Inhibitory Interactions Between Spiny Projection Neurons in the Rat Striatum

MARK J. TUNSTALL, DOROTHY E. OORSCHOT, ANNABEL KEAN, AND JEFFERY R. WICKENS
Department of Anatomy and Structural Biology and the Neuroscience Research Centre, School of Medical Sciences, University of Otago, Dunedin, New Zealand

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Tunstall, Mark J., Dorothy E. Oorschot, Annabel Kean, and Jeffery R. Wickens. Inhibitory interactions between spiny projection neurons in the rat striatum. J Neurophysiol 88: 1263–1269, 2002. 2002.10.1152/jn.00886.2001. The spiny projection neurons are by far the most numerous type of striatal neuron. In addition to being the principal projection neurons of the striatum, the spiny projection neurons also have an extensive network of local axon collaterals by which they make synaptic connections with other striatal projection neurons. However, up to now there has been no direct physiological evidence for functional inhibitory interactions between spiny projection neurons. Here we present new evidence that striatal projection neurons are interconnected by functional inhibitory synapses. To examine the physiological properties of unitary inhibitory postsynaptic potentials (IPSPs), dual intracellular recordings were made from pairs of spiny projection neurons in brain slices of adult rat striatum. Synaptic interactions were found in 9 of 45 pairs of neurons using averages of 200 traces that were triggered by a single presynaptic action potential. In all cases, synaptic interactions were unidirectional, and no bidirectional interactions were detected. Unitary IPSPs evoked by a single presynaptic action potential had a peak amplitude ranging from 157 to 319 μV in different recordings (mean: 277 ± 46 μV, n = 9). The percentage of failures of single action potentials to evoke a unitary IPSP was estimated and ranged from 9 to 63% (mean: 38 ± 14%, n = 9). Unitary IPSPs were reversibly blocked by bicuculline (n = 4) and had a reversal potential of −62.4 ± 0.7 mV (n = 5), consistent with GABAergic-mediated inhibition. The findings of the present study correlate very well with anatomical evidence for local synaptic connectivity between spiny projection neurons and suggest that lateral inhibition plays a significant role in the information processing operations of the striatum.

INTRODUCTION

The basal ganglia are a set of interconnected structures critically involved in operations as diverse as motor activation (Graybiel 1995; Marsden 1982) and reward-related learning (Reynolds et al. 2001). Disorders of these structures underlie major neurological and psychiatric conditions such as Parkinson’s disease and attention-deficit hyperactivity disorder (Hynd et al. 1993). The striatum is the principal input structure of the basal ganglia and is made up of a diverse population of different types of neurons, the great majority of which are GABAergic spiny projection neurons (Bennett and Bolam 1993; Luk and Sadikot 2001; Oorschot 1996; Oorschot et al. 1999; West et al. 1996). The spiny projection neurons are the principal output neurons of the striatum and also the sites at which cortical inputs terminate (Somogyi et al. 1981). Thus they play a crucial role in the input-output operations of the striatum. These neurons not only project to other basal ganglia nuclei but also give rise to an extensive local plexus of collateral branches (Grofova 1975; Preston et al. 1980; Wilson and Groves 1980). Extensive overlap occurs between the axon collaterals and the dendritic trees of adjacent spiny projection neurons, suggesting that synaptic connectivity within the extent of local axonal spread is probable. Ultrastructural evidence has also consistently supported the existence of synaptic connections between spiny neurons (Kitai and Wilson 1982; Somogyi et al. 1981; Wilson and Groves 1980). However, up to now there has been no direct physiological evidence for functional inhibitory interactions between spiny projection neurons.

Early extracellular studies suggested that spiny projection neurons activated by stimulation of their axons may inhibit neighboring neurons (Katayama et al. 1981). However, in these studies there was uncertainty over the identity of the cells from which records were obtained. Another study using axonal stimulation suggested the presence of weak interactions that were effective in reducing synaptic input from presumed distal dendrites (Park et al. 1980). However, these effects may have been mediated by a small population of feedforward interneurons activated by collaterals of corticofugal axons (Koos and Tepper 1999). More recent dual recording studies (Jaeger et al. 1994; Stern et al. 1998) found no direct evidence for inhibitory synaptic interactions among spiny projection neurons. The lack of direct physiological evidence for functional interactions between spiny neurons remains puzzling in the light of the anatomical structure of the striatum.

We made simultaneous intracellular recordings from pairs of spiny projection neurons to test whether inhibitory interactions occur among spiny projection neurons in a striatal slice preparation. The chance of detecting an interaction was optimized by preparing slices using procedures previously shown to maximize the likelihood of detecting synaptic interactions (Thomson et al. 1996) and by making spike-triggered averages of postsynaptic responses to enhance the signal-to-noise ratio. Our experiments have revealed for the first time the existence of GABAergic receptor-mediated synaptic interactions between striatal spiny projection neurons. Preliminary results have been presented in abstract form (Tunstall et al. 2001).

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

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In vitro slice preparation

Experiments were conducted in acutely prepared brain slices of 24- to 28-day-old (65–120 g) male Wistar rats. The exception was one acutely prepared brain slice of a 45-day-old (196 g) male Wistar rat. Each rat was anesthetized by an intraperitoneal injection of 120 mg/kg pentobarbital sodium (Nembutal, Virbac, New Zealand). They were then perfused transcardially for 2 min with ice-cold artificial cerebrospinal fluid (ACSF) of composition (in mM) 124 NaCl, 2.5 KCl, 2 MgSO₄, 2.5 CaCl₂, 1.25 NaH₂PO₄, 26 NaHCO₃, and 11 glucose. This procedure is reported to increase the probability of obtaining functional connectivity (Thomson et al. 1996). The brain was quickly removed and chilled further in ACSF for 3 min. The hemispheres were divided, and one hemisphere placed medial surface down on a chilled surface. A block was prepared by making a parahorizontal cut to the horizontal plane, midway between the anterior and posterior poles. The cut surface of the block was glued to a Teflon chilled surface. A block was prepared by making a parahorizontal cut into one neuron (the “presynaptic” neuron) by injecting continuous current (90 pA) into the microelectrode were digitized at a sampling rate of 10 kHz per channel filtered at between 3 (Axoclamp 2B) and 5 kHz (Haer amplifiers). Data was low-pass filtered at below 10 kHz per channel and recorded to disk using a Digidata 1200 in conjunction with pClamp 6.0 software (Axon Instruments).

Each microelectrode was advanced into the slice using a separate microdrive (Burleigh Inochrom, LSO). The microdrives for each microelectrode were positioned so that the tips could be brought into close juxtaposition within the brain slice. The first electrode was advanced until a spiny projection neuron (identified by its electrophysiological properties, see following text) was stably impaled. The second electrode was then advanced toward the predetermined position of close juxtaposition until a second neuron was impaled. The final distance between the electrode tips during the electrophysiological experiments was estimated geometrically by measuring the distance each microelectrode had advanced into the slice, the initial points of penetration on the surface of the slice, and the angle of each axis of penetration.

Intracellular recording from pairs of projection neurons

Simultaneous intracellular recordings were made from pairs of striatal spiny projection neurons. Conventional sharp microelectrodes (90–120 MΩ) were drawn from glass capillaries and filled with 2 M potassium acetate. Microelectrodes were connected to the headstages of an Axoclamp 2B intracellular recording amplifier. The V2 channel was connected to a 10× band-pass amplifier (Haer). Data was low-pass filtered at 3 (Axoclamp 2B) and 5 kHz (Haer amplifier). The voltage and current outputs from each channel of the microelectrode amplifier were digitized at a sampling rate of 10 kHz per channel and recorded to disk using a Digidata 1200 in conjunction with pClamp 6.0 software (Axon Instruments).

Characterization of inhibitory interactions

The reversal potential of responses was obtained by holding postsynaptic neurons at different membrane potentials and measuring the peak amplitude of the spike-triggered average of the 200 responses evoked at each holding potential. The range of holding potentials chosen was sufficient to cause a reversal in the polarity of the averaged response. Peak amplitudes were plotted as a function of holding potential, and the reversal potential (the holding potential at which the response peak amplitude was 0) determined by interpolation from the equation of the best-fit line through the data points. Curve fitting was performed using the standard simplex algorithm implemented in Sigma Plot (Jandel Scientific). Pharmacological properties of responses were determined using the GABA_A receptor antagonist bicuculline methiodide (100 μM, Sigma-RBI). The effects of this agent were measured by comparing the spike-triggered average of 200 responses evoked under control, treatment, and recovery conditions.

RESULTS

Characteristics of recorded cells

Dual intracellular recordings were obtained from 45 pairs of neurons in slices from 45 animals. All the neurons in the sample (n = 90) exhibited the characteristic electrophysiological properties of spiny projection neurons (Fig. 1) as previously described (Kawaguchi et al. 1989; Kita et al. 1984, 1985; Nisenbaum et al. 1994; Tepper et al. 1993; Wickens and Wilson 1998). These included a resting membrane potential of
-86.2 ± 7.6 (mean ± SD) mV, input resistance of 71.1 ± 5.2 MΩ, evidence of inward rectification in the hyperpolarizing direction, an action potential threshold at 48.1 ± 1.6 mV above rest, and an action potential amplitude of 75.4 ± 4.6 mV.

Incidence of interactions between striatal projection neurons

Unitary postsynaptic responses were detected in averages of 200 traces that were triggered by a single presynaptic action potential. Examples of these responses from different pairs are shown in Figs. 2, A–F, 3B, 4A, and 5A. Unidirectional interactions were detected in 9 of the 45 pairs of neurons recorded. On the basis of this data, the probability of a connection existing between a neighboring pair of neurons is 9/90 or approximately 1 in 10 because in all cases, interactions were tested in both directions. Bidirectional or mutual interactions were not detected in any of the pairs tested. By chance alone, if the probability of a connection in one direction is 0.1, then mutual interactions would be expected in approximately 1 in 100 pairs. Thus failure to find bidirectional interactions does not rule out mutual connectivity, although it does suggest that there is not a strong bias in favor of it.

The cellular properties of the neurons constituting pairs in which interactions were detected were not significantly different from those of noninteracting pairs in the sample (Table 1). There were no significant differences in cellular properties between pre- and postsynaptic neurons of interacting pairs or between these groups and neurons from noninteracting pairs. This indicates that the detection of interactions was not dependent on distinctive cellular characteristics of the pre- or postsynaptic neurons. Interactions were also detected across the full age range of the animals in the sample (24–45 days).

Although the distance between the neurons could not be systematically varied, there was variation in the final distance between the tips of the recording electrodes. This occurred because the second cell of the pair was usually obtained at a different depth from the first. Successful impalements were made at depths ranging from 100 to 250 μm below the slice surface. The estimated distance between interacting pairs ranged from 153 to 445 μm (mean: 264 ± 101 μm, n = 9). The distance between noninteracting pairs ranged from 212 to 509 μm (mean: 335 ± 90 μm, n = 36). The difference between these groups was statistically significant (P < 0.05, unpaired Student’s t-test). The method used to estimate the distance between the tips provided an upper bound on the inter-tip distance and did not allow for bending of the tapered shafts of the electrodes, which would have brought the tips closer. However, such an error would not produce a systematic bias. Thus it appears that closer units are more likely to be functionally connected.

Properties of interactions between projection neurons

In the raw data, the peak amplitude of the unitary IPSPs fluctuated randomly from trial to trial (Fig. 3A). However, noise levels in single traces precluded quantal analysis. Thus the data provided no indication of the number of synaptic sites involved in each functional interconnection. However, the percentage of failures of single action potentials to evoke a unitary postsynaptic response could be estimated by comparing the distributions of synaptic amplitudes for individual responses and the distribution of noise recorded immediately prior to the presynaptic action potential (Fig. 3C). A curve-fitting procedure (described in METHODS) was used to find the parameters of...
mediated IPSPs reported by others (Fitzpatrick et al. 2001; Koos and Tepper 1999; Tepper et al. 1993). Consistent with this, the GABA_A receptor antagonist bicuculline reversibly blocked responses in all interacting pairs tested \((n = 4)\). Blockade of the IPSPs with bicuculline did not change the input resistance of the postsynaptic neuron at the membrane potential from which the responses were elicited (Fig. 5).

**DISCUSSION**

The experiments of the present study provide direct electrophysiological evidence for the existence of local interactions between the spiny projection neurons of the rat striatum. The neurons in the sample were identified by their electrophysiological properties, which were characteristic of spiny projection neurons. The reversal of the postsynaptic potential and its blockade by bicuculline show that the interactions between the spiny projection neurons are GABA_A receptor-mediated IPSPs. To the best of our knowledge, this is the first time that such inhibitory interactions have been described between identified spiny projection neurons in rat striatal slices.
The present study provides the first experimental measure of the probability of inhibitory interactions between neighboring pairs of spiny projection neurons (i.e., 1 in every 10 connections tested). A similar probability (also 1 in every 10) of finding an interacting pair in dual recordings from layer 5 pyramidal neurons has been reported in brain slices of rat somatosensory cortex (Markram et al. 1997). Similarly, inhibitory connections between fast spiking interneurons and pyramidal neurons in slices of adult rat neocortex were found, on average, in 1 in 15 pairs (Thomson et al. 1996). However, Thomson et al. (1996) also noted that both slice thickness and preparation procedure appeared to affect this proportion. Using thick (500 μm) slices prepared from animals that had been perfused transcardially with ice-cold sucrose-containing ACSF, this probability rose to 7 in 32 pairs. The detection of any inhibitory interactions in the present study is, however, in contrast to an earlier study in which inhibitory interactions were not detected (Jaeger et al. 1994). Apart from technical factors, interactions may have previously gone undetected because of the relatively high failure rate of unitary IPSPs that we observed (mean, 38%). Even though the postsynaptic response was sometimes detected in a single sweep, it was only reliably detected by averaging 200 sweeps. The findings of the present study thus help to resolve an apparent contradiction between the known anatomy and physiology of striatal projection neurons. The physiology now correlates very well with the anatomical evidence for local synaptic connectivity between spiny projection neurons (Kitai and Wilson 1982; Somogyi et al. 1981; Wilson and Groves 1980).

### Properties of the unitary IPSP

The observed amplitude of the IPSP evoked by a single action potential in a presynaptic spiny projection neuron is less than that evoked by presynaptic action potential firing of the GABAergic interneurons of the striatum. Koos and Tepper (1999) report that the IPSP evoked in a postsynaptic spiny projection neuron by a single presynaptic action potential in a GABAergic interneuron ranged from 330 to 2,130 μV (mean: 1,060 ± 220 μV, n = 7). This compares with a range of 157–319 μV (mean: 277 ± 46 μV, n = 9) for the IPSP evoked by a single presynaptic action potential in a spiny projection neuron in the present study. The higher amplitude of the IPSP evoked by GABAergic interneurons correlates with the anatomy of their terminals, which are large and probably make multiple terminations on each spiny projection neuron they innervate (Kita 1993). The amplitude of the unitary IPSP is also compatible with those reported in other brain areas. In the cerebral cortex, single axon IPSPs elicited in pyramidal cells by interneurons have been reported to range from 200 to 3,500 μV in amplitude (Thomson et al. 1996). In the latter study, the

![Figure 4. Reversal potential of IPSPs. A: effect of holding potential on amplitude of IPSPs. Changing the holding potential from −51 to −56 mV gave rise to a reduction in the peak amplitude of the IPSPs, and at −70 mV the IPSPs had reversed polarity. Each trace is an average of 200 successive responses. B: a plot of the peak amplitude of each of the responses in A vs. the holding potential shows that the reversal potential of the IPSPs in this case was −62.8 mV.](image)

![Figure 5. IPSPs are mediated by GABA<sub>A</sub> receptors. A: IPSPs recorded under control conditions (top), 5 min after washing on bicuculline (middle), and 15 min after washing off bicuculline (bottom). It can be seen that bicuculline reversibly blocked the IPSPs. Each trace is an average of 200 successive responses. B: current-voltage response of the postsynaptic neuron during the wash-off condition was similar to that of the control and has been omitted for clarity.](image)
connections involving several boutons resulted in the smaller IPSPs recorded, and some connections involving two boutons went undetected. The largest IPSPs recorded might have resulted from 12 to 20 boutons (Thomson et al. 1996). When placed in this context, the amplitude of the unitary IPSP evoked by a single action potential in a presynaptic spiny projection neuron is consistent with the apparently smaller number of boutons involved in the synaptic connection between spiny projection neurons (Somogyi et al. 1982; Wilson and Groves 1980).

Previous studies have used intrastriatal stimulation to characterize inhibitory interactions between spiny projection neurons. Application of focal bipolar electrical stimulation evokes a GABA-mediated IPSP with two components exhibiting a differential sensitivity to GABA B agonists (Seabrook et al. 1991). It has been suggested that the fibers that evoke GABA B agonist-insensitive synaptic potentials originate from the recurrent collaterals of spiny projection neurons (Radnikow et al. 1997). However, in studies using intrastriatal stimulation, it is difficult to separate the effects of spiny projection neurons from the GABA interneurons, which also produce strong inhibitory responses in the spiny projection neurons (Koos and Tepper 1999). Nevertheless, studies using intrastriatal stimulation have shown that, as in other systems, GABA synapses in the striatum are highly modifiable and display short-term activity-dependent plasticity. Both paired-pulse depression of the IPSP evoked by intrastriatal stimulation (Radnikow et al. 1997) and synaptic augmentation (Fitzpatrick et al. 2001) have been described. Thus it should be noted that the characteristics of the synaptic response described in the present study are based on the physiological characteristics of an IPSP evoked by a single action potential, and the response might be increased or decreased by the repetitive firing patterns of the presynaptic neurons.

### Implications for current and future theoretical models of striatal function

The demonstration of synaptic interactions supports previous proposals that inhibitory interactions between spiny projection neurons may be a key determinant of the signal processing operations performed in the striatum (Beiser and Houk 1998; Wickens et al. 1991; Wilson and Groves 1980). One of these proposals is the “winner takes all” model, which is based on the premise that spiny projection neurons are mutually connected (Bar-Gad and Bergman 2001; Gillies and Arbuthnott 2000; Wickens and Oorschot 2000). In the present study, however, the connections found were in one direction only, and no mutually inhibitory interactions were detected. The absence of mutually inhibitory interactions in the current sample does not rule these out as an infrequent chance event, which may have functional significance. However, as the findings indicate no bias toward mutual interactions, such interactions are unlikely to dominate the striatal dynamics.

Many theoretical studies have emerged regarding the role of the basal ganglia in brain function (Amos 2000; Berns and Sejnowski 1998; Redgrave et al. 1999; Suri and Schultz 1999). In these theoretical models, the functional importance of the striatum is paramount, although the principles underlying its operation have so far been based on uncertain anatomical and physiological data. We anticipate that the definitive physiological data on the functional properties of the striatum that we present here will serve to spark a new and influential generation of theoretical models of basal ganglia operation.

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### REFERENCES


### TABLE 1. Cellular properties of interacting and noninteracting neurons

<table>
<thead>
<tr>
<th>Interacting Pairs</th>
<th>Presynaptic neurons</th>
<th>Postsynaptic neurons</th>
<th>Noninteracting pairs</th>
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<tbody>
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<td>$n$</td>
<td>9</td>
<td>9</td>
<td>72</td>
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<tr>
<td>Resting membrane potential, mV</td>
<td>$-87.0 \pm 8.0$</td>
<td>$-85.1 \pm 7.6$</td>
<td>$-86.4 \pm 7.7$</td>
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<td>Input resistance, $\Omega$</td>
<td>68.9 $\pm$ 5.2</td>
<td>73.2 $\pm$ 6.0</td>
<td>71.8 $\pm$ 5.1</td>
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<td>Action potential threshold, mV</td>
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<td>48.0 $\pm$ 1.7</td>
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<tr>
<td>Action potential amplitude, mV</td>
<td>77.3 $\pm$ 4.7</td>
<td>73.6 $\pm$ 5.0</td>
<td>75.2 $\pm$ 4.5</td>
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Values are mean $\pm$ SD.


ORSCHOT DE. Total number of neurons in the neostriatal, pallidal, subthalamic, and substantia nigral nuclei of the rat basal ganglia: a stereological and second-order stereological study. Melbourne, Australia: *Proc. Xth Int Congr Stereol*, 1999, p. 87.


