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CHAPTER 3

In vivo studies of the postnatal development of rat neostriatal neurons

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Introduction

The electrophysiological and morphological properties of the mammalian neostriatum have been the subject of numerous studies over the past 25 years. Of the many different cell types in the neostriatum identified by Golgi staining or intracellular labeling (e.g., Kemp and Powell, 1971a; DiFiglia et al., 1976; Bishop et al., 1982; Chang et al., 1982), the most information is known about the medium spiny neuron, the principal output neuron of the nucleus, and the neuron that comprises up to 95% of the cell population in the rat neostriatum (for review, see Wilson, 1990). The spontaneous activity and synaptic responses of the medium spiny neuron have been shown to be dominated by certain aspects of its passive membrane properties as well as by the morphological and physiological organization of its extrinsic excitatory inputs. The relatively low input impedance and sparse and bursty spontaneous activity of these neurons result in large part from a very considerable fast anomalous rectification (Wilson, 1992, this volume). The characteristic response of neostriatal neurons to stimulation of their principal excitatory cortical or thalamic afferents is complex, consisting of an early EPSP composed of both mono- and polysynaptic components, a subsequent long-lasting hyperpolarization, and a late depolarization, and is virtually identical in all species studied (Buchwald et al., 1973; Wilson et al., 1983; Herrling, 1984; Wilson, 1986; Calabresi et al., 1990).

Despite the considerable literature on the neurophysiology of neostriatal neurons in the adult rat, relatively little is known about the electrophysiological properties of neostriatal neurons in neonates, or the time course of the postnatal development of mature physiology and morphology. There are some data from extracellular recording experiments which suggest that there is little or no spontaneous activity in rat neostriatum prior to postnatal day 10 (P10; Napier et al., 1985; Tepper et al., 1990a,b), and in vitro intracellular recording experiments have revealed important differences in passive membrane properties and synaptic responses to local stimulation in neonates versus adults (Misgeld et al., 1986). There have also been a number of studies of the postnatal development of the basal ganglia in kittens (Adinolfi, 1977; Morris et al., 1979; Hull et al., 1981; Levine et al., 1982); compared to rat, however, the feline basal ganglia is considerably more developed at birth, and displays a more protracted postnatal development.

There have been almost no reports of the postnatal development of the neurophysiological properties of rat neostriatal neurons in vivo. Such information is important for several reasons. The rat is the most common experimental subject for studies of

the anatomy and physiology of the basal ganglia, and more is known about the basal ganglia of the adult rat than that of any other species. In addition, in recent years there has been intensive research in the field of neuronal grafting. The most successful grafting experiments have been performed in rats, in which suspensions of embryonic neurons have been transplanted into the brains of adult hosts (see Gage and Fisher, 1991, for a recent review). Such grafts have been demonstrated to survive in the host, and to establish both afferent and efferent synaptic connections with host neurons (Rutherford et al., 1987; Walsh et al., 1988; Wictorin et al., 1989, 1990; Fisher et al., 1991; Xu et al., 1989, 1991a,b). Although early studies of the neurophysiological and morphological properties of fetal neurons grafted to adult hosts emphasized the similarity of the electrophysiological properties among the grafted neurons and their in situ counterparts (e.g., Wuerthele et al., 1981; Arbuthnott et al., 1985; Rutherford et al., 1987), more recent studies have found significant differences between various types of neurons in situ and those that were transplanted, even after the grafts have been allowed to mature for several months in the host (Walsh et al., 1988; Fisher et al., 1991; Xu et al., 1991b). It is possible that the differences between in situ and graft cells may arise from a functional immaturity on the part of the graft neurons themselves, but before concluding this, a characterization of the physiological and morphological properties of early postnatal neurons in vivo is required. In this chapter, we will review some recent experiments detailing the electrophysiological and morphological characteristics of neostriatal neurons in neonatal rats, and compare and contrast these properties to those observed in the neostriatum of adults.

Methods

The subjects for all experiments were Sprague-Dawley rat pups ranging in age from postnatal day 6 (P6) through P49, and adult male Sprague-Dawley rats. All rats were bred at the Institute of Animal Behavior at Rutgers from stock obtained from Charles River. Both pups and adults were anesthetized with urethane (1.2 – 1.5 g/kg, i.p.). When necessary, adults were supplemented with ketamine (20 – 30 mg/kg, i.m.), and pups were supplemented by inhalation of metofane. Adults and pups greater than 21 days of age were installed into a stereotaxic frame and prepared for intracellular recording by conventional means (see Tepper et al., 1987, for details). Younger neonates were affixed to a modified stereotaxic apparatus by a modification of the method originally described by Nakamura et al. (1987). Briefly, pups were placed on a custom stage designed to hold their head parallel to the stereotaxic frame bars. After removal of the scalp, a small 3 cm stainless steel rod which had short lengths of 3 mm o.d. stainless steel tubing soldered to the ends was affixed to the top of the skull in the coronal plane approximately 3.5 mm anterior to lambda with cyanoacrylate glue and dental cement. Standard stereotaxic earbars were inserted into the hollow tubes, and the pups' four extremities were affixed to the stage with cyanoacrylate glue. To minimize respiratory artifacts and stabilize the preparation, pups were suspended with a small tail clamp. Body temperature was maintained at 37 ± 1°C by a solid state feedback-controlled heating pad.

Microelectrodes were pulled from 2.0 mm o.d. capillary tubing, filled with 1 M potassium acetate containing 3% biocytin (Horikawa and Armstrong, 1988) and possessed in vivo impedances between 75 and 90 MΩ. All other aspects of stimulating and recording have been described in detail previously (Tepper et al., 1987, 1990a,b).

Following the electrophysiological experiments, an overdose of urethane was administered, and rats were perfused with 20 – 50 ml of isotonic saline followed by 50 – 250 ml of 4% paraformaldehyde/0.2% glutaraldehyde in 0.15 M sodium phosphate buffer, pH 7.4. The brains were removed, left in the same fixative overnight, and sectioned on a Vibratome® at 60 μm and reacted for the presence of biocytin by the methods of Horikawa and Armstrong (1988).

Results

Membrane properties

Neostriatal medium spiny neurons recorded in vivo or in vitro display a prominent, fast inward rectification in response to both depolarizing and hyperpolarizing current pulses (Kita et al., 1984; Kawaguchi et al., 1989; Calabresi et al., 1990) that contributes significantly to the electrophysiological responses of these neurons in the adult (see Wilson,

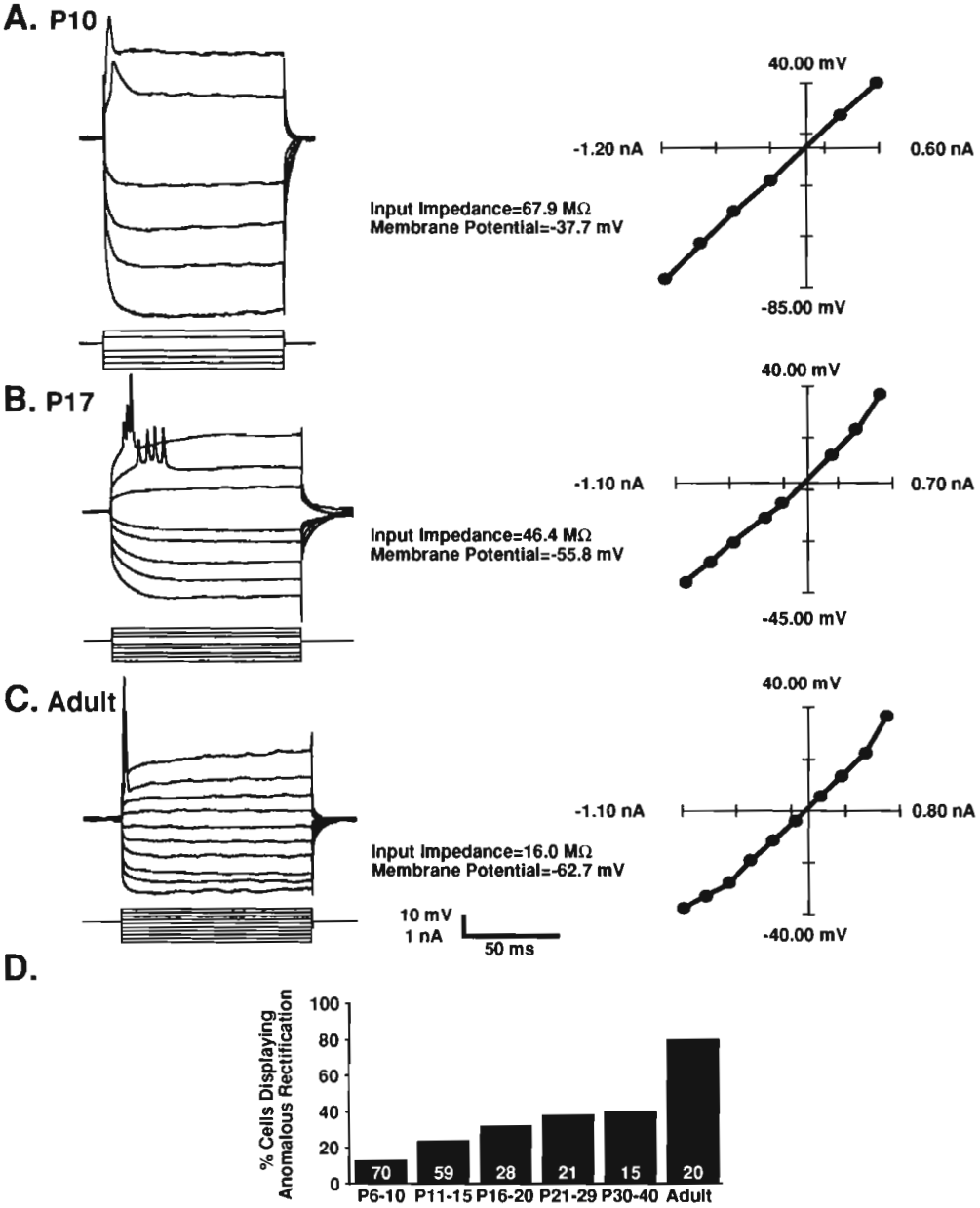


Fig. 1. Neonatal neostriatal medium spiny neurons do not display anomalous rectification. *A*. Typical membrane responses evoked by intracellular current pulses in a P10 pup. Note the linear current – voltage relation. *B*. Neuron from a P17 pup displays a modest anomalous rectification in both depolarizing and hyperpolarizing directions. *C*. Adult medium spiny neuron exhibits marked anomalous rectification. Note trend towards decreasing input resistance and increasing resting membrane potential over development. *D*. Summary graph of the postnatal development of anomalous rectification. Numbers within bars indicate number of neurons per group. Each trace is the average of four single sweeps.

1992, for a recent review). One of the most striking aspects of the membrane characteristics of neonatal neostriatal neurons is the complete absence of this anomalous rectification in a large majority of the neurons (Trent and Tepper, 1991). Prior to P11, less than 13% of neostriatal neurons (n = 70) exhibit the marked inward rectification in either hyperpolaring or depolarizing directions, and most of the neurons display fairly linear current – voltage relations, as illustrated for one representative neuron in Fig. 1*A*. There is steady increase in the proportion of neurons that exhibit anomalous rectification over the next three weeks, with approximately 40% of neurons from the P21 – P29 (n = 21) and P30 – P40 (n = 15) groups showing this property compared to over 80% (n = 20) in adults as summarized in Fig. 1*D*.

A smaller fraction (i.e., 22%) of neostriatal neurons from the first and second postnatal weeks exhibit I – V curves that are dominated by a marked *outward* rectification in both the depolarizing and hyperpolarizing directions. In many cases the outward rectification in response to hyperpolarizing current injections was so extreme that the apparent input resistance rose above 100 MΩ, as illustrated for one neuron in Fig. 2*A*. This was not due to a gross electrode non-linearity as routine extracellular checks of microelectrode linearity performed both prior to impalement and after exiting neurons showed that these electrodes were very linear over a range exceeding ± 1.5 nA. This outward rectification is extremely atypical for adult neostriatal neurons recorded either in vivo or in vitro, as mentioned above, and was never observed in the present studies in animals older than P20.

A third difference in membrane properties between neonatal and mature medium spiny cells is the presence of a transient depolarizing potential (TDR) that appeared as a hump near the onset of intracellularly evoked membrane depolarizations (Trent et al., 1992) as shown in Fig. 3*A*. The mean maximal amplitude of the TDR evoked from rest was 18.1 ± 1.6 mV (mean ± S.E.M.) (n = 40) and mean duration was 23 ± 1 msec (n = 44). The TDR was observed in 56% of neurons from P6 – P10 pups (n = 73), 47% in P11 – P15 pups (n = 68), 6% in P16 – P29 pups (n = 49), and was never observed in older pups or adults as summarized in Fig. 3*C*. The TDR was voltage-sensitive; in vivo it was activated at membrane potentials more negative than – 37 mV, and could be inactivated by holding the membrane potential more positive than – 40 mV. The frequency and maximal amplitude of the TDR was increased by hyperpolarizing prepulses, suggesting that the conductance(s) responsible are partially inactivated at rest. Preliminary

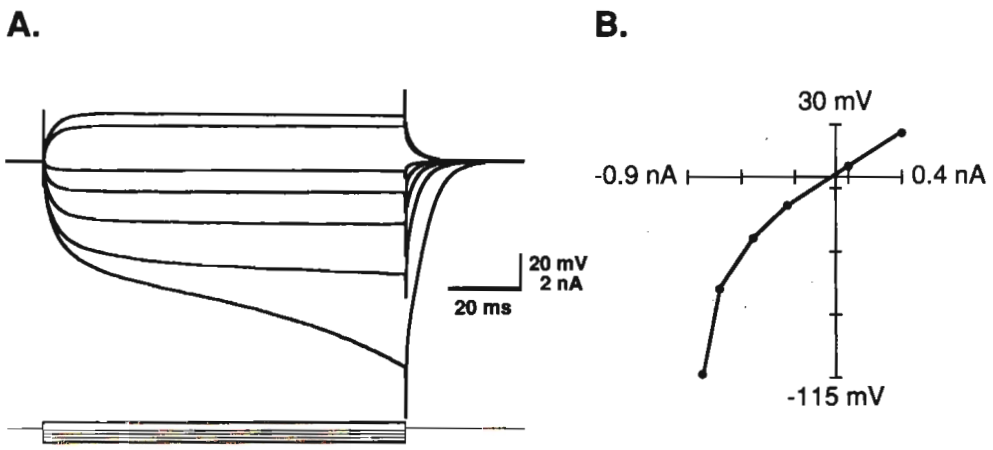


Fig. 2. Less typical type of current – voltage relation displayed by 22% of early neonatal neostriatal neurons. *A*. Responses to intracellular current injections in a P11 pup reveals an extraordinary degree of outward rectification in response to modest hyperpolarizing current pulses. *B*. I – V plot of the data in *A*. Each trace is the average of six single sweeps.

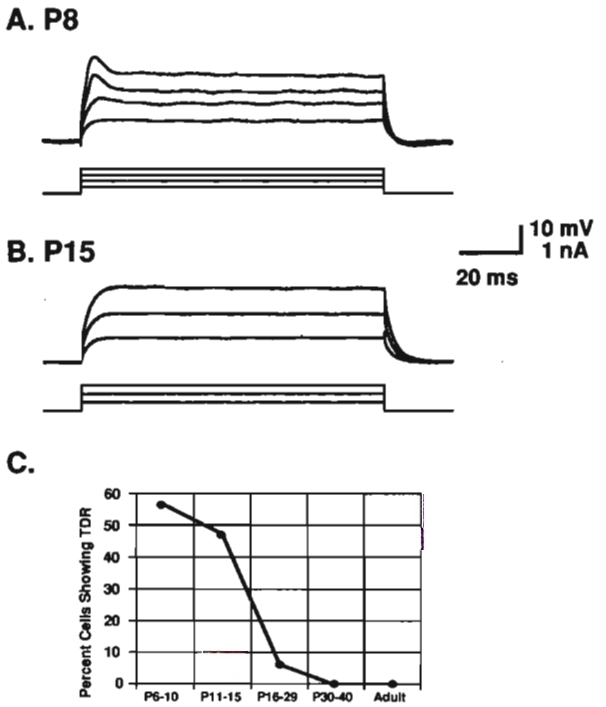


Fig. 3. Neonatal neostriatal neurons possess a transient voltage-dependent conductance that is activated by intracellular injection of depolarizing current. *A*. This transient depolarizing potential (TDR) is indicated by the "hump" present near the start of the pulse in a P8 pup while the same paradigm fails to evoke a TDR in neostriatal neurons from older animals. *C*. The proportion of neostriatal neurons exhibiting the TDR decreases over the first postnatal month and is never observed in animals ≥P30. Each trace is the average of four single sweeps.

results from in vitro experiments indicate that the TDR is not affected by 1 μm tetrodotoxin, but is abolished by 500 μm cadmium, suggesting that it represents an inward calcium current. A calcium-dependent phenomenon very similar to the TDR has been observed in recordings from 10-day-old rat dorsal horn neurons in vitro (Murase and Randic, 1983); the nearly identical amplitude (13.8 + 3.1 mV) and duration (26.5 + 4.0 msec) suggest that the TDR may be a manifestation of a low threshold calcium spike in neonatal neostriatal neurons. It is interesting to note that most neurons that exhibit the TDR also exhibit a long duration spike following relaxation of strong hyperpolarizing current pulses that is similar to the low threshold spike described for mature thalamic neurons in vitro (Jahnsen and Llinás, 1984). Although mature neostriatal neurons do exhibit some calcium-dependent physiological properties (e.g., Galarraga et al., 1989), neither low threshold spikes nor TDR-like phenomena are commonly observed under normal physiological conditions. The apparent loss of the TDR over development may be related to a more general phenomenon in which voltage-activated currents carried by calcium are prominent in the early development of neurons but disappear during pre- and postnatal development (Spitzer, 1982).

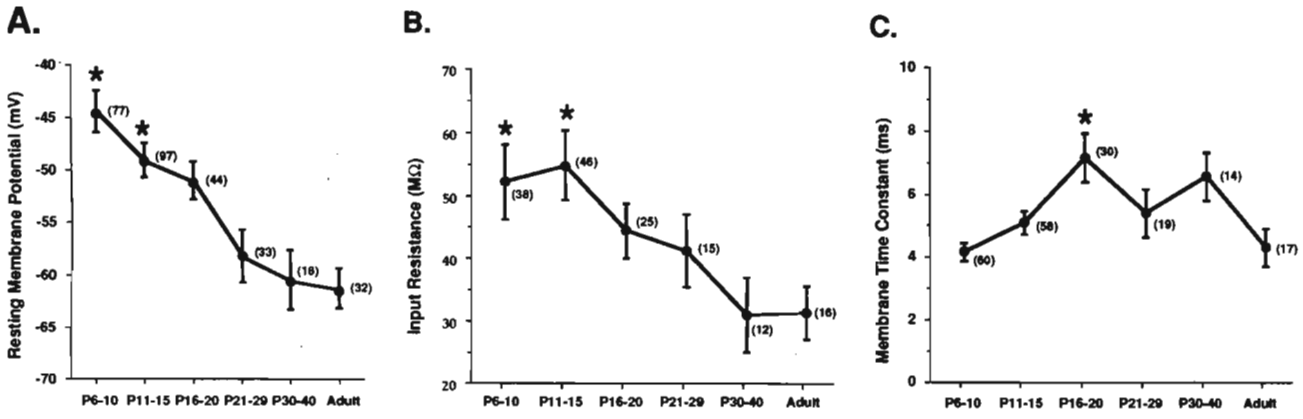


Fig. 4. The resting membrane potential, input resistance and time constant of neostriatal neurons are developmentally regulated. *A,B*. There is a progressive increase in the resting membrane potential over the first three postnatal weeks concomitant with a decrease in membrane input resistance. *C*. The membrane time constant, τ, develops non-linearly. In early neonates, τ initially increases steadily and reaches a peak during the third postnatal week and subsequently decreases to values observed in adults. Numbers within bars indicate number of neurons per group. Error bars represent S.E.M. Asterisks indicate significant difference from adult groups (Schéffé, $P < 0.1$).

The resting membrane potential shows a marked dependence on development, as shown in Fig. 4*A*. From a mean around −44 mV during the first postnatal week, the average resting membrane potential increases by almost 50% by the end of the fourth week when it no longer differs from that of adults. This change in membrane potential and the appearance of anomalous rectification described above appear along with a progressive decrease in membrane input resistance as illustrated in Fig. 4*B*, although this measurement, and that of the time constant may be complicated by the anomalous rectification itself as well as a significant somatic shunt encountered in recording these neurons (Wilson, 1984; Bargas et al., 1988). The membrane time constant, measured by peeling exponents from small intracellularly injected hyperpolarizing transients, also varies significantly during postnatal development from around 4 msec at P6–P10 to a maximum over 7 msec at P16–P20 compared to an adult value around 4 msec, as shown in Fig. 4*C*.

Responses to cortical and thalamic stimulation

Excitatory responses to both cortical and thalamic stimuli could be observed by P6, the earliest age at which good quality intracellular recordings could be obtained. As shown in Fig. 5, the most typical response to cortical or thalamic stimulation in young neonates (< P21) consisted of a relatively simple EPSP, lacking both the subsequent long-lasting hyperpolarization and late depolarization that is characteristic of responses to identical stimuli in the adult (Buchwald et al., 1973; Wilson et al., 1983). Although never observed in animals younger than P12, the long-lasting hyperpolarization and late depolarization were simultaneously first observed near the middle of the third postnatal week in a small proportion (i.e., 15%; $n = 41$) of neurons. The proportion of neurons exhibiting this triphasic response to cortical or thalamic input increased steadily but still had not reached adult frequencies by the end of the sixth postnatal week as shown in Fig. 5*B*.

The initial EPSP itself showed both age-dependent and age-independent features, as illustrated in Fig. 6. Although neither the mean maximal amplitude (about 10 mV) nor duration (about 50 msec) changed as a function of postnatal development, the onset latency was significantly longer in neonates than adults ($F = 17.8$, $df = 5,216$, $P < 0.001$), and the rise time was significantly shorter in pups less than P15 than in older neonates or adults ($F = 3.4$, $df = 5,159$, $P < 0.01$). Even in the youngest neonates, the initial EPSP consisted of both monosynaptic and polysynaptic components as previously described for adult neostriatal neurons (Wilson, 1986). In contrast to the situation obtained in adult neostriatum, the cortically evoked EPSP

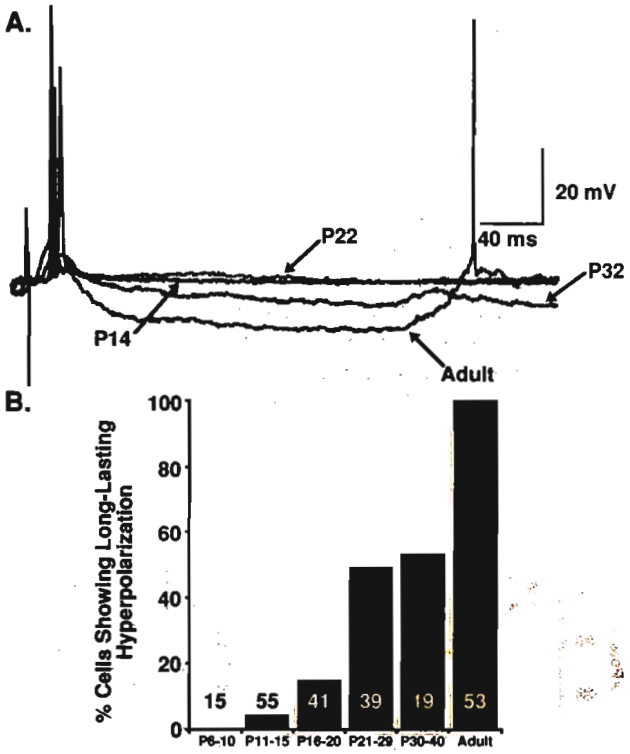


Fig. 5. Cortically evoked responses elicited by neostriatal neurons increase in complexity over the postnatal period. *A*. Stimulation of ipsilateral prefrontal cortex evokes a simple EPSP lacking the late long-lasting hyperpolarization and rebound depolarization in young neonates (P14, P22) that later develops into the characteristic triphasic response (P32, adult). *B*. The late long-lasting hyperpolarization in response to cortical stimulation does not appear in a significant proportion of neurons until the third postnatal week. Numbers within bars indicate numbers of cells tested.

could be completely reversed by intracellular injection of depolarizing current in many neonates.

Approximately one-third of the neostriatal neurons recorded in pups less than P15 (n = 94) exhibited an anomalous hyperpolarizing response to cortical stimulation, as illustrated for three representative neurons in Fig. 7. One of the response's most distinguishing characteristics was its time dependence, illustrated in Fig. 7*A*. If it was observed at all, the hyperpolarization was apparent immediately upon cell penetration. Over the next 5 – 10 min, the amplitude of the hyperpolarizing potential became progressively smaller until it disappeared altogether. This was not due to any apparent deterioration of the recording conditions as the input impedance did not change over this interval, nor to an increase in membrane polarization. It was likewise not dependent on repeated stimulation; the hyperpolarization decayed even if the neuron was not subjected to continuous stimulation from cortex or thalamus.

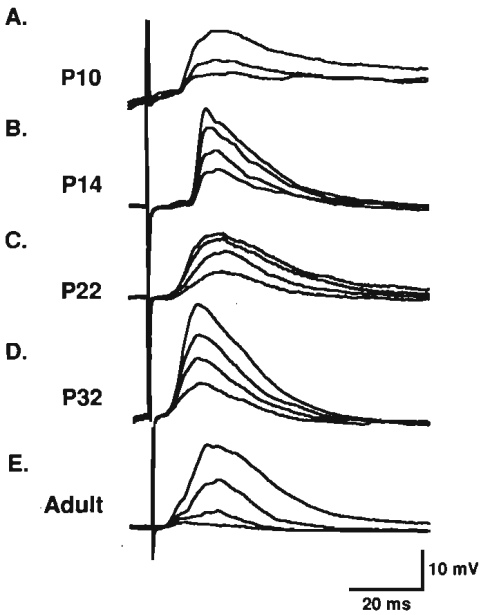


Fig. 6. Power series illustrating that the initial cortically elicited EPSP displays both age-dependent and age-independent characteristics. Although the mean maximal amplitude and duration remained constant over development, the rise time increased and the onset latency decreased as a function of age. Each trace is the average of four single sweeps.

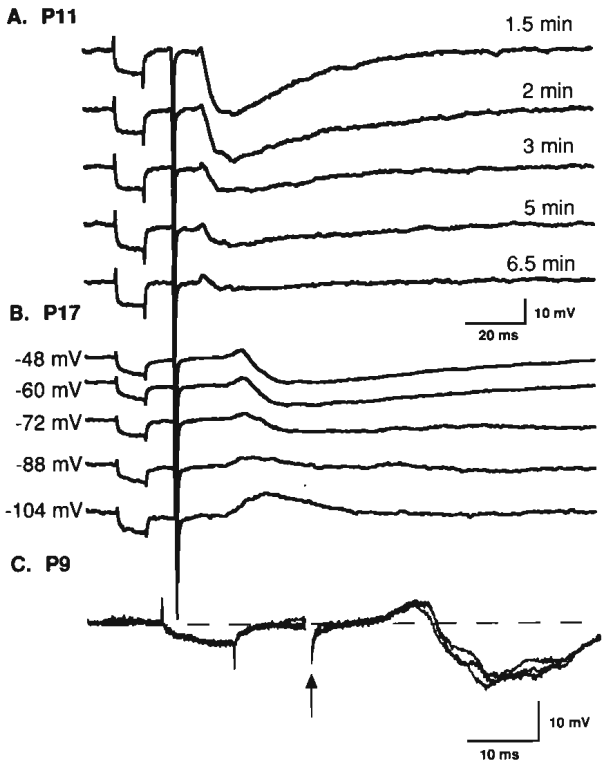


Fig. 7. Many neonatal neostriatal neurons display an anomalous hyperpolarizing response to cortical stimulation. *A*. Cortically evoked hyperpolarizations were transient phenomena that attained maximal amplitude immediately upon impalement and decayed in a time-dependent fashion over the next 5 – 10 min. *B*. The hyperpolarization could be decreased and reversed by intracellular injection of hyperpolarizing current indicating that it is a true IPSP. *C*. In most cases the IPSP was preceded by an EPSP that occurred several milliseconds prior to the IPSP's onset and at the expected latency for a cortically evoked EPSP. In *A* and *B*, each trace is the average of four single sweeps. *C* consists of the superimposition of three single sweeps.

This hyperpolarization was considered to be an IPSP since it exhibited a reversal potential of approximately −68 mV (n = 3), considerably more hyperpolarized than the spike threshold of these neurons, and consistent with its mediation by chloride ions shown in Fig. 7*B*. Most (> 80%) of these hyperpolarizing responses were clearly preceded by a small amplitude EPSP with a mean onset latency (9.1 ± 0.6 msec; n = 22) that was the same as that of the typical cortically evoked EPSP (9.3 ± 0.4 msec; n = 70) shown at high gain in Fig. 7*C*. The IPSP onset latency (13.1 ± 0.8 msec; n = 27) fol-

lowed that of the initial EPSP by approximately 4 msec. The IPSP was observed less and less frequently in older pups, and was never observed in pups greater than P23 or in adults.

Spontaneous activity

In adults, medium spiny neurons fire slowly and irregularly in a bursty pattern (Wilson and Groves, 1981). In vivo intracellular recordings show that the membrane potential alternates between a relatively hyperpolarized state, the "disabled" state around −80 mV and a depolarized or "enabled" state near −50 mV, and it has been argued that these two states result from afferent input and not intrinsic mechanisms (Wilson, this volume). In neonates, there is virtually no spontaneous activity until around P15. When spontaneous activity first appears, it is in the form of randomly occurring single spikes separated by long (up to several minutes) intervals. At this developmental stage, each neuron's resting membrane potential is extremely stable; the prolonged enabled and disabled periods are completely absent, and shorter duration fluctuations in membrane potential are greatly reduced in frequency. Over the next three weeks, the rate of spontaneous activity increases, and the neurons begin to fire in a bursty pattern typical of adult neostriatal neurons. The increase in firing rate and the change in firing pattern is accompanied by a gradual increase in the frequency and amplitude of "spontaneous" shifts in resting membrane potential, and by the fifth postnatal week the spontaneous activity appears essentially as it does in adults.

It is interesting to note that the manifestation of enabled and disabled states is concomitant with the development of the long-lasting hyperpolarization following cortical or thalamic stimulation. That is, through the end of the third postnatal week, the membrane potential remains relatively stable, and, as noted above, the long-lasting hyperpolarization following afferent excitation is absent. Over the next few weeks, some of the neurons begin to exhibit prolonged depolarizing and hyperpolarizing shifts in membrane potential and concomitant bursting activity, while others do not. Invariably, those neurons that displayed the shifts in membrane potential also exhibited a long-lasting hyperpolarization in response to cortical stimulation, whereas those neurons that failed to show prolonged depolarizing and hyperpolarizing episodes also failed to exhibit a long-lasting hyperpolarization, as illustrated for four representative neurons in Fig. 8.

As has been described previously for neostriatal and other central nervous system neurons (Misgeld et al., 1986; McCormick and Prince, 1987; Nakamura et al., 1987; Williams and Marshall, 1987; Michelson and Lothman, 1989; Tepper et al., 1990a,b), both spontaneous and evoked action potentials in early neonates exhibited longer dura-

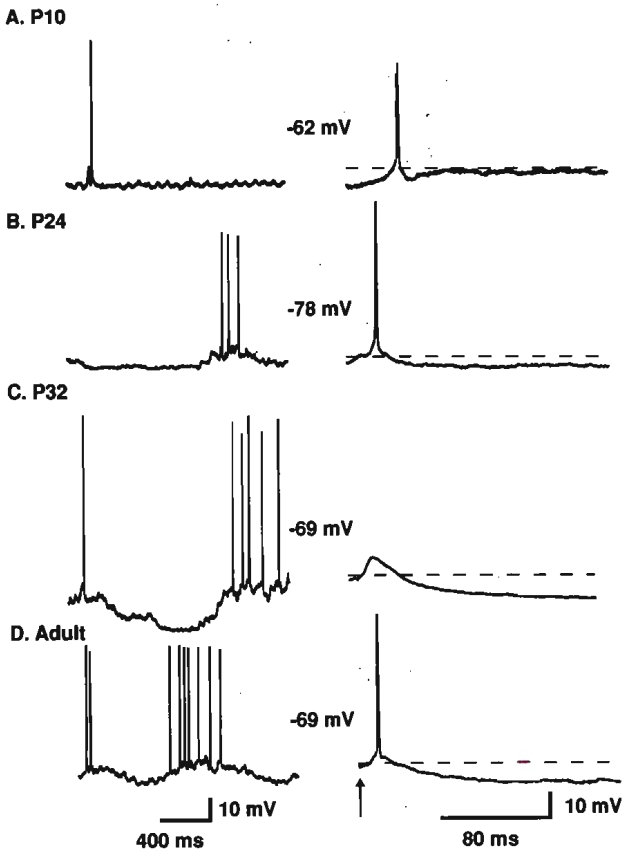


Fig. 8. There is a correlation between the occurrence of enabled and disabled states of membrane potential described by Wilson (this volume) (left) and the manifestation of the long-lasting hyperpolarization following cortical stimulation (right) over development. Arrow denotes application of cortical stimulus. Dashed line indicates pre-stimulus resting membrane potential. Numbers indicate resting membrane potential.

tions and decreased amplitudes compared to those in adults.

Neuronal morphology

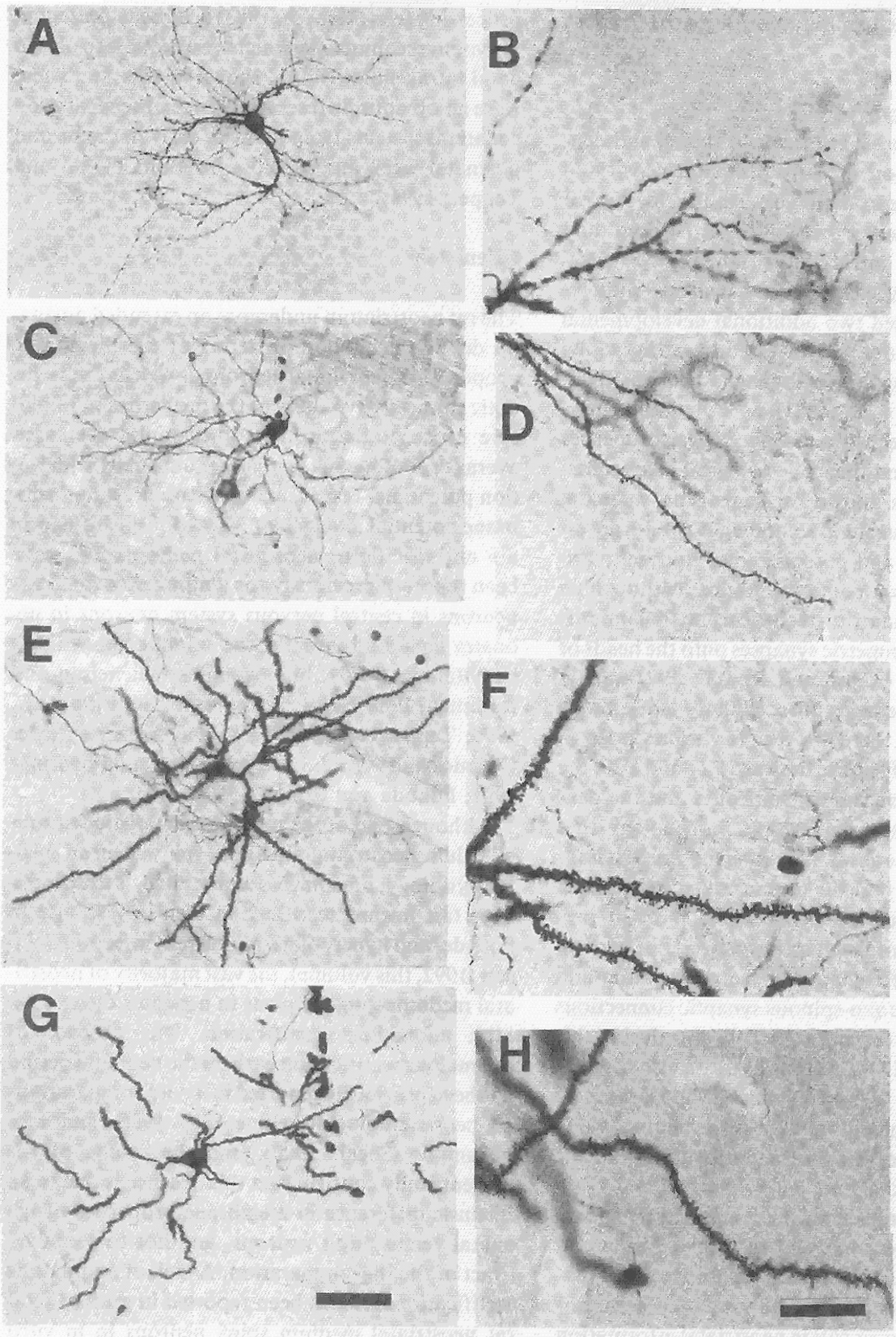
Although the basic cell size and shape, and dendritic configuration of early postnatal neostriatal medium spiny neurons is very similar to that of adults (Kemp and Powell, 1971a; Wilson and Groves, 1980), intracellular labeling of electrophysiologically characterized neurons with biocytin has revealed two additional developmental events that were not well-detected by earlier Golgi studies of developing basal ganglia neurons (Adinolfi, 1977; Hull et al., 1981), as well as allowing a correlation between the morphological properties of the neurons and their physiological characteristics. As has been known for some time, neonatal medium spiny neurons in a number of species are essentially aspiny, and possess rather thin and varicose dendrites compared to adults. Although in adults, the vast majority of cortical and thalamic afferents form asymmetric synapses onto the heads of dendritic spines (Kemp and Powell, 1971b,c), in early postnatal neurons intracellularly labeled with biocytin in vivo, there are very few spines prior to P10 as shown in Fig. 8*A*. Between P11 and P20, the spine density increases dramatically, and the dendrites assume the non-varicose morphology that they possess in the adult, illustrated in Fig. 8. It is interesting to note that the most marked increases in spine density take place between P15 and P20, precisely the time during which the majority of cortical and thalamic afferents reach the neostriatum and form asymmetric axo-spinous synaptic connections (Hattori and McGeer, 1973). It is also during this period that the IPSP response to cortical stimulation disappears, suggesting that the appearance of the IPSP is dependent upon some developmentally transient aspect of the postnatal maturation of the cortical and/or thalamic innervation.

In contrast to the dendritic immaturity of neonatal neostriatal neurons, the local axon collateral plexus that characterizes the morphology of the mature medium spiny neuron and which has been proposed to play a major role in neostriatal information processing (e.g., Groves, 1983) is already present in the youngest animals in which we were able to obtain good intracellular filling, shown in Fig. 8*E*. There were no obvious differences between the local axon collateral systems from neurons early in the second postnatal week and in those in adults (Trent and Tepper, 1991).

Discussion

The rat neostriatum undergoes an extended postnatal development, both in terms of the membrane properties of individual neurons as well as their synaptic connectivity. Many of the properties of immature neostriatal neurons, for example, decreased average spontaneous firing rate, unusually wide action potentials, relatively depolarized resting membrane potential and lack of anomalous rectification are not specific to neostriatal neurons, and have been reported previously for a number of different neurons in central nervous system neurons in neonates including those of the spinal cord (Murase and Randic, 1983), hippocampus (Michelson and Lothman, 1989; Segal, 1990), midbrain (Pitts et al., 1990; Tepper et al., 1990a,b) and neocortex (McCormick and Prince, 1987; Lorenzon and Foehring, 1991; Fukuda and Prince, 1992).

Although one of the most characteristic features of adult neostriatal medium spiny neuron neurophysiology, both in vivo and in vitro, is the prominent fast inward rectification seen in response to both de- and hyperpolarizing current injection (Wilson, 1992, this volume), the vast majority of neostriatal medium spiny neurons in neonates did not exhibit anomalous rectification. The absence of anomalous rectification early in the neonatal period has been previously reported in in vitro preparations of neostriatum (Misgeld et al., 1986) and hippocampus (Segal, 1990), and the results of the present study confirm that this absence is due to an intrinsic difference in membrane properties of neonatal versus adult neurons, and not to some artifact of the slice preparation. A lack of anomalous rectification has also been reported in grafted fetal rat neostriatal medium spiny neurons in in vitro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recording experiments (Walsh et al., 1988), and even after many months' survival in the host brain in in vivo recordings (Xu et al., 1991b). The absence of this type of rectification is likely to contribute to the increased stability of the resting membrane potential of neonatal neostriatal neurons compared to those of adults, and the absence of the long depolarizing episodes which generate bursts of spikes in mature neostriatal neurons (Wilson and Groves, 1980; Wilson, this volume). A secondary effect of the lack of anomalous rectification is that it fixes the membrane time and space constants by removing their dependence on membrane potential (Wilson, 1992), thereby causing the response of neonatal spiny neurons to excitatory afferent input to be essentially independent of the membrane state of the neuron.

Although even the youngest neonatal neurons in this study responded to cortical and/or thalamic input, there were several differences among the responses to afferent excitatory input in neonates and adults. In neonates younger than P12, the characteristic sequence of depolarization – long-lasting hyperpolarization – depolarization was absent, and the most common response to cortical stimulation was a simple EPSP.

Although some have claimed that the long-lasting hyperpolarization seen in adult neostriatal neurons following stimulation of excitatory afferents is an IPSP resulting from activation of the inhibitory recurrent axon collateral system of the medium spiny neuron (e.g., Buchwald et al., 1973; Hull et al., 1973; Levine et al., 1986), there is strong evidence that this phenomenon is due to a disfacilitation of excitatory cortical inputs. Wilson and colleagues have shown that unlike other synaptic

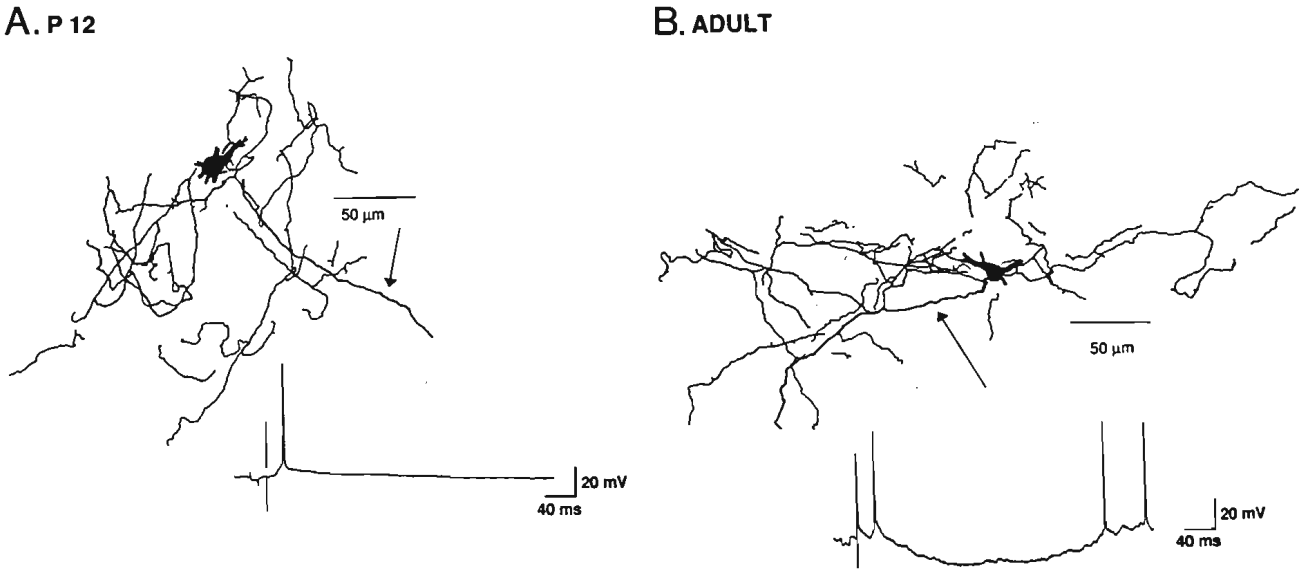

Fig. 10. *A*. The local axon collateral system of the P12 neuron shown in Fig. 9*A* is well-developed as indicated by the partial camera lucida reconstruction (120 μm in depth) and does not differ markedly from that observed in adults (*B*). Despite the well-developed local axon collateral system, cortical stimulation fails to elicit the long-lasting hyperpolarization in the P12 pup, but does so in the adult.

Fig. 9. Neonatal neostriatal medium spiny neurons are morphologically immature and bear thin, varicose and virtually aspiny dendrites as revealed by intracellular labeling with biocytin. *A,C,E,G.* Low magnification photomicrographs of medium spiny neurons intracellularly labeled with biocytin from P12 (*A*), P20 (*C*), P27 (*E*) and adult rats (*G*). *B,D,F,H.* Higher magnification photomicrographs of the dendrites of the neurons shown on the left. The density of dendritic spines increases up through P21 – P29. Note that even at P12, portions of the local axon collaterals are visible as fine beaded processes in *A* and *B*. Calibration marker = 50 μm in *A,C,E* and *G*, and 20 μ in *B,D,F* and *H*.

potentials in neostriatal neurons, the long-lasting hyperpolarization: (1) is accompanied by a small decrease in membrane conductance instead of a significant increase; (2) shows only a minimal dependence on experimentally induced alterations in membrane potential in a direction that indicates a reversal potential that is the same as that for the early and late EPSP components of the response; and (3) is abolished by lesions of the corticostriatal or the thalamostriatal pathways (Wilson et al., 1983). This conclusion is supported by the absence of the two later components of the response to cortical stimulation in early neonates in the present study. Although there are clearly some corticostriatal and thalamostriatal fibers present even in the youngest neonates examined since stimulation of cortex or thalamus could evoke EPSPs in these animals, the bulk of the cortical and thalamic inputs do not arrive, ramify and form axospinous synapses in neostriatum until the middle of the third postnatal week (Hattori and McGeer, 1973). Prior to this time, although a limited number of anterogradely labeled corticostriatal fibers can be observed in neostriatum at the level of the electron microscope, many of the boutons appear to be less densely packed with synaptic vesicles than in the adult, and were not observed in synaptic contact. Of those that do form synapses, the vast majority formed asymmetric synapses with dendritic shafts, not spine heads (Sharpe et al., 1992). Furthermore, in the present experiments, when descending through cortex en route to neostriatum, very little spontaneous activity was detected in cortex until late in the third postnatal week, the time at which the long-lasting hyperpolarization and rebound excitation first appeared in a significant proportion of neurons (cf. Fig. 6). Finally, as discussed below, the axon collateral plexus of biocytin-filled medium spiny neurons is already well-developed at least by the first postnatal week when the neurons do not exhibit the long-lasting hyperpolarization but when others have shown that these neurons do exhibit adult-like sensitivity to GABA agonists (Levine et al., 1990). None of the findings in the present study are consistent with the notion that the long-lasting hyperpolarization following afferent excitation derives from intrastriatal inhibition; rather these data support the disfacilitation hypothesis of Wilson and colleagues (Wilson et al., 1983).

It is of interest to note that the development of the "enabled" and "disabled" states, the prolonged depolarizing and hyperpolarizing shifts in membrane potential that characterize the spontaneous activity of the adult medium spiny neuron, coincided with the appearance of the long-lasting hyperpolarization in response to cortical and/or thalamic stimulation. This observation supports Wilson's contention that these states derive, at least in part, from afferent input from cortex and/or thalamus (Wilson and Groves, 1980; Wilson, this volume). Although they are virtually devoid of spontaneous activity, the neonatal neurons do not appear to be in the "disabled" state, however, since their average resting membrane potential is significantly more depolarized than in adults. It is unlikely that this depolarization derives from tonic cortical or thalamic input, since the lack of disfacilitation following cortical or thalamic stimulation indicates that there is little or no tonic activity in the corticostriatal pathway during the first two postnatal weeks. Furthermore, other types of neurons in neonatal rats also appear to be depolarized early in postnatal development (e.g., Tepper et al., 1990a,b). It may be that the depolarized state arises from intrinsic cellular factors, for example, a generally reduced activity of an electrogenic Na^+/K^+ ATPase in neonates, as has recently been demonstrated for neonatal hippocampal neurons (Atsuo and Prince, 1992).

The prominent IPSP that was evoked by cortical stimulation in neonates is particularly interesting, and very similar to one reported by Misgeld et al. (1986) following intrastriatal stimulation in slices of neonatal rat neostriatum, although they did not comment on its transient nature. This potential was clearly distinct from the long-lasting hyperpolarization that succeeds the initial cortically evoked EPSP in adult neostriatal neurons in that it was of considerably shorter duration, was sensitive to experimentally induced alterations in membrane potential, and exhibited a reversal potential near

that which would be expected for a chloride-mediated IPSP. It is quite likely that this IPSP represents the action of the inhibitory recurrent axon collateral plexus and/or the effects of feedforward inhibition through striatal interneurons. One could speculate that one of the major reasons that this IPSP is so much more apparent in neonates than in adults is because of the low density and sparse nature of the corticostriatal inputs in the neonates in contrast to the more diffuse and widespread innervation in adults (Hattori and McGeer, 1973; Sharpe et al., 1992). This could result in the occurrence of patchy "hot spots" of cortical and/or thalamic input, which would increase the probability of encountering neurons that are less densely innervated directly by cortical and/or thalamic afferents than their nearby neighbors. Under these conditions, the relative input resulting from recurrent or feedforward inhibition may outweigh that from direct excitation allowing the IPSP to be manifest more clearly than in the normal adult striatum. It is interesting to note that this type of innervation pattern has recently been reported for cortical inputs to intrastriatal grafts of neostriatal neurons (Xu et al., 1989; Wilson et al., 1990), and these grafted neurons, but not adjacent host neurons, have also been shown to exhibit cortically and thalamically evoked IPSPs (Xu et al., 1991b) that are similar in many respects to those observed in neonates in situ.

The disappearance of this IPSP over the first few minutes of recording is puzzling; the same transience has been described by Xu and colleagues in grafted neostriatal neurons (Xu et al., 1991b). As they suggest, perhaps the intracellular penetration damages or alters the intracellular environment in such a way as to block the manifestation of the IPSP. In any event, it appears that the physiology of spiny neurons in the immature neostriatum is more significantly affected by intrinsic or feedforward inhibition than that of the mature striatum, and this may contribute to the extremely low levels of spontaneous activity observed in the first few postnatal weeks.

The morphology of neonatal neostriatal medium spiny neurons differs significantly from that of mature medium spiny neurons. Although the size and appearance of the somata is essentially the same as in adults, the most characteristic feature of these neurons, the densely spine-laden dendrites, appear varicose and almost spine-free up through the first two postnatal weeks, consistent with an earlier report based on Golgi staining (Chronister et al., 1976). The dendritic arbor is also less expansive during this time. During the third postnatal week, coincident with the elaboration of the cortical and thalamic afferents and their corresponding synaptogenesis (Hattori and McGeer, 1973), the dendrites become heavily invested with spines and lose their varicose appearance. A similar developmental sequence has been described for feline neostriatal medium spiny neurons, although they are considerably more mature at birth than rodent neostriatal neurons and exhibit a prolonged postnatal maturation compared to that of the rat (Adinolfi, 1977). Nevertheless, even the dendrites of feline medium spiny neurons are only sparsely invested with spines in the first postnatal week (Hull et al., 1981). Based on modeling studies of spiny dendrites (Wilson, 1984, 1992), the paucity of dendritic spines and the lack of anomalous rectification in the early neonatal period should make neonatal medium spiny neurons more electrotonically compact than adult neurons. This is likely the explanation for the relative ease with which cortically evoked EPSPs could be reversed by intracellular current injection in the early neonates in the present study, and suggests that during postnatal development, medium spiny neurons should be more sensitive to the effects of individual synaptic inputs than is the case in the adult. This phenomenon may explain why the magnitude of the maximal cortically evoked EPSP does not change over postnatal development despite the fact that the number of excitatory afferent synapses increases substantially.

One of the more interesting observations to emerge from these studies is the striking similarity (and difference from in situ adult neurons) among many of the electrophysiological and morphological characteristics of neonatal neostriatal neurons and those of fetal neostriatal neurons grafted to ibo-

TABLE I

Comparison among adult, neonatal and grafted neostriatal neurons

	Adult	Neonate	Graft
Anomalous rectification	Yes	No	No
Enabled-disabled states	Yes	No	No
Transient IPSP	No	Yes	Yes
EPSP latency	Short	Long	Long
Spine density	High	Low	Intermediate-low

tenic or kainic acid-treated neostriatum (Zemanick et al., 1987; Walsh et al., 1988; Xu et al., 1991b; see Table I). These similarities include: lack of anomalous rectification, increased membrane resistance, decreased spine density, increased latency to cortically evoked EPSPs, lack of a long-lasting hyperpolarization and subsequent late depolarization following afferent excitation, presence of an early fast IPSP following cortical or thalamic stimulation that decays over time, and absence of discrete enabled and disabled states of the resting membrane potential. Some of these shared features may arise from the reduced extrinsic excitatory input existing in both the immature neostriatum and in grafted fetal neurons and imply that as with nigral dopaminergic neurons (Fisher et al., 1990; Tepper et al., 1990a), grafted neostriatal neurons remain in a developmentally arrested state, both physiologically and morphologically, even several months after transplantation.

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