was compared for controls and paired responses. The controlled current pulse injection and paired injection was separated by 6 s to prevent serial effects on action potential onset from preceding suprathreshold depolarization (Mahon et al. 2000).

**Statistical data analysis**

Data analysis was performed in Origin V6.0, Excel (Microsoft, Seattle, WA), Matlab (MathWorks), and StatView V5.0 (SAS Institute, Cary, NC). Paired comparisons of medians were done using the nonparametric Mann-Whitney U-test, if not stated otherwise. Correlation was estimated by regression analysis. Data are expressed as means ± SE, unless otherwise stated.

**RESULTS**

**Identification of SP neurons and FS interneurons in cortex-striatum-substantia nigra cultures**

We recorded in whole cell configuration from 92 SP neurons and 48 FS interneurons in mature cortex-striatum-substantia nigra organotypic cultures grown for 3–5 wk. SP neurons were identified by a strong inward rectification at hyperpolarized potentials and a long latency to first action potential in response to current injections, whereas FS interneurons had a linear steady-state current-voltage relationship and fired abruptly at high frequencies early during suprathreshold current injection (Fig. 1, A and B) (Blackwell et al. 2003; Farries and Perkel 2000; Kawaguchi 1993; Kawaguchi et al. 1989). The combination of a relatively negative resting membrane potential and long action potential width separated SP neurons from FS interneurons (Fig. 1C; Table 1). Action potential threshold was identical for both neurons, whereas action potential amplitude was ~10 mV smaller in FS interneurons (Table 1). Both populations were also clearly distinguished by the earlier and larger action potential afterhyperpolarization (AHP) in FS interneurons (Fig. 1, D and E; Table 1). Anatomical reconstruction of electrophysiologically identified FS interneurons (7/7) showed the characteristic smooth dendrites and fusiform soma of these neurons (Fig. 1F) (Kawaguchi 1993; Plenz and Kitai 1998b).

**Feedforward connections are stronger, faster, and more reliable than most feedback connections**

In voltage clamp, FS→SP connections had a mean PSC amplitude of $-38.5 \pm 8.5 \text{ pA}$ (median: $-22.4 \text{ pA}$; range: $-9$ to $-119.6 \text{ pA}$) and a slope of $-42.2 \pm 10.2 \text{ pA/ms}$ (median: $-25.3 \text{ pA/ms}$; range: $-6.2$ to $-130.4 \text{ pA/ms}$; $n = 16$; Fig. 2, A, C, and E). Conversely, PSCs from SP→SP connections were $-35$–50% smaller in amplitude and slope, with average values of $-26.5 \pm 5.4 \text{ pA}$ (median: $-17.2 \text{ pA}$; range: $-5.9$ to $-271.5 \text{ pA}$) and $-22.9 \pm 5.2 \text{ pA}$ (median: $-12.6 \text{ pA/ms}$; range: $-2.4$ to $-238.4 \text{ pA/ms}$), respectively ($n = 49$; Fig. 2, A, C, and E). PSC amplitudes were not significantly different between the two synaptic pathways ($P > 0.05$), whereas slope values were significantly different ($P < 0.05$). For both inputs, PSC slope correlated strongly with PSC amplitude ($R = 0.84$ and 0.97, respectively). The mean CV for SP→SP connections was $-0.62 \pm 0.05$ (median: $-0.53$, range: $-1.98$ to $-0.10$) and for FS→SP connections was $-0.66 \pm 0.10$ (median: $-0.67$, range: $-1.79$ to $-0.18$; $P > 0.05$). We encountered no incidence of SP-FS connections (out of $n = 28$ tested) and did not record from FS-FS pairs.

There was a significant difference in the timing between both inputs; on average PSCs from FS inputs were ~0.5 ms earlier in onset and thus reached their peak about 1 ms earlier than SP→SP connections ($1.43 \pm 0.10$ vs. $1.95 \pm 0.08$ ms; $2.77 \pm 0.18$ vs. $3.71 \pm 0.12$ ms; $P < 0.05$, both cases; Fig. 2E). PSCs had a median onset of 1.4 ms (range, 1.0 to 2.8 ms) for FS→SP, whereas onset was 1.9 ms, with a range of 0.8 to 3.4 ms for SP→SP. In regard to time to peak, the median was 2.8 ms (range 1.9 to 4.4 ms) for FS→SP and 3.6 ms (range 2.2 to 6.5 ms) for SP→SP. Finally, the 10–90% rise time was significantly shorter for FS→SP connections (mean: $0.85 \pm 0.09$ ms; median: $0.81$ ms; range: $0.51$–1.85 ms) compared with that of SP→SP connections (mean: $1.20 \pm 0.07$ ms; median: $1.11$ ms; range: $0.58$–3.0 ms; $P < 0.005$). Despite the differences in population means, there was also substantial overlap in synaptic transmission measured for the two pathways. For example, the mean strength of individual connections in each pathway ranged from $-6$ to $-271$ pA for SP inputs and from $-9$ to $-120$ pA for FS inputs (SD
Similarly, the onset and peak time in some SP inputs was as early as seen in FS inputs.

We found comparable results with current-clamp recordings (Fig. 2, B and D). PSP amplitudes and slopes from FS inputs had mean values of $2.82 \pm 0.64$ mV (median: 1.59 mV, range: 0.33–10.65 mV) and $0.73 \pm 0.16$ mV/ms (median: 0.39 mV/ms, range: 0.05–2.54 mV/ms), respectively ($n = 20$). SP→SP connections ($n = 17$) were $\sim50\%$ smaller in amplitude ($1.48 \pm 0.46$ mV, $P < 0.05$; median: 0.57 mV, range: 0.19–6.37 mV) and slope ($0.82 \pm 0.09$ mV/ms, $P < 0.05$; median: 0.16 mV/ms; range: 0.05–1.39 mV/ms). PSP onsets also were significantly earlier in FS inputs compared with SP inputs ($2.9 \pm 0.2$ ms vs. $3.4 \pm 0.2$ ms, $P < 0.05$; median: 2.8 ms; range: 1.5–4.8 ms; median: 3.64 ms; range: 2.1–5.3 ms), but differences in peak response time between the two pathways were lost, presumably because of the dominant effect of the membrane time constant in current-clamp recordings ($P > 0.50$; data not shown). For FS→SP inputs, the time to peak was $7.5 \pm 0.5$ ms (median: 6.9 ms; range: 3.3–12.0 ms), and for SP→SP inputs, $7.6 \pm 0.6$ ms (median: 7.7 ms; range: 4.7–12.1 ms). Similarly, the 10–90% rise time for PSPs was not significantly different for FS→SP connections (mean: $3.55 \pm 0.27$ ms; median: 3.6 ms; range: 1.47–5.8 ms) compared...
with that of SP→SP connections (mean: 4.02 ± 0.4 ms; median: 4.12 ms; range: 1.96—7.55 ms; \( P > 0.05 \)).

We analyzed the reliability of transmission with respect to synaptic conductances \( g_s \) and failure rate for both pathways in voltage clamp. On average, FS inputs failed only one-half as often as SP inputs (10.6 ± 3.9% vs. 22.2 ± 3.5%; Fig. 2F) in accordance with the difference in number of release sites estimated for both types of synapses (Koos et al. 2004). However, this difference was not statistically significant (\( P > 0.05 \)). These suggested differences in the mean resulted from a much wider distribution of failure rates for SP→SP connections. While failure rates were below 30% for most FS→SP connections, SP inputs exhibited failure rates ≤90%, in line with our previous reports in current-clamp mode (Czubayko and Plenz 2002). As expected from differences in PSC amplitudes, the average conductance \( g_s \) was ~14% smaller for SP inputs compared with FS inputs (1.86 ± 0.50 nS; median: 1.09 nS; range: 0.24—24.69 nS) versus 2.16 ± 0.49 nS (median: 1.36 nS; range: 0.48—7.21 nS; Fig. 2F). However, highly reliable SP→SP connections also revealed a high conductance \( g_s \) > 1.5 nS, attaining a strength and reliability in synaptic transmission comparable to that of the FS→FS pathway. The average success probability for FS→FS connections was 89.4 ± 4.0% (median: 95.6%, range: 38.5—100%), whereas for SP→SP connections, it was 77.8 ± 3.5% (median: 86.5%, range: 12.0—100%).

A more quantitative analysis of the PSC time-course for both pathways was performed by fitting the mean synaptic time-course to a biexponential function (see METHODS; Fig. 3). Rise times for FS→SP connections were about 30% shorter compared with those of SP→SP connections (2.01 ± 0.22 vs. 2.88 ± 0.13 ms; \( P < 0.0005 \); Fig. 3A). On the other hand, no differences were found for the decay times, which were 5.47 ± 0.29 and 5.88 ± 0.24 ms for FS→SP and SP→SP connections, respectively. In current-clamp mode, the mean rise times of FS→FS and SP→SP connections were 3.25 ± 0.21 and 3.73 ± 0.25 ms, respectively (median: 3.07 ms; range: 1.85—5.44 ms and median: 3.65 ms; range: 1.99—5.88 ms; \( P > 0.05 \), both cases). In addition, decay times for FS→SP and SP→SP connections were 29.25 ± 3.73 and 38.66 ± 7.7 ms, respectively (median: 29.88 ms; range: 7.53—62.41 ms and range: 31.96 ms; range: 6.13—107.05 ms). Thus synaptic currents decayed within ~6 ms for both pathways, whereas the decay of PSPs during the down-state could last between 50 and 100 ms (Fig. 3B), in line with reports from the acute striatal slice (Koos et al. 2004; Venance et al. 2004). Taken together, feedforward inhibitory connections in the striatal microcircuit on average are ~50—100% stronger, arrive 1 ms earlier, and are about twice as reliable compared with feedforward connections. On the other hand, a significant portion of feedback connections is as strong as feedforward connections.

Given electrotonic attenuation along SP dendrites (Reyes et al. 1998), these findings are consistent with the anatomical demonstration that FS inputs, in addition to targeting dendritic shafts, preferentially target the soma of SP neurons (Bennett and Bolam 1994; Kubota and Kawaguchi 2000), whereas the opposite is true for SP inputs (Oorschot et al. 2004; Smith and Bolam 1990; Wilson and Groves 1980).

**Single FS interneurons provide heterogeneous synaptic inputs to multiple SP neurons**

It has been estimated that one FS interneuron connects to several hundred up to several thousand nearby SP neurons (Bennett and Bolam 1994; Bolam et al. 2006; Koos and Tepper 1999; Kubota and Kawaguchi 2000), but how similar the effect of a single FS interneuron is on many SP neurons is unknown. We found that connections from one FS interneuron to multiple postsynaptic SP neurons differed largely in mean amplitude and failure rate. For example, in the neuron shown in Fig. 4A, the first SP neuron revealed a relatively small PSC amplitude and high failure rate, the second connection exhibited a high PSC amplitude with no failures, and the third connection had a low PSC amplitude and low failure rate. Similar differences were found for two other FS interneurons (Fig. 4B). These differences are unlikely to result from a rundown of the synaptic connection because large amplitude synapses were also recorded after connections with small amplitudes. There was no significant difference between postsynaptic SP neurons that would explain these variations in the feedforward pathway.

These results show that some of the variability in synaptic transmission encountered in the microcircuit is already established at the single neuron level. They are in accordance with the finding that FS interneurons provide multiple innervations to SP neurons (Koos et al. 2004), thereby targeting soma as well as dendrites (Kubota and Kawaguchi 2000). Variation in the number of innervations and innervation location would contribute to the differences in failure rates and synaptic amplitudes observed for single axons.

**Feedforward and feedback connections depress during high-frequency inputs**

In response to action potential bursts, synaptic transmission is subjected to short-term plasticity that regulates the strength of synaptic amplitudes. The plasticity, among other factors, critically depends on the precise timing, number, and frequency of presynaptic action potentials (Thomson 2000). To compare short-term plasticity in both pathways, precisely timed action potential bursts at defined frequencies were used.

FS interneurons can fire action potentials up to several hundred hertz in vitro (Kawaguchi 1993; Koos and Tepper 1999; Plenz and Kitai 1998b) and exhibit high-frequency action potential bursts during spindle activity in vivo (Berke et al. 2004). FS inputs have been found to show short-term depression (Koos and Tepper 1999; Koos et al. 2004; Plenz...
and Kitai 1998b), but the exact frequency relationship is unknown. When testing FS→SP connections at frequencies ranging from 5 to 60 Hz, short-term depression was already well established at 10 Hz and saturated at ~50% for frequencies between 40 and 60 Hz (Fig. 5). The short-term depression was not significantly different in voltage-clamp (n = 13; Fig. 5, A–C) or current-clamp mode (n = 4; Fig. 5, C–E).

SP neurons fire action potentials ≤100 Hz in vitro (Kawaguchi et al. 1989; Kita et al. 1985; Plenz and Kitai 1998b) and are known to exhibit a variety of firing characteristics in vivo ranging from irregular firing, to episodic bursting and brief action potential bursts in urethane-anesthetized and awake animals (e.g., Hikosaka et al. 1989; Wilson and Groves 1981). These firing patterns suggest that short-term plasticity might be

FIG. 2. On average, FS inputs are larger in amplitude, arrive earlier, and are more reliable than SP inputs. A and B: average postsynaptic response (dark gray) and failures (light gray; SE) for a feedback (SP→SP) and a feedforward connection (FS→SP) in voltage and current clamp, respectively. C and D: SP and FS inputs have widely distributed amplitudes with smallest inputs from the feedback pathway and largest inputs from the feedforward pathway. Top: scatter plots. Bottom: box plots of peak amplitude. E: FS synaptic currents have earlier onset (top) and peak earlier (bottom) than SP inputs. F: on average, FS inputs are more reliable than SP inputs, but some SP connections are as strong and reliable as FS inputs.
Furthermore, there was no correlation between the resting membrane potential and whether the target cell exhibited 1) augmentation or depression or 2) the magnitude of augmentation or depression. There was also no correlation between failure rates and the direction of short-term plasticity ($P > 0.7$) or between membrane time constant and the maximum net depolarization at the highest frequency tested ($P > 0.6$). However, augmenting synapses were found to have smaller average PSP amplitudes and PSP slopes compared with depressing inputs ($0.7 \pm 0.15$ vs. $1.9 \pm 0.36$ mV PSP; $0.13 \pm 0.04$ vs. $0.35 \pm 0.08$ mV/ms; $P < 0.05$). We therefore hypothesized that augmenting synapses might be electrotonically remote from the soma, i.e., at dendrites, whereas depressing synapses might be located closer to the soma.

Differences in distances from the soma could make synapses differ in their sensitivity to the dialyzing effect brought about by the whole cell patch technique. Indeed, when studying SP→SP connections in current clamp using the perforated-patch clamp technique with gramicidin D, the majority of feedback connections (20/22) showed $\sim$60–70% short-term depression in current clamp (Fig. 6, C and D), whereas only 2 of 22 feedback connections showed mild augmentation ($<10\%$ at 60 Hz; Fig. 6, C and D). The average short-term depression in current clamp using the perforated-patch technique was about the same as in voltage clamp using whole cell patch recordings ($P > 0.6$). There was no difference in the $R_m$ for SP neurons in perforated patch compared with that in whole cell configuration, reaching on average $330 \pm 25$ MΩ at an average resting membrane potential of $-77.7 \pm 0.7$ mV ($n = 29$; $P > 0.99$). On the other hand, action potentials in SP neurons were $\sim50\%$ longer (1.84 ± 0.08 ms; $n = 29$; $P < 0.001$) and PSP amplitudes were $\sim45\%$ greater in the perforated-patch configuration (1.73 ± 0.27 mV; $n = 22$; $P < 0.05$). Additionally, we fit a biexponential function to the average PSP for each cell using the same approach we did for whole cell and found no significant difference in either $\tau$ rise (4.0 ± 0.5; median: 3.67, range: 2.0–9.8; $P > 0.80$) or $\tau$ decay (50.8 ± 4.2; median: 51.2, range: 11.4–82.8; $P > 0.10$). Last, there was no difference in the membrane time constant of the target SP neuron between whole cell and perforated-patch conditions (13.9 ± 2.4 vs. 12.1 ± 1.9 ms; $P > 0.2$).

These results show that both pathways exhibit significant short-term depression, both in voltage clamp and in current clamp, and that the homogeneity of SP→SP responses is strongly affected by the whole cell recording approach.

**Perforated-patch recordings confirm that synaptic inputs from SP neurons and FS interneurons are depolarizing at rest**

In the gramicidin D perforated-patch recordings, which leave the internal chloride concentration intact (Akaike 1996), feedback inputs to SP neurons were depolarizing at rest. Because $E_{Cl}$ changes with afferent input (Gulacsi et al. 2003), we therefore estimated $E_{Cl}$ in the presence of spontaneous up- and down-state transitions using gramicidin D perforated-patch recordings.

In the absence of DNQX, spontaneous activity in the triple cultures was characterized by irregular, low-frequency synaptic inputs during the down-state interrupted by up-states that lasted for 0.5–2 s and occurred at a frequency of 0.1–0.2 Hz,
as reported previously (Blackwell et al. 2003; Plenz and Kitai 1998b). During the down-state, spontaneous synaptic events were always depolarizing. Similarly, a burst of presynaptic action potentials in the feedback pathway elicited clear depolarizing synaptic events in postsynaptic SP neurons (Fig. 8A). We estimated the reversal potential for four SP→SP and one FS→SP connections from presynaptic action potential bursts delivered at different postsynaptic steady-state current injection levels. Synaptic responses were depolarizing during the down-state and decreased to zero at a membrane potential of –48 ± 3 mV (n = 5; range: –60 to –40 mV). We were unable to reverse these synaptic potentials. SP or FS inputs did not trigger action potentials, even when the membrane potential was held close to firing threshold (Fig. 8C).

Effect of depolarizing feedback inputs during the down-state on subsequent action potential firing

The depolarizing action of feedback inputs during the down-state might facilitate successive excitatory inputs; this mechanism has previously been suggested as highly efficient in the encoding of sequential activity (Plenz et al. 1996) and has been shown in cortical pyramidal neurons (Gulledge and Stuart 2003). More specifically with respect to striatal dynamics, we hypothesized that preceding GABAergic inputs in the down-state might advance action potential firing on a transition into the up-state.

Up-states, elicited by single-shock stimulation to the cortex cultures, were paired with a preceding burst of action potentials in a presynaptic SP neuron. The delay between up-state onset and first action potential ranged between 11 and 32 ms and was precise within 0.1–4 ms (n = 3; averages from 10 up-states each). There was no significant change in delay to the first action potential when up-state transitions were preceded by SP inputs (n = 3). Because the multiple, strong synaptic inputs that drive neurons into the up-state could have masked more subtle changes in action potential firing from single SP inputs, we repeated these experiments using suprathreshold somatic current injections to the postsynaptic neuron instead of cortical stimulation. These experiments using dual perforated-patch recordings were performed in the absence (n = 8) or presence (n = 8) of DNQX. No differences were found between both cases, and results were combined (Fig. 9). A preceding synaptic input delayed action potential onset significantly in 41% of connections tested (7/16), whereas the delay was reduced in 13% (2/16; Fig. 9). No effect was found in 7/16 cases. PSP amplitudes, which ranged from 0.05 to 1.3 mV, did not correlate with amplitude or directional changes in action potential onset (R = –0.05).

These results suggest that the depolarizing action of GABA during the down-state does not consistently change action potential timing during a subsequent suprathreshold depolarization.

DISCUSSION

This study provides a comparative analysis of feedforward and feedback inputs to SP neurons in the striatal microcircuit in organotypic cocultures. Our results show that synaptic transmission in response to single action potentials differs significantly between both pathways. On average, inputs from FS interneurons were about twice as strong and more reliable than inputs from SP neurons, although a subgroup of SP inputs had similar characteristics to FS inputs. We reconciled differences in short-term plasticity reported for both pathways in current- and voltage-clamp mode. Facilitation and augmentation re-
ported for SP inputs in current clamp is absent when the less invasive technique of perforated-patch recording with gramicidin D is used. Under these conditions, SP inputs become similar to FS inputs and show clear synaptic depression by $\pm50-70\%$ in both voltage and current clamp. Finally, our perforated-patch recordings, which left the internal chloride concentration unaltered, clearly showed that both feedforward and feedback inputs depolarize striatal neurons from rest, whether the striatal network is active, i.e., expresses up- and down-states, or is silenced by blocking fast glutamatergic transmission. The effect of such a synaptic depolarization on subsequent suprathreshold depolarization in SP neurons was tested for SP inputs. There was no consistent effect on subsequent action potential timing from preceding down-state inputs, which suggests that both pathways control striatal dynamics predominantly during the up-state.

Comparison of striatal synaptic transmission in cortex-striatum-substantia nigra cocultures to that in the acute striatal slice

Our combined approach of using both current- and voltage-clamp recordings allows for a detailed comparison of striatal

FIG. 5. FS inputs depress significantly in voltage clamp and in current clamp (whole cell recording). A: mean PSC time-course at FS $\rightarrow$ SP synapses for presynaptic action potential burst frequencies ranging from 5 to 60 Hz (population average from $n = 13$ connections; 10 action potentials at each frequency). B: accumulated charge transfer as a function of time for each frequency shown in A. All data normalized to charge transfer at 5 Hz. C: frequency-dependent decrease in total charge transfer (PSC; filled symbols) and total depolarization (PSP; open symbols). D: mean PSP time-course at FS $\rightarrow$ SP synapses for presynaptic bursting frequencies in current clamp (population average from $n = 4$ connections). E: net depolarization as a function of time for each frequency shown in D. All data normalized to net depolarization at 5 Hz. Voltage and current traces aligned to the response onset.
synaptic transmission in the cultures to that in the acute striatal slice. To compare PSP and PSC amplitudes, first the input resistance $R_{\text{in}}$ has to be taken into account. Our mean $R_{\text{in}}$ of 338 MΩ at $V_{m} = -78$ mV reported for cultured SP neurons compares well with the mean values in acute striatal slices from 3- to 5-wk-old rats under similar recording conditions ($R_{\text{in}} = 333–360$ MΩ) (Venance et al. 2004), but is slightly higher compared with other studies (200–360 MΩ; Taverna et al. 2004; <128 MΩ, Koos et al. 2004). It is ~40% lower than what we previously reported (Czubayko and Plenz 2002), which might reflect better culture health in this study.

Our mean amplitude of 1.48 ± 0.46 mV for SP connections at $E_{\text{Cl}} = -59$ mV is above the range of 0.5–1.1 mV estimated by Taverna et al. (2004) for an $R_{\text{in}}$ of 200 MΩ and $E_{\text{Cl}} = -71$ mV. While our mean PSC of $-26.5 \pm 5.4$ pA matches that reported for the acute slice at comparable resting potentials (Taverna et al. 2004; Venance et al. 2004), the use of a relatively high $E_{\text{Cl}}$ in acute slice studies tends to overestimate synaptic currents in these experiments. Accordingly, our mean conductance of 1.86 nS for SP connections is slightly above that in the acute slice (Taverna et al. 2004). These differences most likely reflect differences in the electrotonic compactness between SP neurons from the triple cultures and the acute slice. Cultured SP neurons have somewhat higher input resistances and lower spine densities, because of fewer corticostriatal inputs, compared with SP neurons in the acute slice. An electrotonically more compact neuron will make synaptic conductances and amplitudes of dendritic origin appear larger in somatic recordings. Accordingly, when potassium channels are blocked in SP neurons to make them more compact, the apparent conductance for SP inputs increases severalfold (Guzman et al. 2003; Koos et al. 2004). Higher PSC amplitudes could also arise from a higher incidence of multiple innervations, a higher GABA$_A$ receptor density within synapses, or a preferential somatic innervation of SP→SP connections in the organotypic culture compared with the acute slice.

FIG. 6. Feedback inputs depress significantly in voltage clamp (whole cell recording). A: mean PSC time-course at SP→SP synapses for presynaptic action potential burst frequencies as indicated (population average from $n = 7$ connections; 10 action potentials at each frequency). Current traces aligned to the response onset. B: accumulated charge transfer over time for each frequency shown in A in percentage of total charge transfer at 5 Hz. C: frequency-dependent decrease in total charge transfer (PSC) for SP→SP synapses.
A wide range in failure rate, from 0 to 90%, has previously been reported by our group (Czubayko and Plenz 2002) and was also found in this study in voltage-clamp recordings for feedback connections. Compared with our coculture system, failure rates in acute slices seem to be slightly lower (Koos et al. 2004; Taverna et al. 2004; Tunstall et al. 2002; Venance et al. 2004). Similarly, no electrical coupling was found between spiny projection neurons in contrast to some reports in acute slices (Czubayko and Plenz 2002; Venance et al. 2004; but see Taverna et al. 2004). Dopamine has been shown to change GABA-evoked currents, GABAergic synaptic amplitudes, and paired-pulse responses (Flores-Hernandez et al. 2000; Guzman et al. 2003; Taverna et al. 2005) and to reduce electrical coupling (O’Donnell and Grace 1993). These differences between the cultures and the acute slice might originate from a higher basal level of dopamine in the cortex-striatum-substantia nigra cocultures (Becq et al. 1999; Plenz and Kitai 1998a) compared with the acute slice, in which spontaneous release of dopamine is affected.

Our results on FS interneuron inputs to SP neurons in organotypic cultures are largely comparable with those reported in acute striatal slices. Early reports for both preparations in current-clamp mode and using sharp intracellular recordings showed that FS inputs display large amplitudes, low failure rate, and short-term depression (Koos and Tepper 1999; Plenz and Kitai 1998b). The relatively high PSC amplitude, peak conductance, and lower failure rate compared with feedback connections compares well with a more recent study in the acute slice (Koos et al. 2004).

A consistent finding was the large overlap in synaptic parameters between both pathways. Such overlap makes the attribution of distinct functions for either pathway difficult. The synaptic variability, which for feedforward inputs is already established at the single neuron level, could be solely a characteristic of the culture system. However, this seems unlikely given a similar wide range in values with 10- to 20-fold differences reported in acute slice studies (Koos et al. 2004; Taverna et al. 2004; Venance et al. 2004). Thus while, on average, FS inputs are stronger and more reliable than SP inputs, there is a subpopulation of SP inputs that is as strong as FS inputs, which might represent feedback inputs that target the soma (Smith and Bolam 1990; Wilson and Groves 1980). This interpretation is supported by the finding that the quantal currents for both pathways are similar (Koos et al. 2004).

A robust finding in our study was the early arrival of FS inputs compared with SP inputs. Based on their earlier onset in slope and PSC peak amplitudes, most FS inputs occurred ~1 ms earlier than SP inputs. Because synaptic delays were measured from the peak of the presynaptic action potential, differences in action potential waveform between FS and SP neurons will only marginally affect the estimate of synaptic onset. The difference in synaptic onset could originate from differences in axonal path length and/or conduction velocity between FS→SP connections and SP→SP connections. However, there were no obvious differences in the spatial distance...
between simultaneously recorded pairs of SP neurons or SP and FS neurons. Similarly, different synaptic release properties between the two synaptic pathways (e.g., Hefft and Jonas 2005) could underlie the observed differences in synaptic onset. We suggest that the difference in synaptic onset between FS and SP inputs at least partially originates from a difference in the postsynaptic target innervation. The early arrival of FS inputs is in line with the idea that FS inputs preferentially innervate the soma of SP neurons. Conversely, the late arrival for most SP neurons is in line with the anatomical finding that SP input innervate SP neuron dendrites. These differences in anatomical innervation do not seem to be absolute. For example, we found several SP inputs as strong as FS inputs with respect to their PSC amplitudes and low failure rate. Such SP inputs could reflect multiple synaptic innervations at the SP soma, which has been recently shown at the electronmicroscopic level (Oorschot et al. 2004).

Feedforward and feedback pathways showed significant short-term depression in voltage and current clamp. The depression reported in voltage clamp in this study was slightly larger for SP inputs and compares well with the depression for single burst trials analyzed in the acute slice at high spike frequencies by Koos et al. (2004). While we could repeat our previous findings (Czubayko and Plenz 2002) and those of others (Taverna et al. 2004) regarding short-term facilitation for feedback connections in current clamp using the whole cell patch configuration, the short-term plasticity turned into short-term depression only when the less invasive perforated patch method was used. We suspect that synapses at varying distances from the soma are exposed differently to the relatively invasive approach of the whole cell preparation leading to the heterogeneity observed. Given that we found no differences in the PSP kinetics, as parameterized by \( \tau_{\text{rise}} \) and \( \tau_{\text{decay}} \), results in our preparation suggest differences arose because of either desensitization or adversely affected signaling cascades. Our results on short-term plasticity provide a coherent picture for both pathways in which short-term depression dominates during action potential burst transmission.

**FIG. 8.** SP and FS inputs are depolarizing during the down-state and reveal an estimated reversal potential \( E_{\text{Cl}} \) close to action potential firing threshold. A: simultaneous recording of spontaneous up-state activity in 2 SP neurons (SP1, SP2) in cortex- striatum-substantia nigra cocultures in gramicidin D perforated-patch configuration. A presynaptic action potential burst in SP1 (20 Hz) results in a depolarizing PSP during the down-state in SF2 (arrow). Note synchronous, spontaneous up-states in both neurons and spontaneous action potential firing in the SP2 neuron during the up-state. B: using steady-state current injections, postsynaptic responses to presynaptic action potential bursts (10 action potentials) reduce in amplitude as the membrane potential approaches the action potential threshold (single SP→SP connection; \( n = 5 \) averages each taken during down-state periods). C: summary of mean PSP amplitude at indicated postsynaptic membrane potentials for SP→SP (○) and FS→SP connections (○). Notice disappearance of response at depolarized potentials close to action potential threshold. □, corresponding action potential threshold in SP neurons obtained from spontaneous action potential firing during up-states.
In summary, results in synaptic transmission for both pathways are highly comparable with those obtained in the acute striatal slice, with FS inputs on average being larger in amplitude and more reliable than SP inputs.

**Functional considerations for both pathways in the striatal microcircuit**

Our results led us to the conclusion that both pathways will exert their main control on striatal dynamics during the up-state in a time critical manner. First, the reversal potential for both inputs reported in this study is close to the membrane potential range during the up-state. This value is in accordance with earlier reports using sharp-intracellular recordings (for discussion see Plenz 2003). Thus during the up-state, the temporal dynamics of both pathways is largely described by a fast shunting operation through their respective synaptic currents that decay within ~6 ms. Second, given that both pathways also reveal pronounced short-term depression, which accentuates the temporal aspect of input changes (Abbott et al. 1997; Fuhrmann et al. 2002; Thomson 2000), the striatal microcircuit is well posed for a time-critical processing of synaptic inputs. It has been shown that FS inputs can change the timing of a somatic action potential in the postsynaptic neuron (Koos and Tepper 1999). While such a mode of action is possible for some SP→SP connections, the role of more distal, dendritic SP→SP connections is unclear. Recently, somatic action potentials have been shown to control intracellular calcium transients in striatal SP neurons in distal dendrites through spike backpropagation (Kerr and Plenz 2002). It was also shown that a delay in single action potential timing inversely correlates with dendritic calcium transients through the N-methyl-D-aspartate (NMDA) receptor during an up-state (Kerr and Plenz 2004). While our study shows that some SP inputs were able to change the onset of action potential firing on depolarization from the down-state, the effect was not consistent, which makes functional interpretations difficult. On the other hand, SP inputs at the rather small SP dendrites could control the temporally precise backpropagation of action potentials into SP neuron dendrites (Plenz 2003). At cortical synapses, the coincidence between glutamate bound to the NMDA receptor and a back-propagating action potential controls spike-time dependent plasticity (STDP) (Stuart and Hausser 2001). Similarly, GABAergic inputs to SP neurons, by delaying or advancing action potential firing, might regulate long-term plasticity at corticostriatal synapses based on some form of STDP. Previous experiments that showed the induction of long-term potentia-
tion (LTP) (Kerr and Wickens 2001) or long-term depression (LTD) (Calabresi et al. 1992) in the absence of fast inhibition do not rule out the idea for the involvement of GABAergic inputs in corticostriatal plasticity. Kerr and Wickens (2001) induced LTD in a magnesium-free solution, which abolishes the voltage-sensitive component of the NMDA receptor and thus part of the time critical component in STDP. Calabresi et al. (1992) induced LTD through high-frequency stimulations combined with suprathreshold postsynaptic steady-state current injections, which facilitates uncorrelated action potential firing, a condition that favors LTD under STDP (Feldman 2000). Therefore, feedback connections might primarily control future striatal processing of cortical inputs by regulating corticostriatal plasticity instead of changing the current striatal control of basal ganglia output.

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