Direct Examination of Local Regulation of Membrane Activity in Striatal and Prefrontal Cortical Neurons in Vivo Using Simultaneous Intracellular Recording and Microdialysis

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ABSTRACT

Slice preparations are typically used to study the effects of pharmacological manipulations on the electrophysiological activity of mature neurons. However, the severing of afferent inputs is known to significantly change the natural membrane activity of the neuron. To study the effects of local pharmacological manipulations on neurons in the intact brain, we combined the methods of microdialysis and intracellular recording in vivo. After implantation of a microdialysis probe into the prefrontal cortex (PFC) or striatum, intracellular recordings were conducted within −500 μm of the active surface of the probe. The spontaneous membrane activity, passive membrane properties, and intracellularly and synaptically evoked responses of striatal and cortical neurons recorded during perfusion of artificial cerebral spinal fluid were not different from that of neurons recorded in intact animals. Moreover, in the PFC, local perfusion with glutamate or N-methyl-D-aspartate depolarized neurons and increased spike activity. Conversely, local perfusion of tetrodotoxin hyperpolarized neurons while markedly reducing spontaneous membrane depolarizations and eliminating spike activity. In the striatum, local perfusion of the γ-aminobutyric acidA receptor antagonist bicuculline rapidly depolarized neurons and increased spontaneous spike activity. Given that striatal and PFC neurons recorded in animals undergoing microdialysis in the current study exhibited electrophysiological properties similar to those recorded in intact controls, it is likely that the effects of local microdialysis on ongoing synaptic activity, neuronal excitability, and endogenous neurotransmitter levels are minimal. We conclude that the use of local microdialysis in intracellular recording is a powerful method for studying local receptor regulation of synaptic activity in vivo.

Recent studies directed at understanding the influence of network events on neuronal membrane properties in the central nervous system have, in large part, been carried out using in vitro preparations. Although these isolated preparations are useful for studying the synaptic pharmacology and membrane biophysics of neurochemically and/or visually identified neurons, extrapolation of observations made in vitro to the intact adult system is often problematic. In addition to the potential caveats related to the impact of the specific physical-chemical conditions used in the in vitro preparation on the viability or membrane biophysics of neurons, the disconnection of the neuron from its extrinsic inputs can have a significant impact on the steady-state properties of the neuronal membrane. For example, spiny projection neurons recorded in vivo in the cortex or striatal complex often exhibit characteristic shifts in membrane potential consisting of “up” (depolarized plateau potential between −65 and −48 mV) and “down” (resting potential between −88 and −75 mV) states (Steriade et al., 1993a,b; Wilson, 1993; O’Donnell and Grace, 1995; Wilson and Kawaguchi, 1996; Paré et al., 1998a,b; Onn and Grace, 1999, 2000; West and Grace, 2002). In both the cerebral cortex and the striatum, the up state is driven by glutamatergic inputs (Mahon et al., 2001). In the striatum, neocortical (dorsal striatum; Wilson, 1993; Mahon et al., 2001) and allocortical (ventral striatum; O’Donnell and Grace, 1995) and/or thalamic (Wilson, 1993) afferents seem to interact to produce the up state. In the cortex the up state is primarily dependent upon synchronous activation of cortico-cortical synaptic connections (Steriade et al., 1993b; Cowan and Wilson, 1994; Amzica and Steriade, 1995, 1996; Silberstein, 1995). Consistent with these findings, striatal and cortical neurons recorded in vitro in brain slices do not exhibit bistable membrane activity (Nicolai et al., 2000; Lavin and Grace, 2001). Paré and colleagues have further shown that, in addition to being necessary for spontaneous

ABBREVIATIONS: aCSF, artificial cerebrospinal fluid; GABA, γ-aminobutyric acid; TTX, tetrodotoxin; BIC, bicuculline; NMDA, N-methyl-D-aspartate; PFC, prefrontal cortex; PB, phosphate buffer; PBS, phosphate-buffered saline; TBS, Tris-buffered saline; EPSP, excitatory postsynaptic potential; IR, input resistance.
action potential discharge, the tonic activity of synaptic inputs to cortical pyramidal neurons in vivo is sufficient to maintain a membrane input resistance significantly lower than that observed in vitro, consequently altering other “passive” membrane properties such as excitability (Paré et al., 1998b). Together, these studies indicate that the synaptic activity occurring throughout the dendritic tree of the spiny neurons of the cortex and striatum not only sets the natural firing pattern of these neurons but also has a significant impact on the response of the neuronal membrane to the activation of ligand-gated ion channels. At present, however, little is known about the influence of local neurotransmitters or synaptic activity on the steady-state membrane properties of spiny neurons in vivo.

Given the above-mentioned information, it is likely that the influence of a specific local receptor population on neuronal excitability and spontaneous activity will depend on the ongoing activity within circuits that provide synaptic inputs to the neuron. Thus, studies investigating the influence of local neurotransmitter interactions on neuronal activity in intact systems are critical for understanding the influence of network events on the membrane properties of spiny neurons, as well as the modulation of their activity by local receptor stimulation. Toward this end, the current study was undertaken to determine the viability of the use of local microdialysis for temporally and spatially controlled delivery of pharmacological agents during intracellular recordings in vivo. The potential impact of the microdialysis procedure on the membrane properties and synaptic responses of striatal and cortical neurons was assessed by comparing recordings made in control animals (no probe) to recordings from neurons located within 500 μm of a microdialysis probe continuously perfused with artificial cerebral spinal fluid (aCSF). To test the validity of combining the techniques of intracellular recording and microdialysis in vivo, the influence of local pharmacological manipulations of glutamatergic and GABA-ergic receptors on the membrane properties of cortical or striatal neurons was examined. The effect of eliminating the majority of synaptic input via local perfusion of tetrodotoxin (TTX) on the spontaneous activity and membrane properties of cortical neurons was also assessed.

Experimental Procedures

Materials. Dulbecco’s phosphate-buffered saline, N-methyl-D-aspartate (NMDA), glutamic acid, and tetrodotoxin (TTX) were purchased from Sigma-Aldrich (St. Louis, MO). (−)-Bicuculline (BIC) methohchloride was purchased from Sigma/RBI (Natick, MA). d-Glucose was purchased from Fisher Scientific (Springfield, NJ). All other reagents were of the highest grade commercially available.

Subjects and Surgery. Male Sprague-Dawley or Fisher 344 rats (Hilltop, Scottsdale, PA) weighing 275 to 450 g were used. Before experimentation, animals were housed two per cage under conditions of constant temperature (21–23°C) and maintained on a 12-h light/dark cycle with food and water available ad libitum. All animal procedures were approved by the University of Pittsburgh Institutional Animal Care and Use Committee and adhered to the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health. Additionally, all efforts were made to minimize the number of animals used and their suffering. Before surgery, animals were deeply anesthetized with chloral hydrate (400 mg/kg i.p.) and placed in a stereotaxic apparatus (Narishige, Tokyo, Japan or David Kopf Instruments, Tujunga, CA). The level of anesthesia was periodically verified via the hind limb compression reflex and maintained using supplemental administration of chloral hydrate (80 mg/ml) via a lateral tail vein (approximately 0.2 ml/0.5 h). Temperature was monitored using a rectal probe and maintained at 36–37°C with a heating pad (Fintronics, Orange, CT).

After drilling a burr hole (−2–3 mm in diameter) over the dorsal striatum (coordinates: 0.5–2.0 mm anterior from bregma, 2.0–3.5 mm lateral from the midline) or prefrontal cortex (PFC) (coordinate: 2.7–3.7 mm anterior from bregma, 0.5 to 1.2 mm lateral from the midline), the dura was resected and the cortical surface exposed. A concentric microdialysis probe having 2 (PFC) or 4 (striatum) mm of exposed membrane (320 or 450 μm in diameter, ∼6000 molecular weight cutoff; Bioanalytical Systems, West Lafayette, IN or CMA/Microdialysis, Natick, MA) was then lowered with a micromanipulator (Narishige or David Kopf Instruments) at 3 to 6 μm/s (Fig. 1, a and d). Once the probe reached the targeted region of the PFC (3.5–4.5 mm ventral to brain surface) or the striatum (5.5 or 6.5 mm ventral), it was fixed with dental cement (Kerr, Romulus, MI) to a screw positioned in the skull or remained fixed in the stereotactic carrier for the remainder of the experiment. After implantation, the probe was perfused with aCSF containing 136.9 mM NaCl, 2.7 mM KCl, 0.5 mM MgCl2, 0.9 mM CaCl2, 1.47 mM KPO4, and 8.1 mM Na2HPO4 (Dulbecco’s phosphate-buffered saline), and 10.0 mM d-glucose at a rate of 2 μl/min using a microperfusion pump (Baby Bee; Bioanalytical Systems).

Intracellular Recordings. Intracellular electrodes were pulled from 1.0-mm-o.d. borosilicate glass tubing (World Precision Instruments, Sarasota, FL) using a Flaming-Brown P-80/PC electrode puller. Microelectrodes were filled with potassium acetate (2–3 M) solution containing 2% biocytin using a nonmetallic Microfil syringe needle. Intracellular electrodes used for cortical recordings had impedances of 50 to 90 MΩ, whereas electrodes used for striatal recordings ranged from 30 to 100 MΩ as measured in situ. Electrode potentials were amplified via a headstage connected to a Neurodata IR-183 intracellular preamplifier (Cygnus Technology, Delaware Water Gap, PA). Intracellular current was injected via an active bridge circuit integral to the preamplifier. Continuous or event-triggered collection of data of both voltage and current signals from the amplifier were digitized and stored onto a PC via a Microstar data acquisition board interface (Microstar Laboratories, Bellevue, WA) controlled by custom software (Neuroscope; Brian Lowry, Pittsburgh, PA). Output from the amplifier was simultaneously monitored on a Philips PM3337 storage oscilloscope (Fluka, Eindhoven, The Netherlands), digitized (NeuroData NeuroCorder DR 390; Cygnus Technology), and stored on videotape. Cell penetrations were defined as stable when the cells exhibited a resting membrane potential of at least −55 mV; discharged action potentials having amplitudes of at least 45 mV (range 48–82 mV), a positive overshoot; and fired trains of spikes after membrane depolarization. Data were collected for cells defined as stable when these electrophysiological properties were maintained for a minimum period of 5 min. After experimental manipulations, neurons were injected (−10–60 min) with biocytin using a train of depolarizing current injection pulses (∼0.5 nA, 300 ms, 2 Hz).

Electrical Stimulation. In striatal experiments, twisted-pair bipolar stimulating electrodes (Plastics One, Roanoke, VA) were implanted into the orbital prefrontal cortex (coordinates: 3.7–4.7 mm anterior to bregma, 0.2–2.3 mm lateral to midline, 2.5–4.0 mm ventral to brain surface) ipsilateral to the recording electrode. Stimulation sites in the medial, ventral, and ventrolateral orbital PFC were selected based on the results of striatal retrograde and anterograde tracing studies (Deniau et al., 1996). Single pulses or pairs of electrical stimuli (100-ms interspike interval) with durations of 200 to 250 μs and intensities between 0.1 and 5.0 mA were generated using an S88 stimulator (Grass Instruments, Quincy, MA) and photoelectric constant current/stimulus isolation unit (PSIU6P; Grass Instruments) and delivered at a frequency of 0.2 Hz.
Procedure for Intracellular Recording during Local Pharmacological Manipulations via Microdialysis.

Electrophysiological recordings were initiated approximately 2 to 4 h after probe implantation (Fig. 1). Electrode tips were positioned to enter the brain surface approximately 1 mm lateral or caudal to the probe, and angled at 10° toward the probe. The electrode was then lowered and neurons were impaled at coordinates lying within 500 μm of the active membrane of the probe. After impaling a neuron, the neuron was allowed to stabilize for several minutes until synaptic and/or spike activity reached a steady state. Baseline synaptic activity was then recorded for at least 5 min after which the effects of intracellular injection of hyperpolarizing and depolarizing currents were determined. After steady-state activity and membrane properties were recorded, the aCSF perfused through the probe was switched to a drug-containing solution (500 μM glutamate, 200 μM NMDA, 100 μM BIC, or 10 μM TTX) using a zero dead-volume liquid switch (CMA/Microdialysis or Bioanalytical Systems). Due to the recovery of the probes, the concentration of drug in the tissue immediately adjacent to the probe was estimated to be approximately 10% (for 2-mm probes) or 25% (for 4-mm probes) of the concentration in the perfusion fluid, and substantially less at the soma of the neuron being recorded. Time was allowed for the drug to reach the active surface...
of the probe (dead volume, 6 to 12 μl; time, 3–6 min), after which spontaneous activity and the effects of intracellular current injection were recorded during perfusion of the drug. Effective doses of BIC, TTX, glutamate, and NMDA were derived from previous studies (Karreman and Moghaddam, 1996; West and Galloway, 1997) and were soluble in aCSF.

**Data Analysis.** Changes in neuronal membrane properties, synaptic activity, and spike activity were analyzed using custom software (Neuroscope; Brian Lowry). Resting membrane properties and spike characteristics (Tables 1 and 2) were determined for control neurons (no probe) and for neurons proximal to the dialysis probes before and after addition of a drug to the perfusion fluid. Baseline resting membrane potential, input resistance, and current threshold were determined from 30- to 60-s epochs taken from traces occurring after at least 5 min of stable recording and not more than 7 min before the onset of drug perfusion. Input resistance of each neuron in the “down” membrane potential state was calculated by injecting a voltage midway between the spike threshold and peak. For striatal cells, spike duration was measured from the spike threshold to the point where the falling phase of the action potential returned to the membrane potential at spike threshold. To maximize our power to detect differences between neurons in control and perfused animals, individual Student’s t tests were conducted on each dependent variable. Potential effects of the microdialysis procedure on the proportion of spontaneously active neurons were assessed via comparisons between control and probe groups using the Fisher’s exact test.

**Histology.** After experimentation, animals were deeply anesthetized and perfused transcardially with ice-cold saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4. Brains were postfixed in 4% paraformaldehyde in PB for 1 to 7 days then cryoprotected with 25% sucrose in PB. Brains were then sectioned into 60- to 80-μm coronal slices and processed to reveal biocytin using a standard, commercially available avidin/biotin procedure kit (Vector Laboratories, Burlingame, CA). Specifically, sections were washed 2 × 5 min in PB, 10 min in 0.2% hydrogen peroxide in PB, 2 × 5 min in PB, and 1 × 5 min in 0.2% Triton X in 0.1% phosphate-buffered saline (PBS). After these rinses sections were incubated for 1 to 10 h in the avidin/biotinylated horseradish peroxidase solution (VECTASTAIN Elite ABC at 1 drop each of reagent A and B per 5 ml of 0.2% Triton in PBS). Sections were then rinsed 2 × 5 min in PBS and 3 × 5 min in 0.05 M Tris-buffered saline (TBS, pH 7.6) and incubated in 0.4% diaminobenzidine-4 HCl/3% nickel ammonium sulfate in TBS for 10 min alone and 10 additional minutes with hydrogen peroxide added (0.05–0.1%). The reaction was quenched with TBS (3 × 5 min). Sections were mounted from water onto gelatin-coated glass slides. After drying, sections were counterstained with a mixture of neutral red/cresyl violet (8:1), dehydrated, and coverslipped. Neurons were identified as striatal medium spiny or cortical pyramidal on the basis of the morphology of the dendrites and were confirmed to lie within 500 μm of the probe track. In brains in which neurons were not filled or recovered, the recording location relative to the probe track was estimated from the 3,5-diaminoben-
zidine tetrachloride product that had reacted with the small amounts of blood adjacent to the electrode and microdialysis probe tracks.

**Results**

In vivo intracellular recordings were made from 29 striatal neurons in 26 rats and 27 cortical neurons in 25 rats (total = 51 rats).

**Electrode and Microdialysis Probe Placement.** For striatal recordings, all stimulating electrode tips implanted into the cortex were confirmed to lie in the PFC between 3.2 and 4.7 mm anterior to bregma, 0.4 and 2.2 mm lateral to the midline, and 2.8 and 4.3 mm ventral to the dural surface (Paxinos and Watson, 1986). All dialysis probe tips were confirmed to lie within the dorsal striatum between 0.1 mm posterior and 1.7 mm anterior to bregma, 2.0 and 4.5 mm lateral to the midline, and 5.0 and 7.7 mm ventral to the dural surface (Paxinos and Watson, 1986). The majority of recording electrode tracks was observed to pass and terminate within 500 μm of the observed probe track (Fig. 1d).

Three biocytin-filled neurons were identified in the central and dorsolateral striatum proximal to the dialysis probe track (Fig. 1e). A fourth biocytin-filled neuron was recovered in a control animal. Three of these neurons were unequivocally identified as medium-sized spiny neurons; the remaining neuron exhibited morphological and physiological characteristics consistent with medium spiny cells.

For PFC recordings in control rats, electrode tracks were confirmed to terminate and/or biocytin-filled cells were observed to lie within the prelimbic, infralimbic, or ventral orbital cortex according to the atlas of Paxinos and Watson (1986). In dialyzed rats, the tracks of the dialyzed portion of the probe lay, on average, at 10° anterior-to-posterior angle, such that the dorsal end of the dialysis membrane lay in the dorsal medial orbital cortex (3.8–4.0 mm anterior to bregma, 0.5–0.9 mm lateral to the midline, and 1.5–2.0 mm ventral to the brain surface) and the ventral tip lay in the prelimbic or infralimbic cortex (2.7–3.2 mm anterior to bregma, 0.5–0.9 mm lateral to the midline, and 3.0–5.0 mm ventral to the brain surface; Fig. 1). The majority of the recording electrode tracks and/or biocytin-filled neurons seemed to lie within 500 μm of the dialysis probe membrane, with none extending beyond 1000 μm of the probe track. Five biocytin-filled neurons were identified as pyramidal neurons in the prelimbic or infralimbic cortex lying within 500 μm of the probe track (Fig. 1b). Two neurons were identified as pyramidal-like spiny neurons lying within the medial/ventral orbital or dorsal peduncular cortex (Paxinos and Watson, 1986).

**Electrophysiological Properties and Spontaneous Activity of Striatal and Cortical Neurons Recorded in Tissue Perfused by a Microdialysis Probe.** For all recordings, qualitative factors that had the greatest impact on the viability of the neurons were the rate at which the microdialysis probe had been implanted, the duration of the equilibration period, and the overall health of the animal while under anesthesia. Lowering the probe at a rate greater than 500 μm/min markedly decreased the probability of finding a healthy neuron proximal to the probe. The minimum equilibration period seemed to be approximately 2 h, with most recordings occurring more than 3 h after probe insertion. In addition, recording stability was particularly susceptible to the deleterious effects of increases in body temperature or difficulties in respiration. These problems were minimized with a combination of injections of saline through...
the tail vein, hydrating the air around the snout, and holding the body temperature at 36°C.

In both the PFC (Table 1) and striatum (Table 2) the membrane properties, including resting membrane potential, input resistance, and current threshold, did not differ between neurons recorded from dialyzed or control rats. Moreover, characteristics of spontaneous and/or current-evoked action potentials were similar between control and dialyzed rats (Tables 1 and 2). In both control and PFC-dialyzed subjects, 40% of PFC neurons exhibited spontaneous spike firing. Moreover, the average firing rate of spontaneously active neurons did not differ between groups (Table 1). Neurons in both control and PFC-dialyzed rats displayed spontaneous shifts from resting potentials (−75 ± 1.7 mV) to a plateau potential 7 to 14 mV more depolarized than the resting state (Fig. 2a). Membrane responses to intracellular current injection in neurons recorded proximal to the dialysis probe were also similar to those observed in the intact (control) animal (Fig. 2b).

In the striatum, the majority of recorded neurons in both control (7 of 12 cells) and dialyzed (12 of 17 cells; p > 0.05) groups was not spontaneously active. In spontaneously active neurons, the basal firing rate was generally low (<0.5 Hz, range 0–2.6 Hz; Table 2) and did not differ between control and probe groups (p > 0.05; Table 2). Striatal neurons in both control and probe groups often exhibited spontaneous shifts in membrane potential from a hyperpolarized state to a depolarized plateau (Fig. 3a), as indicated by the bimodal distribution of membrane potentials over time (Fig. 3b). In neurons from both control and dialyzed rats, the membrane response to depolarizing current injection consisted of a graded depolarization from which the action potential was initiated. A prominent after-hyperpolarization typically followed the action potential (Fig. 3c).

**Synaptically Evoked Activity in Striatal Neurons.** In striatal neurons recorded in both control and probe groups, postsynaptic potentials and, in some cases, spikes could be evoked by single pulses or pairs of electrical stimuli delivered to the PFC (Fig. 4). To compare the effects of PFC stimulation on cells from control and probe groups, a series of single pulses (0.2 Hz) of electrical stimuli were delivered at gradually increasing stimulus intensities (0.1–3.0 mA). For all cells, the first excitatory postsynaptic potential (EPSP) elicited by PFC stimulation having an amplitude of at least 10 mV was analyzed. Statistical analyses of recordings from cells in control and probe groups revealed no significant differences in the average membrane potential before electrical stimulation, EPSP onset latency, amplitude, duration, or current intensity required to evoke an EPSP greater than 10 mV in amplitude (Table 3).

**Pharmacological Manipulations.** Perfusion of TTX into the PFC increased membrane input resistance from an average of 27 ± 12 to 71 ± 10 mΩ (mean ± S.E.M.), abolished spike activity, and markedly reduced spontaneous subthreshold depolarizations, resulting in an average membrane potential similar to that of the down state (n = 2; Fig. 5). In
contrast, seven of seven PFC neurons showed a depolarization of the membrane and increase in spontaneous or current-evoked spike activity during perfusion of glutamate or NMDA via the probe (Fig. 6). In some cases, when cells could be held for a sufficient period \((n = 2)\), the excitatory effects of glutamate or NMDA were observed to wash out after reperfusion with ACSF (Fig. 6b). Interestingly, NMDA had variable effects on input resistance \((\text{IR with ACSF} = 34.8 \pm 11.2 \, \text{mO}, \text{IR with NMDA} = 26.6 \pm 7.5 \, \text{mO}; \text{mean} \pm \text{S.E.M.})\), seeming to have less of an effect on neurons with high \((> 50)\) input resistances. Nonetheless, NMDA significantly decreased current threshold and increased the number of spikes evoked per unit of depolarizing current injected intracellularly (Fig. 6).

In the striatum, the influence of local tonic GABA_\text{A} receptor activation was assessed by recording from neurons during perfusion of ACSF and after addition of BIC to the ACSF. Intrastriatal BIC \((100 \, \mu\text{M})\) infusion depolarized the membrane potential of single striatal neurons and in some cases induced spontaneous spiking activity (Fig. 7a). Comparisons of time histograms of the membrane potential constructed from the same neuron recorded during separate periods of ACSF and BIC infusion revealed an overall depolarizing shift in the minimum, maximum, and average membrane potential, revealed as a rightward shift in the time spent at a given membrane potential (Fig. 7b). The average membrane potential of striatal neurons was also significantly more depolarized after 5 to 6 min of intrastriatal BIC infusion (Fig. 7c; \(n = 4, p < 0.05, \text{paired} \, t \text{test})\).

**Discussion**

In this study, cortical and striatal neurons located in proximity to a microdialysis probe were found to exhibit passive membrane properties and intracellularly and synaptically evoked responses indistinguishable from neurons recorded without microdialysis. In most cases, the neurons were estimated to be located less than 500 \(\mu\text{m}\) from the dialysis probe. The responsiveness of the spiny neurons to local pharmacological manipulations suggested that the dendritic fields...
and/or somata of the neurons were within the perfusion volume of the microdialysis probe. Additionally, because the majority of neurons labeled in this study exhibited large dendritic fields that extended into the neuropil for hundreds of micrometers, it is likely that the above-estimated distance that a dialyzed drug would need to diffuse to influence a given neuron is a conservative one.

**Influence of Local Microdialysis on Spontaneous and Evoked Activity.** Implementation of this technology also revealed for the first time essential information regarding the state of neurons and neural tissue proximal to the microdialysis probe. Thus, cortical and striatal neurons recorded in control subjects versus animals undergoing the microdialysis procedure described herein demonstrate no significant differences in multiple measures of passive membrane properties, spontaneous activity, or intracellular current and synaptically evoked responses. These observations are of significant interest for the interpretation of microdialysis studies monitoring extracellular neurotransmitters levels, particularly when considered in the light of recent concerns raised regarding the functional state of the tissue surrounding the dialysis probe. Specifically, it has been suggested that local microdialysis traumatizes the tissue and creates a functional dead space around the probe (Lu et al., 1998), which may extend for several hundred micrometers. Although previous studies have explored the impact of microdialysis procedures on the regulation of neurotransmitter efflux (DeBeor and Abercrombie, 1996; cf. Moore et al., 1996) and used reverse microdialysis or micropipette infusions to deliver drugs locally near an intracellular recording electrode (Paré et al., 1998a,b; Castro-Alamancos, 2000), the present study is the first to examine the impact of microdialysis on the membrane activity of neurons in the vicinity of the probe. If the probe implantation or dialysis procedure had severely disrupted synaptic activity either mechanically or by altering the diffusion of locally released neurotransmitters, it is likely that significant differences in natural membrane activity, input resistance, and spike characteristics of neurons recorded proximal to the probe would have been observed compared with intact controls (Wilson, 1993; O’Donnell and Grace, 1995). Moreover, the presence of the bistable membrane potential pattern in cortical and striatal neurons, as well as their responses to afferent stimulation and TTX, showed that synaptic inputs to these neurons remained intact in the presence of the dialysis probe. Given that stable neurons recorded in animals undergoing microdialysis in the current study (some of which were located less than 50 μm from the dialysis probe) exhibited electrophysiological properties similar to those recorded in intact controls, it is likely that the effects of local microdialysis on ongoing synaptic activity and neuronal excitability are minimal. Additionally, the finding that reverse dialysis of the GABA_A receptor an-

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*Fig. 5. Effect of local perfusion of TTX on PFC neuron physiology. a, left, spontaneous activity exhibited by the neuron during aCSF perfusion. This intrinsically bursting neuron displayed characteristic up and down states with action potentials that discharged during the up state. Inset, at a faster time base, it can be seen that the burst-firing pattern is characterized by spikes with gradually decreasing amplitudes, similar to that reported previously by Steriade et al. (1993a). Right, proportion of time (30-s epoch) that the neuron spent at a given membrane potential. The bimodal distribution indicates that the membrane potential was distributed primarily between two modes, one at approximately −70 mV, the other at −61 mV. b, left, membrane activity during reverse dialysis of TTX (5 min). After the addition of TTX (10 μM) to the aCSF, the bistable membrane potential pattern was depressed and action potentials were eliminated. Instead, the neuron rested in a hyperpolarized state. Right, time-membrane potential histogram shows that in the presence of TTX, the membrane potential is shifted to the left (hyperpolarized) and is distributed around a single mode at approximately −81 mV. c, left, membrane activity after 10 min of reverse dialysis of TTX. The membrane potential hyperpolarized further and depolarized plateau potentials were nearly absent. Right, time-membrane potential histogram shows that after 10 min of TTX infusion, the membrane potential had become more hyperpolarized and unimodal.*
tagonist BIC depolarized striatal neurons indicates that the neuron is receiving tonic GABA-ergic stimulation and, therefore, the dialysis procedure does not significantly deplete via washout endogenous neurotransmitter surrounding the recorded neuron. This observation is also supported by our previous studies demonstrating that intrastriatal infusion of dopamine D1 and D2 receptor antagonists decrease and increase, respectively, the excitability of striatal neurons recorded proximal to the dialysis probe (West and Grace, 2002). Moreover, the effects of DA antagonists were observed in both within- and between-subject experiments, indicating that the duration of microdialysis and other time effects did not influence the responsiveness of the neuron to local drug infusion (West and Grace, 2002). Our previous studies showing that striatal microdialysis does not alter the striatonicgiral efferent regulation of midbrain dopamine cells (West and Grace, 2000) also suggests that the activity of striatal output neurons is not significantly affected by the microdialysis procedure. These data demonstrate that, provided probe implantation is done with sufficient care, the neuronal environment proximal to the probe is not severely disrupted.

**Responsiveness of Frontal Cortical and Striatal Spiny Neurons to Local Pharmacological Manipulations.** In the present study, local pharmacological manipulations executed using reverse dialysis showed that in vivo, the “resting” membrane characteristics and spontaneous patterns of membrane activity are determined in large part by afferent activity. The effects of local perfusion of TTX in the PFC observed in the present study are similar to the findings of Paré et al. (1998b), demonstrating that cortical neurons recorded in vivo in the presence of TTX showed an increase in input resistance and hyperpolarization of the membrane. This is also consistent with previous studies showing that neurons recorded in slice preparations have significantly higher input resistances and more hyperpolarized resting membrane potentials than those recorded in vivo (Paré et al., 1998b). These studies indicate that excitatory afferent activity in the cortex normally provides a tonic level of membrane conductance in the pyramidal neuron; functionally maintaining a relatively low input resistance and depolarized membrane potential. Moreover, loss of the spontaneous shifts in membrane potential states after TTX demonstrates conclu-
sively that synaptic input is necessary for the expression of bistable membrane potential properties of spiny neurons, a finding consistent with the effects of lesioning specific afferents in vivo (Steriade et al., 1993b; Wilson, 1993; Amzica and Steriade, 1995; O’Donnell and Grace, 1995) or eliminating extrinsic afferents as with an in vitro preparation (Pare et al., 1998; Lavin and Grace, 2001). Furthermore, the increased excitability produced by NMDA indicates that even in anesthetized subjects, in most cortical neurons there is sufficient excitatory afferent activity to permit the voltage-dependent activation of NMDA receptors. However, in a subset of neurons receiving less excitatory input (i.e., those with higher input resistance), the effects of NMDA activation are revealed only upon intracellular injection of depolarizing current.

Intrastriatal infusion of the GABA\textsubscript{A} receptor antagonist BIC depolarized striatal neurons and in some cases induced spontaneous firing. This is the first demonstration in vivo that local GABA-ergic tone exerts a considerable inhibitory influence over the natural membrane activity of bistable neurons. Consistent with our findings, previous studies using intracellular recordings in vivo have shown that local microiontophoretic application of BIC reduced inhibitory postsynaptic potentials evoked after electrical stimulation of the sensorimotor cortex or substantia nigra (Calabresi et al., 1990). Additionally, local pressure-ejected BIC reduced the stimulation threshold for spike discharge in striatal neurons recorded in vivo and antagonized paired pulse inhibition in a subpopulation of these cells (Nisenbaum and Berger, 1992). Multiple studies using brain slice preparations have also demonstrated that the generation of action potentials in spiny neurons during synaptic activation is greatly influenced by GABA-ergic tone on striatal GABA\textsubscript{A} receptors (Nisenbaum et al., 1992; Köös and Tepper, 1999). Interestingly, we have also observed that local perfusion of TTX (\sim 5 min) produced a depolarization of the average membrane potential of a striatal neuron to a similar degree as BIC; however, the TTX-induced depolarization was associated with an absence of spike activity and a decrease in the amplitude of subthreshold membrane potential fluctuations (our unpublished observations). Taken together with the BIC data, these findings indicate that there is a physiologically significant level of tonic inhibitory input onto medium spiny neurons in the striatum. It is possible that this TTX-mediated depolarization is, in part, a result of decreased inhibitory GABA-ergic tone. Although the activation of GABA-ergic

![Fig. 7](image-url)
interneurons by corticostriatal inputs is thought to contribute to GABA-ergic transmission in the striatum (Kawaguchi, 1993; Kita, 1993; Plenz and Kita, 1998; Köös and Tepper, 1999), the current findings demonstrate that under conditions with relatively low spontaneous activity in the cerebral cortex (Table 1), tonic GABA_A receptor activation in the striatum is a significant factor in determining the resting membrane potential and firing pattern of striatal medium spiny neurons recorded in vivo.

Although the responsiveness of the recorded cortical and striatal neurons to local pharmacological manipulations indicated that the neurons were located within the range of the infused drug, it is likely that in some cases the activity of the recorded cell may have been altered indirectly as a result of infused drug actions on local feedback circuits. In both the cortex and striatum, spiny neurons emit local collaterals that are thought to influence the activity of other projection cells via lateral feedback processes (Park et al., 1980; Somogyi et al., 1981; Bishop et al., 1982; Penny et al., 1988; Kita, 1993). Additionally, multiple classes of interneurons exist in both the cortex and striatum (Kawaguchi and Kubota, 1996; Kawaguchi, 1997) and have been shown to modulate the activity of spiny projection neurons in a feed-forward manner (Jaeger et al., 1994; Köös and Tepper, 1999). Even though many of the observed effects of local drug infusion were rapid in onset (30–300 s), we cannot rule out the potential involvement of local circuits. Regardless of the direct or indirect nature of the drug effect, the results from the current study demonstrate that local drug infusion via the dialysis probe is an effective means of studying the modulation of network properties of spiny neurons by local and extrinsic afferents.

Acknowledgments

We thank Nicole MacMurdo and Christy Wanyt for excellent technical assistance, Aline Pinto for help with the photomontage, and Brian Lowry for the development of software (Neuroscope) used in data acquisition and analysis.

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