I. INTRODUCTION

One of the areas in which our understanding of the structure and function of the striatum is undergoing the most rapid expansion and change is in the identification and characterization of striatal interneurons, their synaptic connectivity and the role that they play in the organization and control of striatal output. First shown by strong uptake of radiolabeled GABA (Bolam et al., 1983), and subsequently by immunoreactivity for GABA and/or GAD (Bolam et al., 1985; Cowan et al., 1990; Kita, 1993), a population of striatal GABAergic interneurons comprising only a few percent of all the striatal neurons was identified. These interneurons have become the focus of a large number of anatomical and, more recently, electrophysiological studies. Results from these experiments, most of them performed over the past 10 years, have led to the current view that although few in number, striatal GABAergic interneurons play a predominant role in regulating spike timing in the spiny output neurons through feedforward inhibition (for reviews, see Tepper et al., 2004, 2008). Although Golgi studies have suggested that there are as many as 9 morphologically distinct neuron types in the rodent striatum (e.g., Chang et al., 1982), the current consensus view is that in addition to the spiny projection neurons that comprise up to 97.7% of striatal neurons in rodents (Rymar et al., 2004), the striatum is composed of one type of cholinergic interneuron (see Chapter 7), and three types of neurochemically and electrophysiologically distinct GABAergic interneurons (Kawaguchi, 1993; Kawaguchi et al., 1995; Tepper and Bolam, 2004; Tepper et al., 2008). However, recent physiological studies in striatum, and by analogy with findings from the cortex and hippocampus over the past 15 years, this view is almost certain to underestimate the true diversity of striatal GABAergic interneurons. This review will focus on the anatomy and physiology of the three well-known GABAergic interneuronal cell types of the rodent striatum, as well as recent advances in striatal...
neuroanatomy and neurophysiology which suggest that a re-evaluation of the number of distinct striatal GABAergic interneuron subtypes is overdue.

II. PARVALBUMIN-IMMUNOREACTIVE INTERNEURONS

A. Neurocytology

Parvalbumin-immunoreactive (PV+) striatal interneurons are medium to large sized, averaging 16–18 μm in diameter. These were the striatal GABAergic neurons originally identified by their high affinity uptake of GABA (Bolam et al., 1983) and subsequently by the strongest immunoreactivity for GAD67 and GABA of any striatal neuron (Bolam et al., 1985; Cowan et al., 1990; Kita, 1993; Kubota et al., 1993). PV+ neurons do not express any of the other markers that identify the other types of striatal GABA interneurons, i.e., calretinin, nitric oxide synthase (NOS), somatostatin (SOM) or neuropeptide Y (NPY). Striatal PV+ interneurons issue 5–8 aspiny, often varicose, dendrites that taper rapidly and branch relatively sparsely to form a restricted dendritic arborization only 200–300 μm in diameter. The axon issues from the soma or proximal dendrite and branches repeatedly forming an extremely dense, highly varicose arborization that overlaps and extends well beyond the limits of the dendritic field of the cell of origin. The axonal arborization is the densest of all the striatal dendrites that taper rapidly and branch relatively sparsely to form a restricted dendritic arborization only 200–300 μm in diameter. The axon issues from the soma or proximal dendrite and branches repeatedly forming an extremely dense, highly varicose arborization that overlaps and extends well beyond the limits of the dendritic field of the cell of origin. The axonal arborization is the densest of all the striatal dendrites.

B. Afferents and Efferents

PV+ interneurons receive a substantial monosynaptic input from the cortex but in contrast to the spiny cells, which receive only one or two synapses from each cortical afferent (Kincaid et al., 1998), single cortical axons make multiple contacts with PV+ interneurons (Ramanathan et al., 2002). This may account, in part, for the greater responsivity of PV+ interneurons to cortical stimulation (Parthasarathy and Graybiel, 1997) compared to spiny neurons (Mallet et al., 2005). In contrast to this powerful cortical input, PV+ interneurons appear to receive few synaptic inputs from thalamus (Kita, 1993).

Striatal PV+ interneurons also receive GABAergic input from PV+ globus pallidus neurons that specifically target interneurons in the striatum (Bevan et al., 1998). In addition, there is a cholinergic input from striatal cholinergic interneurons (Chang and Kita, 1992), and a dopaminergic input, presumably from substantia nigra (Kubota et al., 1987).

By far the predominant target of the PV+ interneuron is the spiny projection neuron with about 50% of striatal PV+ boutons synapsing pericellularly, on the soma or proximal dendrites of spiny neurons (Kita, 1993; Bennett and Bolam, 1994a). Other PV+ boutons (some of which likely originated in globus pallidus) were observed making synapses with spiny dendrites as well as with varicose dendrites (Kita, 1993) belonging to PV+ and nitric oxide synthase (NOS)-immunoreactive interneurons (Bevan et al., 1998).

C. Basic Membrane Properties

The earliest descriptions of the electrophysiological properties of the PV+ interneuron were provided by Kawaguchi (1993) who used visually guided whole cell recordings in slices from young (16–20 days) rats and targeted somata larger than those of spiny cells to attempt to record from interneurons. The first class of interneuron he described had the shortest duration action potentials of all striatal neurons (0.29 ± 0.04 ms) at half amplitude), a rapid (1.3 ± 0.27 ms) time to peak and brief spike afterhyperpolarization and the lowest input resistance of all GABAergic interneurons (86 ± 38 ms). These interneurons were, like spiny neurons, hyperpolarized (resting membrane potential ~ −80 mV) and displayed no spontaneous activity at rest. Of 11 neurons with these electrophysiological characteristics tested, 10 were immunopositive for PV (Kawaguchi, 1993).

Subsequent experiments in acute slices from older rats revealed a class of fast-spiking (FS) interneurons with identical properties including a nearly linear IV response, low input resistance, hyperpolarized resting membrane potential and narrow action potentials with a rapid, large amplitude and brief duration spike afterhyperpolarization (Kóos and Tepper, 1999, 2002; Bracci et al., 2002, 2003; Taverna et al., 2007; cf Fig. 8.1C).

PV+ interneurons in striatum have been shown to exhibit gap junctions at the electron microscopic level.
GABAergic Interneurons of the Striatum

(Kita et al., 1990, Kita, 1993). In the first report of paired recordings between striatal FS interneurons, clear evidence of electrotonic coupling was found (Koós and Tepper, 1999; cf. Fig. 8.1E). The coupling ratio ranged between 3 and 20%, and although the coupling was not strong enough to induce spiking per se, it was sufficient to synchronize depolarization-induced spiking in electrically coupled neurons such that the variability between spikes in the two neurons in response to current injection, when they did occur, was extremely small (cf Fig. 8.1F). This suggests that groups of FS interneurons may form an inhibitory syncytium capable of exerting powerful and synchronous inhibitory control over spike timing in a large number of spiny neurons, thereby influencing the temporal relationship of their spike trains leading to the formation of behaviorally relevant functional pools of cells (Koós and Tepper, 1999).

**FIGURE 8.1** Striatal PV+/ fast-spiking interneurons. A. Typical morphology of an immunocytochemically striatal GABAergic PV+ interneuron stained with an antibody against parvalbumin from adult rat striatum. B. Drawing tube reconstruction of a biocytin labeled rat striatal FS interneuron that was presynaptic to a spiny neuron (not shown). Note the extremely dense axonal arborization (red) that extends beyond the simple, aspiny and compact dendritic arbor (black). C. 1. Typical response of mouse striatal FS interneuron to intracellular injection of de- and hyperpolarizing current pulses. Note the low input resistance, the linear IV relation and the characteristic non-linear current-spiking relation where the minimum suprathreshold stimulus elicits a short burst of three spikes (black trace), the next greater current injection elicits a 120 ms burst of non-accommodating spikes followed by a silent period followed by intermittent spiking (green trace) and a slightly larger current injection evokes longer periods of intermittent spiking. Sufficiently greater current injection elicits sustained firing in excess of 200 spikes/sec (not shown). C2. Note extremely narrow action potential and deep and rapid onset spike AHP. D. Striatal FS interneurons are silent at rest (3). As they are depolarized small subthreshold membrane oscillations occur (2) that eventually give rise to intermittent spiking (red arrows in 1). E. Left two panels show a pair of FS interneurons that are electrotonically coupled. Intracellular current pulses injected into FS #1 (right lower panel) induces much smaller, electrotonic responses in FS #2 (upper right, black traces) distorted by the membrane capacitance of the intervening dendrites. F. Spiking induced by intracellular depolarization in a pair of electrotonically coupled FS interneurons is nearly synchronous due to the effects of the coupling. All recordings were obtained from neurons in acute brain slices in vitro. Sources: B: Modified from Tepper and Bolam, 2004; D: Modified from Koós and Tepper, 2002; E: Modified from Koós and Tepper, 1999. (see Color Plate Section to view the color version of this figure)
Electrotonic coupling with similar properties has been observed between pairs of PV+ fast spiking interneurons in cortex and hippocampus (Freund and Buzsáki, 1996; Galaretta and Hestrin, 1999, 2001, 2002), although the highest coupling ratios in the cortical interneurons appear larger than in striatum, but this could be an artifact of the relatively small striatal sample size.

D. Firing Characteristics

One of the most characteristic and consistently reported properties of striatal PV+ FS interneurons is a non-linear spiking response to intracellular depolarization. Lower amplitude stimuli of increasing strength produce only passive depolarizing responses but at a certain level, a tiny increase in current results in the appearance of short bursts of spikes interrupted by periods of no spiking (Kawaguchi, 1993; Koós and Tepper, 1999, 2002; Kubota and Kawaguchi, 2000; Narushima et al., 2006). Stronger depolarizing pulses in striatal FS interneurons elicit high, sustained firing rates (>200 spikes/sec) with little spike frequency adaptation (Koós and Tepper, 1999, 2002; Plotkin et al., 2005; cf Fig. 8.1C).

During the non-spiking periods intercalated between episodic firing, prominent subthreshold membrane oscillations can be observed (Koós and Tepper, 1999, 2002; Bracci et al., 2002, 2003; Taverna et al., 2007; cf Fig. 8.1C1, D1). The oscillations are clearly voltage dependent and are not present when the neuron is hyperpolarized or at rest. They are 2–3 mV in amplitude and exhibit a peak in power near 40 Hz (Bracci et al., 2003). The oscillations are able to induce episodes of firing and appear to be responsible for the stuttering, intermittent firing pattern of striatal FS interneurons. The oscillations and the intermittent firing pattern were resistant to blockade of Ca2+ channels, SK channels or intracellular Ca2+ chelation. The oscillations were, however, completely eliminated by TTX, and it has been suggested that they are due to an interaction between a persistent Na+ conductance and voltage gated K+ conductances (Bracci et al., 2003).

Whole cell recordings from striatal FS interneurons in slices from mice reveal that they express virtually identical properties to those described for rat FS interneurons (Centonze et al., 2003; Narushima et al., 2006; Técuapatla et al., 2009b). These electrophysiological characteristics are very similar to those of parvalbumin-expressing fast spiking interneurons of adult mouse cortex (Galaretta et al., 1999, 2001, 2002) and hippocampal basket cells (Freund and Buzsáki, 1996). The short duration action potential, lack of spike frequency adaptation and large spike afterhyperpolarizations are likely related to the expression of Kv 3.1, a slowly inactivating delayed rectifier channel that exhibits rapid activation and deactivation kinetics, and that is selectively expressed in striatum in PV+ FS interneurons (Lenz et al., 1994).

Interestingly, in another study in which PV+ FS interneurons were identified in striatal slices of young (12–18 day old) BAC transgenic mice engineered to express enhanced green fluorescent protein (EGFP) controlled by the PV promoter (Freiman et al., 2006), although fluorescent (PV+) fast spiking interneurons were in several ways qualitatively similar to FS interneurons previously described, they did not exhibit the intermittent firing or subthreshold oscillations seen in juvenile and adult rats or in adult mice, and also exhibited a more depolarized resting membrane potential (∼63 mV) and wider action potential duration (0.70 ms at half amplitude) than reported by others (Freiman et al., 2006). As these neurons were unquestionably PV+ FS interneurons, these differences may be attributable to the younger age of the mice, lowering (room) temperature recording, and/or other aspects of the recording conditions. There is also the intriguing possibility that there are actually several subtypes of striatal PV+ interneurons that each have distinct physiological properties, as is known to be the case, for example, in the amygdala (e.g., Woodruff and Sah, 2007).

E. Synaptic Connectivity

Synaptic responses in spiny neurons resulting from spiking of FS interneurons were first reported by Plenz and Kitai (1998) in one out of four paired recordings of an FS interneuron and a spiny cell in an organotypic co-culture of cortex, striatum and substantia nigra. This recording showed that a single spike in the presynaptic FS interneuron elicited an IPSP several mV in amplitude. Soon after, paired whole cell recordings of FS interneurons and spiny neurons were obtained from acute slices of striatum from adult rats. These revealed unusually strong unitary IPSPs in the spiny cells from single spikes in FS interneurons. IPSPs were blocked completely by bath application of bicuculline indicating that the FS-spiny neuron synaptic response is mediated predominantly or exclusively by GABA_A receptors. The synaptic connections were always unidirectional, from the interneuron to the spiny cell but never in the other direction, and were observed in approximately 25% of the paired recordings when the two cells were within 250 μm of each other. The synaptic connection was remarkably
reliable and for most pairs exhibited a failure rate of less than 1% (Koós and Tepper, 1999, 2002).

Early estimates of the convergence values of FS interneurons and spiny cells were based on the measured volume of the axonal arborization of four biocytin-filled FS interneurons (6.65 ± 1.19 × 10^{-7} mm\(^3\)) assuming 3-dimensional isotropic density, a cell density in striatum of 84,900 cells/mm\(^3\) (Oorschot, 1996) that corresponds to 541 ± 101 spiny cells within the volume of the axonal arbor of a FS interneuron. Assuming that all spiny cells within the arborization are innervated and if FS interneurons make up 5% of the cells in striatum, the upper limit of convergence is 27 FS interneurons per spiny cell (0.05 × 541). The lower limit was estimated by assuming that only 25% of the spiny cells are innervated (the probability of finding a synaptically connected pair) and only 3% of the striatal cells are FS interneurons and was calculated to be four interneurons per spiny cell (541 × 0.25 × 0.03; Koós and Tepper, 1999). However, in subsequent reports from other labs and more recent paired recordings from our lab where the pre- and postsynaptic neurons were in closer proximity to one another, the incidence of connectivity was significantly higher, with a lower limit around 50% (Tavarna et al., 2007). In terms of convergence, the increase in the lower limit of synaptic connectivity is offset by more accurate estimates of the number of PV+ neurons from stereological cell counts that suggest that the proportion of PV+ neurons in the rat striatum is actually only 0.7% (Rymar et al., 2004). This would give a lower limit of convergence of 2 FS interneurons per spiny cell (541 × 0.5 × 0.007) and an upper limit of 4 FS interneurons per spiny cell. Note that this assumes that all FS interneurons are PV+, an assumption that may prove incorrect (see below).

Average unitary FS-Spiny IPSPs recorded in hyperpolarized spiny neurons were over 0.4 mV in amplitude, and when measured when the spiny cell was just subthreshold, averaged greater than 1 mV in amplitude. Temporal summation in response to bursts of 2–5 spikes in the FS interneuron within 10–50 ms led to compound IPSPs that could be up to 7 mV in amplitude. The effectiveness of these IPSPs was evident by the ability of unitary IPSPs to significantly delay the timing of depolarization-evoked spikes in spiny neurons and, in the case of short bursts of presynaptic spikes, to completely block spiking in the spiny neuron (Koós and Tepper 1999).

When compared to IPSPs arising from the local axon collaterals of spiny neurons (Czubayko and Plenz, 2002; Tunstall et al., 2002; Tepper et al., 2004; Tecuapetla et al., 2009) (see Chapter 5), the feedforward interneuronal IPSP appeared significantly larger and had a significantly lower failure rate than the collateral IPSP under a number of different experimental conditions (Guzman et al., 2003; Koós et al., 2004; Tecuapetla et al., 2005, 2009; Gustafson et al., 2006). Quantal analysis revealed that whereas individual FS-Spiny and Spiny-Spiny synapses were in fact biophysically similar, the differences in average IPSP/C amplitude and failure rate could be attributable to a more proximal location and larger number of synapses formed by FS inputs to spiny neurons than from collateral inputs from other spiny neurons (Koós et al., 2004).

F. In Vivo Recordings

Each of the striatal interneurons make up such a small proportion of the cells in the striatum that in vivo recordings from unambiguously identified GABAergic interneurons are relatively rare. Intracellular recordings from a neuron identified post-hoc by horseradish peroxidase staining as a likely PV+ FS interneuron based on the large soma size and varicose dendritic arborization exhibited EPSPs in response to cortical stimulation. The EPSPs gave rise to short bursts of high frequency spikes (Kita, 1993), as would be predicted from the in vitro responses of striatal PV+ FS interneurons.

More recently, extracellular single unit recording studies in vivo in anesthetized rats putatively identified FS interneurons on the basis of their short duration action potentials and a short, high frequency (>300 Hz) burst of 3–5 spikes, very similar to that reported by Kita (1993), in response to cortical stimulation (Mallet et al., 2005, 2006). These neurons exhibited spontaneous firing rates of around 0.5 spikes/second during slow wave sleep and 3.5 spikes/second at other times. When neurons with these characteristics were juxtacellularly stained with biocytin and then tested for parvalbumin immunoreactivity, all neurons tested were immunopositive for parvalbumin, thus unequivocally identifying them as PV+FS interneurons (Mallet et al., 2005, 2006). Comparison of the responses of spiny neurons to those of FS interneurons following cortical stimulation showed the FS interneurons to be more responsive during periods of cortical desynchronization than during slow wave sleep whereas the opposite was true for spiny neurons (Mallet et al., 2005). On average FS interneurons were more responsive to cortical stimulation than spiny neurons (Mallet et al., 2006), consistent with results from previous immediate early gene expression experiments (Parthasarathy and Graybiel, 1997). Local application of picROTOXIN increased the spiking of spiny neurons in response to
cortical stimulation, particularly under conditions favoring the activity of FS interneuronal activity strongly suggesting that strong feedforward inhibition of spiny neurons by FS interneurons normally occurs in vivo as well as in vitro (Koós and Tepper, 1999, 2002; Koós et al., 2004; Mallet et al., 2005).

Presumed FS interneurons have also been identified in vivo in unanesthetized behaving rats. Tetrode recordings revealed a population of neurons that were tonically active with average firing rates of 5–30 Hz spikes/sec during waking that displayed narrow duration waveforms and high frequency bursts during slow wave sleep, and an anatomical distribution very similar to that reported for PV+ FS interneurons (Berke et al., 2004; Berke, 2008). The presumed FS interneurons were more active when the animals were awake than during slow wave sleep, consistent with the results from the anesthetized animals. These neurons were found to be entrained by high voltage spindle activity that occurred principally while rats were immobile but interestingly, even nearby FS interneurons failed to exhibit correlated firing while rats were performing a radial maze task (Berke, 2008), suggesting that feedforward inhibition of individual spiny neurons may be comprised of inputs from neurons with very different firing rates and/or behavioral correlates.

G. Pharmacology

Striatal FS interneurons are innervated by striatal cholinergic interneurons (Chang and Kita, 1992), and express both nicotinic and muscarinic receptors. The two types of cholinergic receptors have opposing effects on the feedforward inhibition mediated by FS interneurons.

Local or bath application of nicotinic cholinergic agonists in vitro depolarizes striatal FS interneurons by up to 40 mV, evoking episodes of irregular bursty firing in the normally silent neurons, thereby increasing feedforward inhibition of spiny neurons (Koós and Tepper, 2002; Fig. 8.2). The cholinergic excitation persists during bath application of carbachol and is insensitive to high concentrations of the Type 1 nicotinic receptor antagonist, methyllycaconitine (MLA), but can be completely blocked by mecamylamine. This profile strongly suggests that the receptor responsible is one of the heteromeric, non-desensitizing nicotinic receptor subtypes (Alkondon and Albuquerque, 1993). It has been suggested that disfacilitation of FS interneurons as their nicotinic excitation is transiently reduced during the brief and stereotyped pause in striatal cholinergic interneurons that accompanies behaviorally relevant stimuli (Aosaki et al., 1994) may play a role in relaying the pause to the spiny neurons with high temporal fidelity (Koós and Tepper, 2002).

Acetylcholine also acts through pirenzepine-sensitive muscarinic receptors located on axon terminals of striatal FS interneuron to presynaptically inhibit GABA release and reduce the feedforward inhibition of spiny cells by FS interneurons as illustrated in Figure 8.2E,F (Koós and Tepper, 2002). The balance of this dual cholinergic regulation of FS interneurons may depend on behavioral state and the level of interneuronal activity with the direct excitatory nicotinic effects predominating when FS neurons are relatively inactive during periods of cortical synchrony and the presynaptic inhibitory effects predominating during cortical desynchronization when the FS interneurons are highly active.

FS interneurons are also excited by dopamine (Bracci et al., 2002). The excitation is accompanied by a decrease in membrane conductance and, like nicotinic stimulation, is sufficient to generate spiking. These effects were blocked by SCH-23390 but not by quinpirole, suggesting that they were mediated by a D1-like dopamine receptor. Subsequent experiments revealed that SCH-23390-sensitive dopamine-induced excitation persisted in D1 knockout mice, indicating that it is mediated by dopamine D1 receptors that have been shown to be co-expressed with parvalbumin in striatal FS interneurons (Centonze et al., 2003). Interestingly, unlike D1 expressing direct pathway spiny neurons that express presynaptic D1 receptors on their axon terminals that facilitate GABA release (Misgeld et al., 2007), or striatopallidal neurons that express presynaptic D2 receptors that inhibit GABA release (Tecuapetla et al., 2009), the evidence for presynaptic modulation of the FS to spiny neuron synapse by dopamine, particularly by the D2 receptor known to be expressed by these neurons, is considerably weaker and more equivocal (Bracci et al., 2002; Guzman et al., 2003; Tecuapetla et al., 2007).

III. SOMATOSTATIN/NOS/NEUROPEPTIDE Y INTERNEURONS

A. Neurocytology

The discovery of somatostatin (SOM)-immunoreactive interneurons in striatum was followed by demonstrations that SOM, NPY and NADPH-diaphorase/nitric oxide synthase (NOS) were all co-expressed in the same neuronal population (Vincent and Johansson, 1983; Chesselet and Graybiel, 1986) and that these neurons were distinct from
those that expressed parvalbumin or calretinin (Kubota et al., 1993). At first these interneurons did not appear to be GABAergic because unlike the other striatal GABAergic interneurons, somatostatin-immunoreactive neurons did not appear to express GAD mRNA (Chesselet and Robbins, 1989) or immunoreactivity for GABA or GAD 67 (Kubota et al., 1993). However, subsequent immunocytochemical labeling following colchicine treatment revealed that all NOS-reactive cells were strongly immunopositive for GAD 67 (Kubota et al., 1993) and intracellular labeling followed by electron microscopic post-embedding immunogold labeling for GABA or GAD showed that their synaptic boutons were strongly GABA immunopositive (Kubota and Kawaguchi, 2000).

SOM/NPY interneurons have medium-large somata, 15–25 μm in diameter, and are the second largest cell in the striatum after the cholinergic interneuron. The neurons emit 2–5 thick, rapidly tapering aspiny primary dendrites that branch within 50 μm of the cell body, become varicose, and give rise to a relatively simple and unbranched dendritic arborization up to 600 μm in diameter (Difiglia and Aronin, 1982; Vincent and Johannson, 1983; Vincent et al., 1983; Kawaguchi, 1993; Aoki and Pickel, 1988; see Fig. 8.3). The axonal arborization of SOM/NPY interneurons is the sparsest of any striatal neuron and is also the longest, tending to course in straight lines for up to 1 mm. Some of these neurons appeared to give rise to two main axons (Kawaguchi, 1993).
Unbiased stereological counts of striatal neurons immunostained for SOM (21,300) and NPY (14,355) differ (Rymar et al., 2004), consistent with the results of a double and triple labeling study that showed that only about 80% of SOM neurons also expressed NPY and that only 73% of neurons immunoreactive for SOM, NOS, NADPH-diaphorase or NPY expressed all four peptides (Figueroed-Cardenas et al., 1996). Therefore the proportion of striatal neurons comprised of PLTS cells would be somewhere between 0.55 and 0.8%. It is unclear if the different combinations of co-expression of SOM, NOS, NADPH-diaphorase and NPY are associated with different electrophysiological and/or morphological phenotypes.

B. Afferents and Efferents

Striatal PLTS neurons receive a monosynaptic excitatory input from the cortex (Kawaguchi, 1993) as well as a dopaminergic innervation, presumably from substantia nigra (Kubota et al., 1988; Li et al., 2002). They are also the target, along with PV/FS interneurons, of PV/FS afferents from globus pallidus (Bevan et al., 1998) and cholinergic inputs from striatal cholinergic interneurons.

C. Basic Membrane Properties

SOM/NPY interneurons were first described by Kawaguchi in whole cell recordings from young rats (Kawaguchi, 1993). These cells were initially distinguished from spiny neurons and from the other GABAergic interneurons by the presence of a low threshold Ca\(^{2+}\) spike (LTS) that could be elicited by intracellular depolarization or synaptic stimulation delivered at the resting membrane potential, and by the expression of long-lasting depolarizing plateau potentials (P) that occurred following depolarizing current injections, sufficiently strong excitatory synaptic stimulation or as a rebound upon cessation of a hyperpolarizing current injection and were termed PLTS neurons (Kawaguchi, 1993; cf Fig. 8.3D,E). PLTS cells were further characterized by a very high input resistance, more than seven times greater than that of the FS interneurons (638 ± 245 M\(\Omega\)), a resting membrane potential more than 20 mV more depolarized than that of the FS interneurons (−56.4 ± 15.7 mV), and long duration action potential (1.0 ± 0.41 ms at half amplitude; Kawaguchi, 1993; Kubota and Kawaguchi, 2000; Centonze et al., 2002).

Although Kawaguchi (1993) originally termed these cells PLTS interneurons, more recently most authors, including Kawaguchi and colleagues, have dropped the P from the electrophysiological acronym for the striatal NPY-NOS immunoreactive neurons that display an LTS and plateau potentials, and refer to them simply as LTS neurons (e.g., Kawaguchi et al., 1995; Kubota and Kawaguchi, 2000; Centonze et al., 2002).

D. Synaptic Connectivity

The only available data on the connectivity of PLTS neurons comes from a few recordings of synaptically connected PLTS interneurons and spiny neurons. These recordings show that intracellular stimulation of the PLTS neuron that evokes a single spike elicits an IPSC in the spiny cell that shows very little amplitude variability, perhaps suggesting that each PLTS neuron makes only one or a very limited number of synapses with target spiny neurons (Koós, 2000; Tepper and Bolam, 2004; Tepper et al., 2008; Fig. 8.3G), a suggestion that is consistent with the sparse and longitudinal morphology of the PLTS axonal arborization (Fig. 8.3F).

A recent in vitro study showed that stimulation of striatal cholinergic interneurons, or a single cholinergic neuron leads to activation of a recurrent network that results in IPSCs in the stimulated cholinergic neuron (Sullivan et al., 2008). Pharmacological studies showed that the IPSCs were GABA\(_A\) IPSCs. Furthermore, the IPSCs were eliminated by repetitive stimulation and/or by antagonists of nicotinic receptor \(\beta_2\) subunits, suggesting that the cholinergic interneuron activated a striatal GABAergic neuron via a \(\beta_2\)-subunit containing nicotinic receptor that then synapsed back onto the cholinergic interneuron and inhibited it. FS interneurons, but not spiny neurons are known to express nicotinic receptors; however these are non-desensitizing unlike the receptor mediating the recurrent feedback to the cholinergic interneuron. Thus, the GABAergic intermediary is some type of striatal GABAergic interneuron other than the FS interneuron. Although this interneuron is still unidentified, there are cholinergic inputs to the PLTS interneuron (Vuilet et al., 1992), whose other properties make it an excellent candidate for the GABAergic interneuron responsible for the recurrent inhibition in the cholinergic interneuron (Sullivan et al., 2008).

E. Spontaneous Activity

There are scant data on the spontaneous activity of PLTS interneurons. Despite their depolarized resting membrane
FIGURE 8.3 Striatal SOM/NOS/NPY GABAergic PLTS interneurons. A,B,C. Fluorescent immunocytochemistry of mouse striatum with an antibody against NOS. D. Typical PLTS neuron recorded in slice from an adult mouse displays the very high input resistance, depolarized resting membrane potential, sag in response to hyperpolarizing current injections and the LTS and sustained plateau depolarizations (arrow) following relaxation of hyperpolarizing current injections that are characteristic of PLTS neurons. E. The same neuron, held hyperpolarized at -80 mV, responds to a depolarizing current injection with a plateau depolarization (arrow). F. Drawing tube reconstruction of PLTS neurons from rat striatum filled with biocytin after recording shows that sparsely branching, mostly linear axonal arborization previously described for these neurons. Soma and dendrites are in black and the axonal arborization is in red. G. Paired recording of the cell reconstructed in F with a spiny neuron showing a monosynaptic connection with low failure rate and little amplitude variability. H. Typical PLTS neuron recorded in slices from adult rat striatum exhibits same characteristics illustrated in the mouse PLTS neuron shown in D. Compare with the LTS neuron illustrated in I. The LTS neuron recorded from a slice of adult rat striatum has approximately one fourth the input resistance of the PLTS neuron, lacks the prolonged plateau potentials, and has a narrower duration spike with a biphasic AHP. J. Short burst of 3 spikes in a presynaptic LTS interneuron delays or completely aborts depolarization-evoked spiking in a postsynaptic spiny neuron. Sources: F. Modified from Tepper et al., 2008; G, I: Modified from Tepper and Bolam, 2004; J. Modified from Kocsis and Tepper, 1999. To view a color version of this image please visit http://www.elsevierdirect.com/companion/9780123747679.
potential, they are not spontaneously active in vitro, and there is no way to identify them from extracellular recordings in vivo.

F. Pharmacology

PLTS interneurons are depolarized by bath application of dopamine, an effect that is blocked by the D1-like antagonist, SCH-23390. The depolarization is sufficient to trigger spiking and is associated with a decrease in membrane conductance (Centonze et al., 2002). More than 75% of striatal SOM+ neurons are also immunoreactive for the D5 dopamine receptor (Rivera et al., 2002) whereas most show no D1 mRNA expression and those that do express only low levels of the message (Le Moine et al., 1991). Thus, like the PV+ interneurons, the excitatory effects of dopamine on PLTS interneurons are mediated by D5 receptors.

IV. LTS NEURONS

Another striatal interneuron that expresses low threshold Ca\(^{2+}\) spikes has also been described, and termed an LTS interneuron (Koós and Tepper, 1999). The principal differences between the PLTS and LTS neurons were the absence of the persistent plateau depolarizations in the LTS neuron, either upon depolarization or following the cessation of a hyperpolarizing current injection, and a significantly lower input resistance (\(>600\, \text{M} \Omega\)) for PLTS [Kawaguchi, 1993] vs. \(<200\, \text{M} \Omega\) for LTS). In addition, these authors also occasionally observed PLTS neurons with the same characteristics described by Kawaguchi (1993) in the same slices as those in which LTS neurons were recorded. Only a few examples of this cell type were recorded, and none was recovered so their morphology and peptide expression remain unknown. Although it is possible that the LTS neurons and the PLTS neurons represent the extremes of a distribution of properties of a single neuronal type as pointed out by Koós and Tepper (1999), the differences were significant enough to classify the LTS as a different cell type, as can readily be seen by comparing panels H and I in Figure 8.3.

A. Synaptic Connectivity

Like the PV+ FS interneurons, paired recordings between LTS interneurons and spiny neurons revealed that LTS interneurons exerted a particularly strong inhibitory effect on spiny neurons. Small depolarizing pulses to LTS neurons evoked brief, high frequency (\(>200\, \text{Hz}\)) bursts of spikes riding on an LTS. These bursts were capable of delaying or aborting depolarization-induced spiking in postsynaptic spiny neurons (Koós and Tepper, 1999; Fig. 8.3J). These characteristics suggest that along with PV+ FS interneurons, LTS cells play a significant role in mediating powerful feedforward inhibition of spiny neurons.

V. CALRETININ INTERNEURONS

Calretinin (CR)-expressing interneurons are medium sized (12–20 μm in diameter). They issue a small number of smooth, aspiny dendrites that branch sparingly and taper into very thin processes (Bennett and Bolam, 1993). All the anatomical information we have is from immunocytochemical studies as there have been no recordings or biocytin labeling, so little is known about the detailed neurocytology of rat CR+ neurons. Examples are shown in Figure 8.4. CR+ interneurons make up a similar proportion of neurons in the rodent striatum as the PV+, FS and SOM/NPY GABAergic interneurons, about 0.8% (Rymar et al., 2004). However, in primates including humans, the proportion of CR+ neurons is much greater and outnumbers that of PV+ and SOM/NPY interneurons by 3 or 4 to 1 (Wu and Parent, 2000). Furthermore, in the human striatum, there are four morphologically distinct types of neurons that express CR (Prens et al., 1998). Neonatal hypoxia results in the neurogenesis of CR+ striatal interneurons in rats that persists for at least 5 months after induction. Interestingly the neurogenesis appears limited to the CR+ interneurons since there is no neurogenesis of striatal neurons that express markers for any of the other striatal interneurons or projection neurons (Yang et al., 2008).

Almost nothing is known about the neurophysiology of the calretinin interneurons. There have been no recordings of these cells in vitro to date, and no way to identify them from in vivo recordings.

VI. OTHER GABAERGIC INTERNEURONS:
TYROSINE HYDROXYLASE-IMMUNOREACTIVE NEURONS

In addition to the classically recognized striatal interneurons described above, there appears to be (at least one) additional population of neurons in the striatum. Originally identified in the striatum of adult monkeys by tyrosine
hydroxylase (TH) immunocytochemistry (Dubach et al., 1987), striatal TH-immunoreactive (TH+) neurons have subsequently been reported in a number of other species including rat (Tashiro et al., 1989a,b; Meredith et al., 1999), mouse (Mao et al., 2001; Petroske et al., 2001), monkeys (Betarbet et al., 1997; Mazloom and Smith, 2006) and man (Cossette et al., 2005; Huot and Parent, 2007).

In addition to TH, others have reported the existence of striatal neurons immunoreactive for l-aromatic acid decarboxylase (AADC) in normal rats (Tashiro et al., 1989a; Mura et al., 1995, 2000; Meredith et al., 1999), as well as neurons immunoreactive for dopamine itself. Investigators who have examined two or more of these markers generally agree that there are more TH+ neurons than AADC+ or DA+ neurons in the striatum of rodents (Mura et al., 1995, 2000; Meredith et al., 1999), suggesting that not all striatal TH+ neurons are dopaminergic, and further, that there are at least two distinct populations of striatal TH+ neurons.

It is difficult to summarize the literature on the morphology and incidence of the striatal TH+ neurons because the description of both are surprisingly variable, ranging from “in the tens of thousands” (Dubach et al., 1987) to a low of about 1 TH+ neuron per section in humans (Huot et al., 2007). Other estimates range from to several 10s of neurons per 30–60 μm section in rat (e.g., DA+ neurons in Mura et al., 2000) or monkey (TH+/DA+ neurons in Tandé et al., 2006) to very large numbers in some studies (>450,000 TH+ neurons/striatum in primate; Palfi et al., 2002).

Reports concerning the somatic size and neurocytology of striatal TH+ neurons are equally disparate with cells being reported as small as 6–12 μm in diameter (Meredith et al., 1999; Jollivet et al., 2004), 8–12 μm (Dubach et al., 1987; Mazloom and Smith, 2006) or up to 20 μm in diameter (Tashiro et al., 1989b). There is further disagreement about the nuclear envelope, which is often used to identify neurons as projection neurons or interneurons since the spiny projection neurons always have smooth, non-invaginated nuclei whereas the PV+, CR+, and NOS/NPY GABAergic interneurons described above consistently show invaginated nuclei (Bolam et al., 1983). Some investigators claim that striatal TH+ neurons have the invaginated nuclei of interneurons (e.g., Dubach et al., 1987; Mazloom and Smith, 2006) while others claim that they possess the smooth nuclear envelopes of spiny projection neurons (Meredith et al., 1999). While most of the papers cited above report that striatal TH+ neurons exhibit smooth, sometimes varicose aspiny dendrites characteristic of other striatal interneurons, one claims that striatal TH+ neurons are spiny, express substance P or enkephalin, and are therefore spiny projection neurons (e.g., Darmopil et al., 2008).

In addition, striatal TH+ neurons are often described as immunoreactive for the dopamine transporter (DAT) as well as GABA or GAD67, suggesting that they are both dopaminergic and GABAergic (e.g., Betarbet et al., 1997; Cossette et al., 2005; Mazloom and Smith, 2006; Tandé et al., 2006; Huot and Parent, 2007). In primates, 8% of the striatal TH+ neurons colocalized CR+ (Mazloom and Smith, 2006).

Some investigators argue that TH+ neurons are not present in the striatum of control animals at all, but only appear after 6-OHDA or MPTP lesions of the nigrostrial system in rat (Meredith et al., 1997; Lopez-Real et al., 2003; Darmopil et al., 2008) or monkey (Mazloom and
Smith, 2006), while still others argue that they are not present at all in control or lesioned rat or mouse striatum, but only in primates (Dubach et al., 1987; Betarbet et al., 1997; Yang et al., 2008).

It is obviously difficult to know what to make of these disparate findings. Some of the discrepancies can almost certainly be attributed to species differences, while others may be the result of different technical artifacts associated with fixation, preservation and the vagaries of immunocytochemistry. It is clearly difficult to pick out TH-immunostained somata in the striatal neuropil from the background of dense staining of TH/H_1001 nigrostriatal dopaminergic axons and terminals, and this undoubtedly contributes to the discrepant results. There is, however, a general consensus, at least based on the most recent primate data, that striatal TH+ neurons do exist, and represent a novel class or classes of striatal interneuron that also express the dopamine transporter (DAT) as well as GAD, and are therefore likely both dopaminergic and GABAergic. These neurons appear to be distinct from the other, previously defined striatal PV+/CR+ or NOS/NPY+ GABAergic interneurons (Betarbet et al., 1997; Cossette et al., 2005; Huot and Parent, 2007).

A. Striatal EGFP-TH+ Interneurons

Until recently, there has been no way to identify striatal TH+ neurons in brain slices in order to obtain recordings from them that would allow description of their electrophysiological properties, afferent or efferent connectivity, or detailed morphology of the dendritic or axonal arborization. With the advent of strains of mice genetically engineered to express enhanced green fluorescent protein (EGFP) under the control of cell-type specific promoters such as TH, ChAT or PV (Gong et al., 2007), one can identify almost any cell type desired in a brain slice and then use IR-DIC optics to patch that neuron cell and record.

Using this approach in mice engineered to express EGFP in neurons that express TH, we have identified a population of EGFP-TH+ neurons in normal mouse striatum (Ibanez-Sandoval et al., 2007, 2008). These neurons appear to be distinct from the other, previously defined striatal PV+, CR+ or NOS/NPY+ GABAergic interneurons (Betarbet et al., 1997; Cossette et al., 2005; Huot and Parent, 2007).

**FIGURE 8.5** Striatal EGFP-TH interneurons. A. Low magnification fluorescence photomicrograph of a section through the striatum of a BAC transgenic mouse that expresses EGFP under the control of the TH promoter. Arrows point to some of the EGFP-TH+ neurons visible. B, C. Higher magnification photomicrographs of striatal EGFP-TH+ neurons. D. Drawing tube reconstruction of a striatal Type II EGFP-TH+ neuron filled with biocytin after recording in vitro. Note the varicose dendrites (black) and axonal arborization (red). E. Responses to current injections in a Type I EGFP-TH+ neuron. The extreme spike frequency adaptation leading to complete spike failure, the sag in response to hyperpolarizing current pulses and the high input resistance (upper inset) and wide duration action potential (lower inset) are all characteristic of this, the most common of the striatal EGFP-TH+ neurons. F. Some Type I neurons exhibit spontaneous activity in vitro. G. Local electrical stimulation (arrow) elicits GABA_A IPSPs sufficient to block spiking (left panel, red traces) as well as glutamatergic EPSPs sufficient to elicit spiking (middle panel, red trace) in Type I neurons. To view a color version of this image please visit http://www.elsevierdirect.com/companion/9780123747679

**Corpus callosum**

M

L

D

20 μm

V

+10 μM bicuculline + 10 μM DNQX

Local stimulation control

+10 μM bicuculline

+ 10 μM DNQX

EF Type I

Type I spontaneous activity

D

200 ms

2 ms

−69 mV

0 pA

100 pA

20 mV

20 mV

100 pA

20 mV

20 mV

20 mV

20 mV

20 mV

20 mV
Two of these cell types, Types II and III are a type of FS interneuron, whereas Type I neurons are characterized by a very strong spike frequency adaptation that leads to complete spike failure after 100 ms or so in response to strong depolarizing current injections. Type IV neurons fire low threshold spikes, but do not display the prolonged plateau depolarizations that characterize the NOS/NPY PLTS neuron described by Kawaguchi and others (Kawaguchi, 1993, Kawaguchi et al., 1995). This cell type is very similar to the LTS neuron described by Koós and Tepper (1999).

All the EGFP-TH+ interneurons are well integrated into the functional architecture of the striatum. Local stimulation elicits EPSPs and IPSPs and paired recordings show that the most common efferent target is the spiny projection neurons. Evoked spiking in striatal EGFP-TH+ neurons produces potent IPSP/Cs in spiny neurons sufficient to delay spikes evoked by intracellular depolarization. The IPSP/Cs are blocked by picrotoxin or bicuculline demonstrating that the EGFP-TH+ neurons are also GABAergic (Ibanez-Sandoval et al., 2008). Their dopaminergic nature has yet to be conclusively verified.

VII. SUMMARY AND CONCLUSIONS

Striatal GABAergic interneurons participate in a powerful feedforward inhibitory circuit that is likely the primary mechanism by which the spike timing of the striatal output neurons is controlled. Of the three types of striatal GABAergic interneurons that have been recognized for some time, we have the most information about the PV+ FS interneurons and the NOS/NPY+ PLTS interneurons, since they have been identified, recorded and intracellularly labeled in vitro. Although we tend to consider the PV+ fast spiking interneuron as the striatal fast spiking interneuron, there are clues from various studies in striatum of rodents and primates, both older and more recent, that there may well be more than a single type of striatal FS interneuron, and perhaps even more than one type of PV+ striatal fast spiking interneuron.

Similarly, it is unclear if the neuron originally described by Kawaguchi (1993) as the PLTS interneuron is the striatal GABAergic interneuron that fires low threshold Ca²⁺ spikes and whether or not all such neurons express the same neurochemical makeup. Recent data from several sources suggest otherwise.

We know very little about the physiological role and detailed neurocytology of the CR+ interneuron, since as of this writing, no one has yet succeeded in recording from these cells and filling them. This will undoubtedly change in the very near future as soon as a BAC transgenic mouse that expresses EGFP selectively in CR+ neurons is created. Nevertheless, the finding that there are 4 morphologically distinct types of CR+ neurons in human striatum indicates the possibility that there is more than one functional subtype of striatal CR+ GABAergic interneuron.

A BAC transgenic mouse that expresses EGFP selectively in neurons with the TH promoter has recently allowed the description of four electrophysiologically distinct subtypes of striatal EGFP-TH+ interneurons, three of which display electrophysiological characteristics that are completely unlike those of any previously described striatal neuron.

It is becoming clear that the striatum enjoys a much richer diversity of GABAergic interneurons than was once thought. We are only now beginning to discover the functional differences between the varieties of these interneurons. As new molecular, anatomical and physiological techniques continue to become available, particularly more strains of transgenic mice that selectively label neurons that express calretinin and other peptides, receptors and neuroactive agents expressed by striatal neurons with fluorescent markers such as EGFP, it is almost certain that the number of functionally different striatal GABAergic interneurons, currently numbering at least 8, will continue to grow.

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