Developmental Brain Research, 54 (1990) 21–33
Elsevier

BRESD 51081

Postnatal development of the electrical activity of rat nigrostriatal dopaminergic neurons

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(Accepted 16 January 1990)

Key words: Antidromic response; Development; Dopaminergic neuron; Electrophysiology; Neonate; Substantia nigra

Extra- and intracellular recordings were obtained in vivo from dopaminergic nigrostriatal neurons in rat pups ranging in age from postnatal day (PD) 1 to PD28, and in adult rats. Neurons from PD1–3 rats were active at very low rates in a random pattern, rarely showed bursting activity, and often exhibited long periods of up to several minutes of silence. Spontaneous spikes were of relatively low amplitude and long duration. The mean firing rate increased and became more regular over time, and short bursts consisting of only 2 spikes were observed. By the second postnatal week, the initial segment component of the spontaneous spike resembled that seen in adults, but the somadendritic component was still relatively small, and there was often a very marked temporal delay between the two. Near the end of the second postnatal week, neurons exhibited a transient phase of pacemaker-like activity. Mean firing rates continued to increase with time, as did the incidence and complexity of bursting activity. The spontaneous firing rate, pattern and spike morphology approached adult values by the fourth postnatal week. Antidromic responses from neostriatum were obtained as early as PD1, and consisted of a significantly greater proportion of full initial segment-soma dendritic spikes compared to nigrostriatal neurons from adult rats. There was usually a long delay between the initial segment and somadendritic components of the spike. Mean antidromic latency and mean antidromic threshold did not vary significantly from PD1–3 to adults. Axonal conduction velocity reached maximal adult values by PD16–21. Neostriatal-evoked orthodromic responses consisted principally of a poststimulus inhibition whose duration decreased from PD1 through adulthood. Pure excitatory responses were very rarely observed at any age. Intracellular recordings from PD2, PD3 and PD5 rats revealed striatal-evoked inhibitory postsynaptic potentials in non-dopaminergic nigral neurons with a mean onset latency (9.8 ± 3.8 ms) which did not differ from that previously reported for adult rats.

INTRODUCTION

Monoaminergic neurons are among the first in the brain to differentiate, migrate and send axons to telencephalic terminal fields[27,31]. In rat, midbrain dopaminergic neurons begin to express tyrosine hydroxylase activity by the 14th day of gestation[32], and become organized into discrete cell groups similar to that seen in the adult midbrain during the first postnatal week[39]. The dopaminergic nigrostriatal projection develops even before this, with tyrosine hydroxylase-immunoreactive axons present in the neostriatal anlage at around the same time that the enzyme can be detected in the midbrain[32].

In part because of their early development, much interest has been generated in the possible role that monoaminergic systems play in synaptic plasticity. A large body of work by Kasamatsu and associates has demonstrated important modulatory functions of noradrenergic afferents and noradrenaline in the physiological expression of synaptic plasticity in the kitten visual cortex[15,16,28], and ultrastructural studies have shown an inhibitory effect of norepinephrine on synapse formation in neonatal rat visual cortex[1]. A similar role for dopaminergic afferents to neostriatum has as yet been unexplored.

Although previous investigators have reported on the postnatal development of basal ganglia neurons, including substantia nigra neurons in neonatal kittens[6,21,24], the feline nervous system is more developed at birth than the rat nervous system, and for the past several years, a large majority of neurophysiological, neuroanatomical and neuropharmacological studies have used rats as their principal subjects. Pitts et al.[29] reported preliminary electrophysiological data from dopaminergic neurons from 4-week-old rats, and found no significant differences in spontaneous activity or conduction velocity between these animals and adults. Thus far, there have been no reports, either in vitro or in vivo concerning the early ontogeny of electrical activity of rat midbrain

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dopaminergic neurons. This information is relevant, not only to aid in our understanding of the role that dopaminergic afferents may play in the functional development of the neostriatum and other dopaminergic terminal fields, but also with respect to the rapidly accumulating physiological and anatomical data on fetal mesencephalic transplants of dopaminergic neurons to the dopamine-depleted striatum (e.g. ref. 5). The present experiments were designed to characterize the in vivo electrophysiological properties of nigral dopaminergic neurons in neonatal rat pups, and to chart the changes in these properties during postnatal development. Portions of this work have appeared in abstract form previously[34,38].

MATERIALS AND METHODS

Subjects

Subjects consisted of 68 Sprague–Dawley pups derived from pregnant dams (obtained from Charles River or The Institute of Animal Behavior at Rutgers). Pregnant females were checked daily for the presence of new litters, and the day of birth was considered to be postnatal day 1 (PD1). The ages of the rat pups used ranged from PD1 to PD28, and their weights varied from 6.5 to 74.5 g. Pups were anesthetized by i.p. injection of urethane (1.3 g/kg b. wt.), and supplemented by inhalation of metofane (methoxyfluorane) if necessary. The procedure for installation of neonatal rats into a stereotaxic device has been described in detail previously[25]. Body temperature was maintained at 37 ± 1 °C by a solid state heating pad.

For purposes of comparison with nigrostriatal dopaminergic neurons from adult rats, 12 male Sprague–Dawley rats, over 75 days of age (weights ranging from 240 to 460 g) were anesthetized with urethane (1.3 g/kg, i.p.) and installed in a stereotaxic frame according to standard procedures[35].

Electrical stimulation

Bipolar stimulating electrodes with a tip separation of approximately 150 μm were constructed from enamel-coated stainless steel wires, 100 μm in diameter (California Fine Wire), and possessed in vitro impedances of approximately 10 KΩ. A small burr hole was drilled overlying the anterior–lateral neostriatum (coordinates 0.5–0.7 mm anterior to bregma, 2.4–3.5 mm lateral to the midline) and an electrode was inserted to depths ranging from 2.2 to 3.4 mm below the cortical surface. In adult rats, a stimulating electrode was positioned in the anterior–lateral dorsal neostriatum at coordinates 1.0 mm anterior to bregma, 3.7 mm lateral and 4.0 mm below the cortical surface. Constant current stimuli, 0.1–5.0 mA at a pulse duration of 500 μs, were delivered at a rate of 0.67 Hz by a Winston A-65 timer/stimulator and Winston SC-100 stimulus isolation units.

Recording

A burr hole approximately 1.5 mm in diameter was drilled overlying the substantia nigra at coordinates 0.8–1.5 mm anterior to lambda and 0.7–1.5 mm lateral to the midline for neonates and 2.1 mm anterior to lambda and 2.0 mm lateral to the midline for adults. Single unit extracellular discharges were recorded with glass micropipettes filled with 2% Pontamine sky blue in 2 M NaCl, possessing in vitro impedances of 5–10 MΩ, amplified with a Neurodata IR183 preamplifier and displayed on a Tektronix 5113 storage oscilloscope. Typical filter settings were 100 Hz or 1 kHz low pass and 10 kHz or 30 kHz high pass. All data were recorded on magnetic tape for off-line analysis. In a few cases, intracellular recordings were obtained with glass micropipettes filled with 2 M potassium methyl sulfate and possessing in vivo impedances of 40–75 MΩ. Intracellular data were digitized on-line with a Nicolet 4094C digital oscilloscope interfaced to a Macintosh II computer and stored on disk for off-line analysis.

Data analysis

Spike trains were played back from tape off-line and input to a Macintosh II microcomputer through a National Instruments MIO16L multifunction board. Spontaneous activity was analyzed for firing rate, and first order interspike interval histograms and autocorrelograms were constructed. Spike trains were also analyzed for the occurrence of burst discharges, defined as two or more spikes occurring with an interval between the first two spikes of 80 ms or less and terminating with the first interspike interval greater than 160 ms[12]. Analyses of spike waveform and duration were obtained by averaging 5–10 action potentials.

Peristimulus time histograms were constructed from spike trains following neostriatal stimulation at threshold for each neuron (the current that evoked 100% antidromic responding) in order to assess the orthodromic effects of the neostriatal stimulus. The Macintosh was programmed to calculate the mean number of spikes per bin from user-specified regions of the histogram. Analyses were performed using bin widths of 5 ms. Poststimulus effects were calculated by comparing the number of poststimulus spikes per bin to the prestimulus baseline values. Deviations from baseline values of ±10% or greater were considered to represent effects of the stimulus.

For purposes of statistical analysis, data from rats were pooled and assigned to one of the following groups: PD1–3, PD4–6, PD7–10, PD11–15, PD16–21, PD22–28, and adult. Parameters were analyzed by a one-way analysis of variance by group. Where appropriate, differences between specific age groups were tested with Scheffe's F-test at the $P < 0.1$ level. For nominally grouped (non-parametric) data, frequency distributions were tested with Chi square at a level of $P < 0.05$.

Histology

At the end of each experiment, the neostriatal stimulation site was marked by a small lesion created by passing 0.5–1.0 mA DC for 1 s through the stimulating electrode. The last recording site was marked by iontophoresis of Pontamine sky blue through the recording electrode by passing –20 μA for 8–20 min. Animals were perfused with 10–20 ml normal saline followed by 50–70 ml of 10% formalin or 4% paraformaldehyde containing 0.2% glutaraldehyde in 0.15 M sodium phosphate buffer at pH 7.4. The brains were removed, postfixed in 10% formalin for 1–7 days, and 80–100-μm sections cut on a vibratome. Sections were stained with Neutral red and each stimulation site and the site of the last recording marked by a Pontamine sky blue dot were noted, photographed and/or drawn at 1× with a Nikon Optiphot microscope equipped with a drawing tube.

In some cases, 40–60-μm vibratome sections were reacted for immunocytochemical visualization of tyrosine hydroxylase using a primary antiserum from Eugene Tech International and the ABC method of Vector Laboratories with 3,3′-diaminobenzidine as the chromagen.

RESULTS

Neuronal identification

Extracellular recordings were obtained from 206 antidromically driven neurons in 68 rat pups. Neurons in this study were assumed to be dopaminergic provided that they could be antidromically activated from ipsilateral neostriatum with latencies greater than 9 ms, and provided that later histological analysis using either Neutral red-stained sections or sections reacted for tyrosine hydroxylase immunocytochemistry indicated

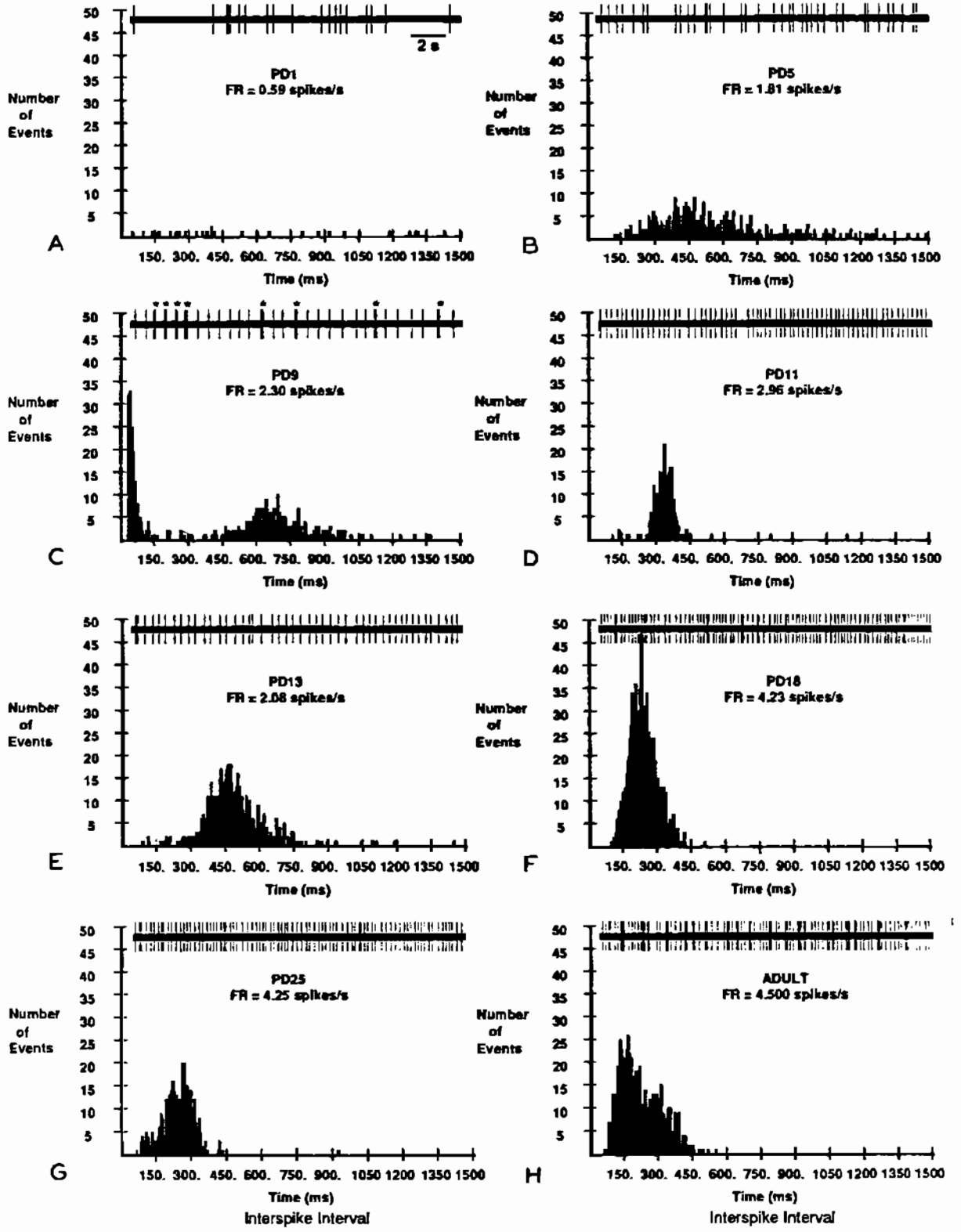

Fig. 1. Representative first order ISI histograms and examples of the spike trains from which they were constructed illustrating changes in the spontaneous firing patterns of nigrostriatal neurons during development. A: neuron from a PD1 rat fires essentially at random (mean ISI ± S.E.M. = 1212 ± 183 ms). Note long silent periods in the spike train. B: neuron from a PD5 rat fires with greater regularity (553 ± 57 ms). C: neuron from a PD9 rat shows a bimodal ISI histogram reflecting the occurrence of short bursts consisting of 2 spikes with a mean ISI of approximately 60 ms imbedded within a more regular firing pattern (434 ± 51 ms). Bursts, each consisting of only 2 spikes, are marked with asterisks in the spike train. D,E: neurons from a PD11 rat (D; 336 ± 32 ms) and a PD13 rat (E; 480 ± 46 ms) exhibiting the very regular, pacemaker-like firing patterns typical of the latter part of the second postnatal week. F: PD18 rat (236 ± 22 ms). G: PD25 rat (235 ± 30 ms). H: neuron from an adult rat (220 ± 22 ms). FR, firing rate. Bin width = 5 ms. Each histogram was compiled from 3–5 min of spontaneous activity. Spike trains consist of the initial 30 s of each record.

that the neurons were located within the region of the substantia nigra, pars compacta. Evoked responses were considered antidromic provided that they collided with appropriately timed spontaneous spikes, or, for neurons that exhibited little or no spontaneous activity, could follow twin pulse stimulation with interstimulus intervals corresponding to a train of 200 Hz. Neurons encountered in the vicinity of the substantia nigra, pars compacta that were not antidromically driven were excluded from study. Of the 206 antidromically activated neurons, 165 were histologically localized to the substantia nigra and were tentatively identified as dopaminergic nigrostriatal neurons.

The remaining antidromically activated neurons were found to be located in thalamus, in the posterior regions of the centromedian–parafascicular complex, and will not be discussed in detail in this report.

Intracellular recordings were obtained from an additional 10 neurons: of these, 2 were antidromically activated from neostriatum at latencies appropriate to nigral dopaminergic neurons and were located in the substantia nigra, pars compacta. The remaining 8 neurons could not be antidromically driven, and reconstruction of their electrode tracks showed that they were located in the substantia nigra pars reticulata. These neurons are henceforth described as 'unidentified' nigral neurons, although it is quite probable that they are non-dopaminergic nigral pars reticulata GABAergic neurons.

Spontaneous activity

Spontaneously active nigrostriatal dopaminergic neurons could be recorded as early as PD1. The mean firing rate of nigrostriatal dopaminergic neurons in neonates increased as a function of age from PD1–3 through PD22–28 ($F = 30.2$, d.f. = 6,138, $P < 0.001$), with a concomitant decrease in the mean interspike interval (ISI) over this developmental span ($F = 11.99$, d.f. = 6,135, $P < 0.001$). By PD22–28, both mean firing rates and mean ISIs no longer differed from those in adults. Mean firing rates and interspike intervals are listed by age group in Table I.

The pattern of spontaneous activity also changes significantly over development, as illustrated in Fig. 1. Dopaminergic neurons from the earliest postnatal group often displayed only sporadic spontaneous activity into which was embedded long periods (up to several minutes but typically on the order of 5–45 s) of silence. The occurrence of the characteristic bursting firing pattern was essentially absent in cells from PD1–3 neonates. Over the next week, mean firing rate increased and the first signs of what appeared to be the precursor of adult-type bursting activity became evident. At this stage (Fig. 1, PD5), some neurons began to exhibit short bursts, almost always consisting of just two spikes with a relatively constant interspike interval around 60 ms (c.f. Table I), in an otherwise tonic or random firing pattern, resulting in a bimodal interspike interval histogram. The interval between the first two spikes in a burst remained extremely constant throughout development. The spontaneous firing rate continued to increase over the next week. During this stage, the neurons most often displayed a very regular, almost pacemaker-like firing pattern, with a low incidence of bursting. This firing

TABLE I

Developmental changes in spontaneous activity of nigrostriatal neurons

	PD1–3	*PD4–6*	*PD7–10*	*PD11–15*	*PD16–21*	*PD22–28*	*Adult*
Mean firing rate (spikes/s)	0.921 ± 0.14*	1.37 ± 0.19*	1.34 ± 0.19*	2.64 ± 0.19*	2.29 ± 0.35*	3.99 ± 0.30	4.68 ± 0.34
	(14)	(19)	(19)	(29)	(13)	(19)	(26)
Mean ISI (ms)	1234 ± 173*	1034 ± 202*	947 ± 119*	473 ± 54	715 ± 177	227 ± 27	251 ± 23
	(13)	(18)	(18)	(29)	(13)	(19)	(26)
Random/pacemaker	76.9/23.1	83.3/16.7	83.3/16.7	51.7/48.3**	76.9/23.1	57.9/42.1	73/27
Spike duration (ms)	5.15 ± 0.67*	3.57 ± 0.31	2.85 ± 0.14	2.99 ± 0.14	3.45 ± 0.26	3.02 ± 0.13	3.36 ± 0.07
	(12)	(18)	(19)	(29)	(14)	(19)	(21)
Percent cells firing at	23.1	16.7	5.6	18.5	30.8	30.0	57.7**
least one 3-spike burst	(13)	(18)	(18)	(27)	(13)	(20)	(26)
Percent cells firing at	7.7	5.5	0	7.4	7.7	5.0	26.9**
least 10% spikes in bursts	(13)	(18)	(18)	(27)	(13)	(20)	(26)
Burst duration (ms)	60.1 ± 2.2*	75.6 ± 9.8*	61.0 ± 2.8*	68.3 ± 2.3*	115.8 ± 20.4	108.4 ± 17.1	167 ± 28.7
	(10)	(11)	(10)	(14)	(5)	(11)	(17)
1st burst ISI (ms)	59.5 ± 2.45	61.1 ± 1.9	60.4 ± 2.6	65.7 ± 2.0	65.3 ± 6.4	66.7 ± 2.5	63.5 ± 1.7
	(10)	(11)	(10)	(14)	(5)	(11)	(17)
N spikes/burst	2.10 ± 0.10*	2.16 ± 0.09*	2.00 ± 0.01*	2.04 ± 0.01*	2.45 ± 0.18	2.33 ± 0.14	3.43 ± 0.44
	(10)	(11)	(10)	(14)	(5)	(11)	(17)

* Significant difference from adult group (Scheffé, $P < 0.1$). ** Cell distribution significantly different from remainder of age groups χ^2, $P < 0.05$).

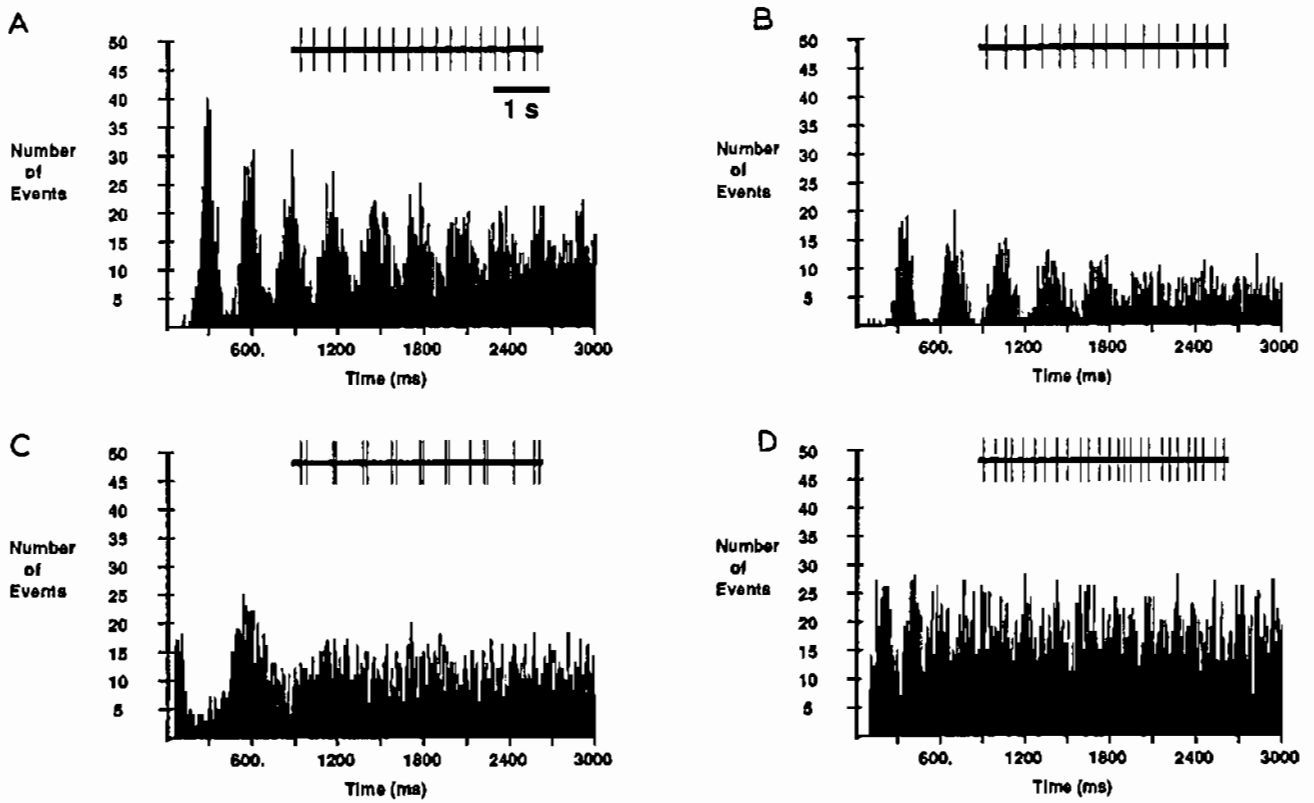

Fig. 2. Autocorrelation histograms of nigrostriatal neurons from selected developmental phases. A,B: nearly half of the nigrostriatal neurons from the PD11–15 age group fired in a highly regular pacemaker-type mode. Both histograms show prolonged initial troughs indicating robust postfiring inhibition. Peaks in histogram in A from a PD11 pup are regularly spaced at 300 ms; firing rate = 3.33 spikes/s. In B, peaks are spaced at 340 ms; firing rate = 2.82 spikes/s. Note complete absence of bursting in these neurons. C: neuron from PD9 rat at transition between initial burst firing mode in which bursts consist of only 2 spikes with a very stereotyped interspike interval (61 ms, first peak in histogram) and the repetitive firing mode (note weakly rhythmic nature of latter part of histogram). D: typical autocorrelogram from an adult showing mostly random firing with some bursting activity. Bin width = 5 ms. Each histogram was compiled from 3–5 min of spontaneous activity. Spike trains consist of the initial 5 s of each record.

pattern is clearly apparent in autocorrelograms consisting of a repeating series of peaks at constant intervals, as shown in Fig. 2. Almost half of all neurons from the PD11–15 age group fired in this mode. Over the next week, the occurrence of the doublet bursts became more frequent and longer bursts were occasionally seen as the firing pattern became less regular. The rate and pattern of spontaneous activity, as well as the average duration and number of spikes per burst became largely indistinguishable from that in adults by early in the fourth postnatal week. However, the proportion of neurons that fired at least one burst containing 3 or more spikes, or that fired at least 10% of their total spikes in bursts of at least 2 spikes was significantly greater in the adult group than in any of the neonatal groups.

The morphology of the extracellularly recorded spontaneous action potential waveform also changed significantly during neonatal development, as shown in Fig. 3. Neurons from PD1 animals exhibited action potentials of relatively low amplitude (i.e. the signal-to-noise ratio was usually poor), and the amplitudes of individual action potentials from a single neuron tended to be more variable than in adults. The duration of spontaneous spikes was measured in averages of 5–10 digitized spikes from the point at which the initial segment (IS) spike first deviated from baseline to the point at which the soma-dendritic (SD) spike afterpotential returned to baseline. Spike waveforms were of significantly greater duration in the neonates compared to adults ($F = 7.99$, d.f. = 6,131, $P < 0.001$), largely due to a broadening of the SD component of the spike in neurons from the two youngest groups. The duration of the IS spike did not change significantly from PD1–3 through adulthood. During the first postnatal week, the amplitude of the spikes increased due to an increase in the amplitude of the SD component of the spike. By PD7, the amplitude of the IS component did not appear significantly different from that seen in adults. At this stage, it was observed that the amplitude of the IS component of the spike was often equal to, or of greater magnitude than that of the SD component, and that there was often an unusually long delay between the IS and SD spikes. Over the second

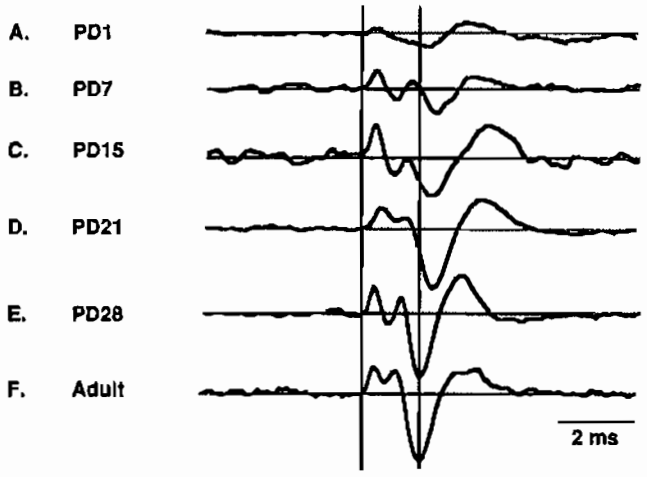

Fig. 3. Developmental changes in the morphology of extracellularly recorded spontaneous action potentials of nigrostriatal neurons. A: dopaminergic neurons from early postnatal rats exhibit low amplitude broad spikes. The IS and SD components are not well defined. B: by the end of the first postnatal week, the IS and SD components are clearly defined, and there is a significant IS-SD delay. At this stage, the IS spike has reached maximum amplitude, and is approximately equal to or greater than the SD spike. C: over the next week, the SD spike grows larger, while the IS spike does not change appreciably. D–F: by the end of the third postnatal week, spontaneous spikes closely resemble those from mature rats. The duration of the spike decreases with development. Each spike is the average of 10 spontaneous spikes from one cell. All are represented at the same gain, with low pass filtering at 1 kHz and high pass filtering at 10 kHz. Each horizontal line represents baseline for the spikes. The onset of each initial segment spike is aligned with the first vertical line, and the second vertical line is drawn through the peak of the SD spike of the adult to illustrate changes in overall spike duration. In this and all subsequent figures, positivity is upwards.

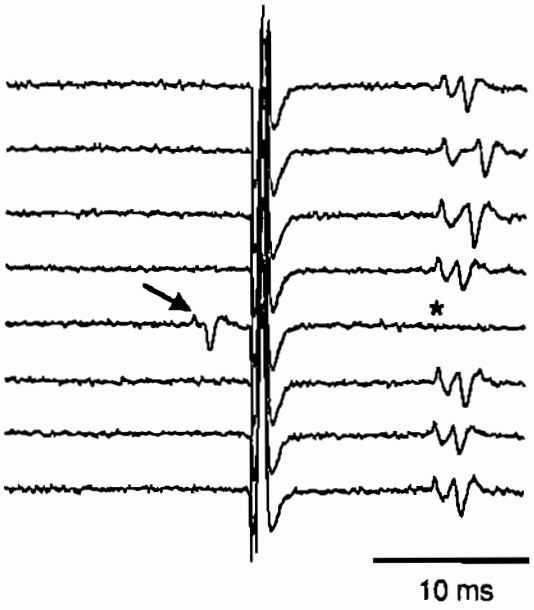

Fig. 4. Eight consecutive oscilloscope sweeps illustrating antidromic responses of a PD1 nigrostriatal dopaminergic neuron elicited by neostriatal stimulation at threshold. A collision is shown due to a spontaneous spike in the third trace (arrow) and the antidromically elicited response is extinguished (asterisk). Note the unusually high proportion of full IS-SD antidromic responses, and the long and variable delay between the IS and SD components of the antidromic spike.

postnatal week, the SD spike amplitude continued to increase, and the recovery from the spike afterpotential occurred more quickly. By the end of the third postnatal week, the morphology of the extracellularly recorded spike waveform was not significantly different from that of nigral neurons in adult rats.

Although not examined rigorously, sensory stimulation in the form of air puffs and light touches to the skin of the back, hand claps, and turning on and off of the room lights was applied while recording from several neonatal nigrostriatal neurons. There were no obvious responses to these stimuli.

Antidromic response properties

Antidromic responses elicited by neostriatal stimulation could be obtained from dopaminergic neurons in pups as little as 6–10 h postpartum, as shown in Fig. 4. As is typical of adult nigrostriatal dopaminergic neurons[4,9,14,35], antidromic responses in neonatal rats most often consisted of the IS spike only, with the action potential failing to invade the SD portion of the neuron even at

TABLE II

Developmental changes in antidromic response properties of nigrostriatal neurons

	PD1–3	*PD4–6*	*PD7–10*	*PD11–15*	*PD16–21*	*PD22–28*	*Adult*
AD latency (ms)	15.5 ± 0.88 (18)	17.1 ± 0.43 (42)	17.08 ± 0.78 (32)	18.88 ± 0.69* (37)	15.76 ± 1.2 (15)	15.5 ± 0.93 (20)	14.95 ± 0.32 (66)
AD threshold (mA)	2.18 ± 0.28 (19)	2.54 ± 0.21 (42)	2.65 ± 0.31* (34)	2.13 ± 0.23 (37)	2.73 ± 0.50 (15)	1.96 ± 0.37 (23)	1.61 ± 0.16 (67)
Conduction velocity (m/s)	0.26 ± 0.027* (18)	0.24 ± 0.011* (42)	0.32 ± 0.027* (32)	0.31 ± 0.022* (37)	0.46 ± 0.032 (15)	0.46 ± 0.023 (20)	0.49 ± 0.011 (66)
Percent IS-SD AD spikes	47.5 ± 8.4 (18)	60.9 ± 5.9* (38)	34.7 ± 5.8 (27)	31.1 ± 4.7 (34)	46.5 ± 9.8 (15)	29.7 ± 3.7 (19)	29.2 ± 2.3 (60)

* Significant difference from adult group (Scheffé, $P < 0.1$).

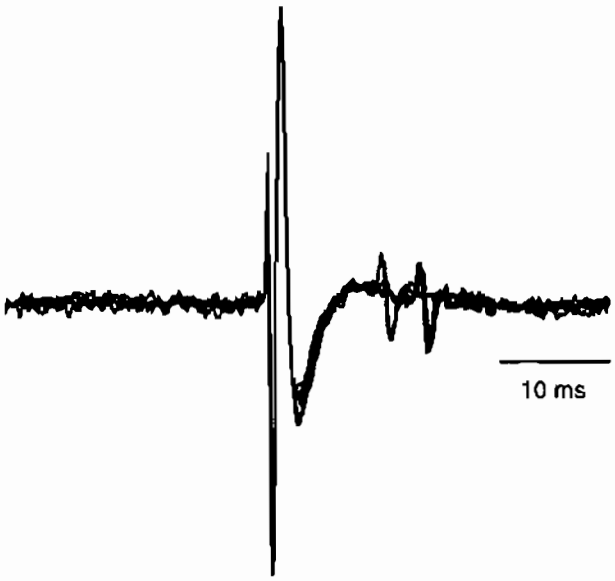

Fig. 5. Neonatal dopaminergic neurons display multiple, discrete antidromic latencies to striatal stimulation. Six consecutive traces obtained at constant stimulating current (0.59 mA) from a PD4 rat are superimposed. Two responses occur at the shorter latency of 10.6 ms and 4 occur at the longer latency of 13.7 ms. Responses at both latencies were observed to collide with appropriately timed spontaneous spikes (not shown).

modest rates of stimulation. However, the proportion of antidromic spikes consisting of a full IS-SD spike, calculated for each neuron by dividing the number of IS-SD antidromic responses by the total number of antidromic responses at threshold (typically 50–150), was significantly greater in neurons from neonates than from adults ($F = 6.41$, d.f. = 6,210, $P < 0.001$). When IS-SD antidromic responses were elicited, there was often an unusually long delay of up to a few milliseconds between the IS and SD components of the spike, particularly in neurons from the youngest animals. As previously reported for adult nigrostriatal neurons[3,35], many neonatal nigrostriatal neurons (53.2 ± 7.7%) exhibited multiple, discrete antidromic latencies, even at constant stimulus currents, as illustrated for a neuron from a PD4 rat in Fig. 5. There were no significant developmental changes in the proportion of neurons exhibiting multiple antidromic latencies.

Neither the mean antidromic latency nor the mean antidromic threshold current (minimum current necessary to elicit 100% antidromic responding on non-collision trials) exhibited a significant developmental trend. Mean estimated conduction velocities reached adult levels by PD16, reflecting nearly a doubling over that of the PD1–3 group. Antidromic response parameters are listed in Table II.

Orthodromic response properties

Peristimulus time histograms for neostriatal stimuli were constructed from 50–250 stimulus trials delivered at the antidromic threshold at 0.67 Hz for each spontaneously active neuron. Responses were classified as pure inhibition (I), pure facilitation (F), inhibition followed by facilitation (IF), facilitation followed by inhibition (FI) or no effect (NE). The most common type of response to neostriatal stimulation was inhibition followed by a rebound facilitation (IF). The next most common response type was a pure inhibition. Together, these two types occurred in 86.67% of the neonatal neurons and 82.9% of the adult neurons. There was no significant developmental component in the breakdown of response types. The duration of the inhibitory period was measured from the time that the number of spikes per bin decreased by at least 10% from prestimulus values until the difference was less than 10%. The mean poststimulus inhibitory period was significantly greater in neonates than adults ($F = 12.6$, d.f. = 6,119, $P < 0.001$). Instances of pure excitatory responses or FI responses were less common (c.f. Fig. 6B), only being observed in 16 of 140 cases (11.4%), with no clear age dependency. Sample peristimulus time histograms for representative neurons are shown in Fig. 6, and a summary of orthodromic responses by age is listed in Table III.

Intracellular recordings

Intracellular recordings were obtained from 2 antidromically activated pars compacta neurons (1 each at PD2

TABLE III

Developmental changes in orthodromic response properties of nigrostriatal neurons

	PD1–3	*PD4–6*	*PD7–10*	*PD11–15*	*PD16–21*	*PD22–28*	*Adult*
Response breakdown IF/I/FI/F/NE (%)	33/33/0/17/17 (6)	30/42/6/3/18 (33)	50/44/6/0/0 (18)	64/25/6/0/6 (32)	60/26/0/7/7 (15)	61/17/17/5/0 (18)	70/20/3/2/5 (59)
Duration of inhibition (ms)	353 ± 161 (4)	359 ± 56* (23)	450 ± 56* (18)	141 ± 17 (28)	366 ± 49* (13)	158 ± 37 (16)	137 ± 10 (53)
Onset of inhibition (ms)	21.96 ± 2.26 (4)	19.85 ± 1.13 (23)	15.70 ± 1.19 (18)	19.72 ± 1.05 (28)	18.74 ± 1.84 (13)	15.73 ± 1.29 (16)	16.92 ± 0.59 (53)

* Significant difference from adult group (Scheffé, $P < 0.1$).

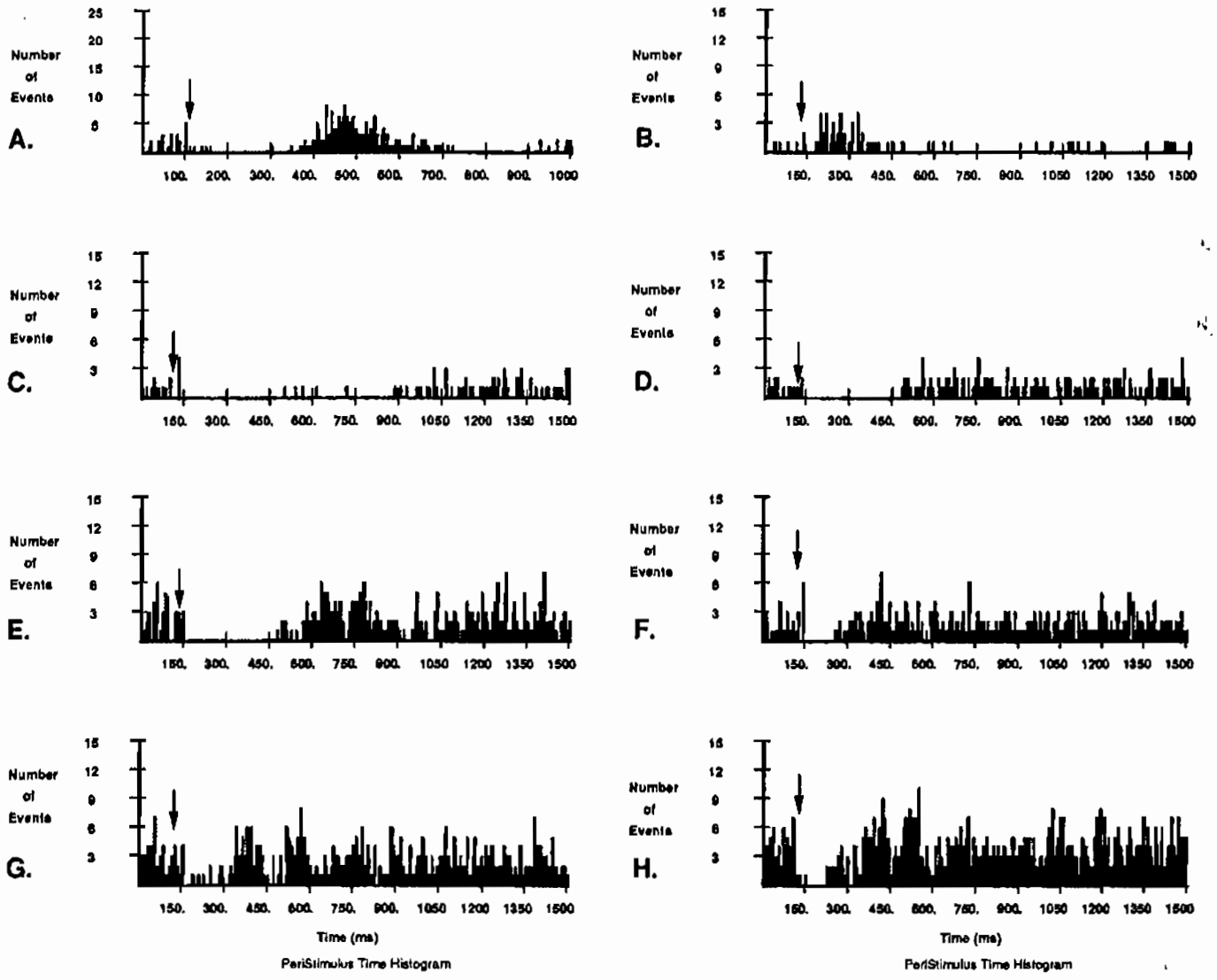

Fig. 6. Representative peristimulus time histograms illustrating various types of responses elicited by neostriatal stimulation in nigrostriatal neurons as a function of developmental stage. A: PD2 rat showing the most common response of inhibition followed by rebound excitation. B: PD4 rat exhibiting an atypical pure facilitatory response. C: PD5 rat. D: PD7 rat. E: PD15 rat. F: PD21 rat. G: PD28 rat. H: Adult rat. Note that the duration of the poststimulus inhibition decreases over development. Arrows indicate delivery of neostriatal stimulus. Bin widths = 5 ms. Each neuron was stimulated at its antidromic threshold current.

and PD5) and 8 unidentified nigral neurons (2 neurons at PD2, 4 at PD3 and 2 at PD5) located ventral to the region of the pars compacta. Intracellular recordings were considered stable if the membrane potential was greater than −35 mV and spontaneous or evoked spike amplitudes exceeded 40 mV. Sample intracellular traces are shown in Fig. 7. The nigrostriatal neurons (Fig. 7A,B) responded to neostriatal stimulation with antidromic action potentials that consisted entirely of full IS-SD spikes, and which failed to display a very prominent afterhyperpolarization. In addition, although the neostriatal stimulation evoked antidromic responses, synaptic potentials were not observed in either of the two nigrostriatal neurons. Conversely, although none of the unidentified, presumed non-dopaminergic pars reticulata neurons could be activated antidromically, all responded to neostriatal stimulation with clear hyperpolarizing potentials, as shown for a representative neuron by the averaged sweeps in Fig. 7C. The mean onset latency to the striatal-evoked hyperpolarizing potentials was 9.8 ± 0.38 ms, which did not differ significantly from that previously reported for non-dopaminergic nigral pars reticulata neurons from mature rats[37].

DISCUSSION

Identification of nigrostriatal dopaminergic neurons

Since at the outset of this study we could not assume that nigral dopaminergic neurons from neonatal rats would display the same electrophysiological and/or phar-

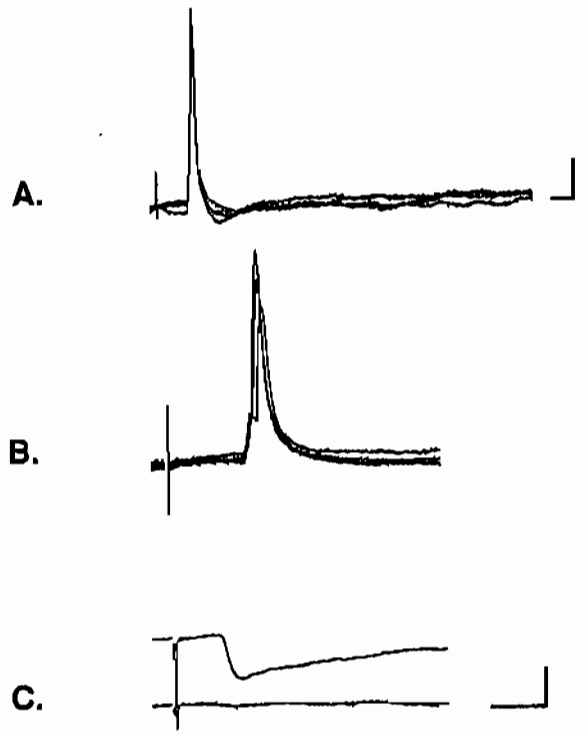

Fig. 7. Striatal-evoked intracellular responses in neonatal nigral neurons. A: four superimposed antidromic responses of a dopaminergic neuron to neostriatal stimulation in a PD2 rat. B: four superimposed antidromic responses of a dopaminergic neuron in a PD5 rat. Note that all responses in A and B consist of full IS-SD responses, and no orthodromic effects are apparent. C: average of 4 sweeps showing a hyperpolarizing synaptic potential obtained in an unidentified nigral neuron in response to neostriatal stimulation in a PD2 rat. Note absence of antidromic response. Lower trace is extracellular control. Both calibration markers = 10 mV, 10 ms. Lower calibration applies to B and C.

macological properties that have been well-documented for nigral neurons in adult rats[2,9,14], identification was not attempted on the basis of criteria such as low rates of spontaneous activity, wide action potential waveform, bursting activity or inhibition of neuronal activity in response to apomorphine or amphetamine. Indeed, almost all of the neurons encountered in the neonatal brain, particularly in PD1–5 rat pups exhibited extremely slow rates of spontaneous activity, or did not fire spontaneously at all, and possessed action potentials of durations equal to or greater than 1.75 ms. Instead, neurons were tentatively identified as dopaminergic nigrostriatal neurons provided that they could be antidromically activated from ipsilateral neostriatum, and provided that subsequent histological analysis indicated that the recording site was localized to the substantia nigra.

For neurons recorded in the vicinity of the substantia nigra, only two types have been shown to project out of the nucleus: dopaminergic neurons located mainly in pars compacta but also to a lesser extent in pars reticulata which project primarily to neostriatum, and non-dopaminergic neurons located mainly in pars reticulata which project primarily to tectum and thalamus, but occasionally to neostriatum[4,14]. In adults, discrimination between dopaminergic and non-dopaminergic nigrostriatal neurons is unequivocal since dopaminergic neurons exhibit antidromic latencies some 2–4 times greater than non-dopaminergic neurons, and because the antidromic response of dopaminergic neurons usually consists of IS spikes, even at low rates of stimulation (0.67 Hz) such as those employed in the present study[14]. The nigrostriatal neurons recorded in the present study exhibited long-latency antidromic responses that usually consisted of only the IS spike, consistent with their identification as dopaminergic neurons. Furthermore, in several cases when sections through substantia nigra were reacted for tyrosine hydroxylase immunoreactivity, the recording site was always found to be located amidst a dense cluster of tyrosine hydroxylase-positive cell bodies. Thus, although a firm neurochemical identification of the nigrostriatal neurons reported in this study, as by double labeling with an intracellular marker and tyrosine hydroxylase, was not performed, it is doubtful that these neurons could be anything but nigrostriatal dopaminergic neurons.

It is unlikely that the differences between neonatal and adult nigrostriatal neurons are attributable to recording from damaged neurons. The electrophysiological properties of these neurons were stable over recordings lasting upwards of 1 h, something that is inconsistent with electrode-induced cell damage. It is also unlikely that these characteristics were due to a differential sensitivity to the urethane anesthetic, since when supplements were given, there was no marked change in any of the electrophysiological parameters.

Spontaneous activity

The spontaneous firing rate of nigrostriatal neurons increased steadily from birth through the third postnatal week with a concomitant decrease in the mean interspike interval. This trend and its time course closely resembles that previously reported for locus coeruleus neurons in the rat[25], and confirm a preliminary report that the firing rates of nigrostriatal neurons from 4-week-old rats have already reached adult levels[29]. It is interesting to note that the speed of maturation of spontaneous activity of rat nigrostriatal neurons seems to be much greater than that of rat striatal neurons, which do not exhibit spontaneous activity even by the 9th day after birth[26].

One notable difference between the neonatal development of noradrenergic and dopaminergic neurons is that fetal and neonatal noradrenergic neurons are remarkably responsive to sensory stimulation, exhibiting very little spontaneous activity in the absence of sensory

input in the first few postnatal days[17,18,25,30]. Early postnatal nigrostriatal neurons, on the other hand, exhibited more frequent spontaneous activity at a higher rate than did locus coeruleus neurons, and did not appear to respond to similar types of sensory stimulation.

During the early postnatal period, many neurons were encountered in which the IS spike appeared similar to that in the adult, but in which the SD spike followed at an unusually long delay, and was often equal to or smaller in amplitude than the IS spike. A similar phenomenon is often obtained in spontaneous spikes of dopaminergic neurons from adult rats that occur near the end of long bursts, when the somadendritic membrane is relatively depolarized[9,10]. During the second postnatal week, half of the dopaminergic neurons displayed a very regular pacemaker-type firing pattern, one that is not seen in such high proportions among dopaminergic neurons from adult rats in vivo, although this type of very regular firing pattern is typical of nigral dopaminergic neurons recorded in an in vitro slice preparation[8].

Many of the physiological characteristics of neonatal nigrostriatal neurons are also similar to those obtained from fetal mesencephalic neurons grafted into the dopamine-depleted striatum[5]. For example, Fisher and colleagues reported that grafted dopaminergic neurons display mean spontaneous firing rates below those of dopaminergic neurons in situ from adult rats, exhibit atypically long-duration action potentials, display a high frequency of pacemaker-type firing patterns, and infrequent burst activity that is largely constrained to the occurrence of two spikes with interspike intervals in the order of 75 ms. Many of these parameters appeared to change to reflect more adult-type properties as the graft matured from 2 months to 9 months postoperatively. However, some characteristics such as the spike duration and frequency and complexity of bursting activity did not appear to change with time, and remained 'neotenized' even after maturation for 9 months, perhaps reflecting a type of arrested development[5]. These findings suggest that studies of the electrophysiological properties of striatal grafts of fetal dopaminergic neurons are more appropriately compared to studies of neonatal dopaminergic neurons in situ than to neurons from animals whose age is equivalent to the postgraft maturation period.

Thus, there appear to be a number of similarities in many of the physiological properties of neonatal dopaminergic neurons, neostriatal grafts of fetal mesencephalic neurons rich in dopamine cells, and pars compacta neurons in vitro. Since all these different preparations have in common a reduced density of functional inputs compared to the in situ mature substantia nigra (see below), it may be that this reduced input is responsible, at least in part, for the altered physiological properties.

Antidromic responses

The antidromic conduction times of nigrostriatal neurons to neostriatal stimulation remain relatively constant from PD1 through adulthood. A similar phenomenon has also been noted for noradrenergic coeruleocortical axonal conduction in neonatal rats[25]. The conservation of latency over development suggests that whatever the roles played by the nigrostriatal dopaminergic system in neonatal neuronal development and adult motor functioning, the timing of dopaminergic nigrostriatal neurotransmission, presumably in relation to that of other afferents to neostriatum, would seem to be an important parameter.

Antidromic responses consisted of full IS-SD spikes significantly more often in neonates than adults. When full IS-SD antidromic responses occurred, there was often a very long delay (up to several ms) between the IS and the SD components, similar to that seen in spontaneous spikes described above. The delay was sometimes so great that the waveforms of these neurons in some ways resembled the waveforms of two coupled cells, previously reported to occur in nigral dopaminergic neurons in adults[7,11]. That this phenomenon does not represent coupled neurons in the present results is argued by the large temporal variability between the two spike components, the consistent obliteration of both components of the spike during collision with spontaneous spikes, the failure to observe single spike waveforms of 'normal' morphology representing the firing of one member of the pair during spontaneous activity, and the absence of an IS-SD break on the initial component of either of the two 'spikes'. This phenomenon did not seem to be an artifact of cell damage, as it was obtained in neurons that were recorded for up to 45 min without showing any signs of deterioration. Rather, the high proportion of IS-SD antidromic spikes as well as the increased delay between the two components may reflect a state of relative somadendritic depolarization[9,10]. Truly coupled neurons, as previously reported[7,11] were not encountered.

Orthodromic responses

Peristimulus time histograms of nigrostriatal neurons following neostriatal stimulation revealed that the most common type of evoked response consisted of inhibition followed by rebound excitation. The duration of the poststimulus inhibitory period decreased as a function of development, concomitant with an increase in spontaneous activity. Similar to the nigrostriatal conduction latency, there was no developmental change in the latency to poststimulus inhibition.

Although they did not report on the duration of caudate-evoked inhibitory periods, Fisher and colleagues[6] found that in young kittens, caudate stimulation often produces an initial excitatory response, the inci-

dence of which declines with age such that exclusively inhibitory responses are observed in adult cats. In neonatal rats, however, we rarely observed excitation from striatal stimulation at any age, and there was no effect of age on the frequencies of the different response types. The reasons for this apparent difference are not immediately obvious, and may simply be related to the difference in species.

Although it is possible that the age-dependent reduction in the duration of the striatal-evoked inhibitory period that we observed suggests a declining inhibitory influence of the striatonigral projection, it is also possible that it results from a relative increase in the spontaneous activity of striatonigral and pallidonigral afferents over development. If striatal and pallidal neurons are relatively inactive in early postnatal life, then the inhibitory 'tone' of the nigrostriatal neurons may be decreased, giving rise to a more pronounced effect of these afferents when they are driven by stimulation. Conversely, later in development when the spontaneous activity of striatonigral and pallidonigral neurons has increased, the effects of striatal stimulation would appear weaker since they would be superimposed upon a certain level of non-stimulated inhibitory synaptic activity. There is good support for this hypothesis since rat striatal neurons do not show any spontaneous activity prior to 9 days of age, and only minimal spontaneous activity by 18 days of age[26], and in cat, the spontaneous activity of caudate and pallidal neurons also shows a significant increase from birth through maturity[21].

Neither of the two intracellular recordings from nigrostriatal dopaminergic neurons revealed a significant striatal-evoked postsynaptic potential, even at stimulus intensities sufficient to elicit antidromic responding. In contrast, all 8 of the non-dopaminergic neurons responded with clear inhibitory postsynaptic potentials (IPSPs), although they did not respond antidromically. These results, including the IPSP onset latency, are precisely the same as we previously reported in intracellular recordings from nigral neurons in adults[36,37]. The lack of an orthodromic effect in the nigrostriatal neurons is somewhat surprising, since clear evidence of orthodromic effects was apparent in the prestimulus time histograms constructed from extracellular recordings. In spite of the paucity of neonatal intracellular data, since this phenomenon has also been observed in adults, one may speculate that these conflicting results could indicate presynaptic effects of the striatal input on dopaminergic neurons and/or a distal site of termination of striatal inputs evoking a type of remote inhibition.

Functional inferences

Several of our findings suggest that nigrostriatal neurons in the neonatal rat exist in a more depolarized or inactivated state than in adult rats, especially during the early postnatal period. The recordings tended to be relatively noisy, and the spikes were usually of lower amplitude than that seen in the adult. Although in nigrostriatal neurons from adult rats there is often a notch or inflection on the initial positive component of the spike corresponding to an IS-SD break, in both extra- and intracellular recordings, the amplitude of the SD component is almost always greater than that of the IS component[9,10,14,35]. However, in neonates, it often occurred that the initial segment component was equal to or larger than the somadendritic spike in amplitude, which followed the IS spike with a considerable and variable temporal delay. The longer temporal delays between the two spike components is not usually encountered in dopaminergic neurons from mature animals[14,35], except during spikes occurring late in bursts, when, as the soma becomes progressively more and more depolarized, the SD component of the action potential grows progressively smaller and increases in duration, presumably due to increasing inactivation of soma dendritic sodium channels[9,10]. Finally, although the majority of neonatal dopaminergic neurons exhibited antidromic spikes consisting of the IS spike only as in adults[8,9,14,35,37], the proportion of full IS-SD antidromic spikes was significantly greater in early neonates, and decreased over development to normal adult proportions by the third postnatal week. Although only a few intracellular recordings were obtained from nigral neurons in the neonatal rats, all antidromic responses were full spikes and these data are also consistent with a relatively depolarized state of neonatal nigrostriatal neurons. In vivo intracellular recordings from nigral dopaminergic neurons in adults indicate that the proportion of full IS-SD antidromic spikes can be increased by direct depolarization of the somadendrite[9,10].

Thus, several lines of evidence point towards the conclusion that dopaminergic nigral neurons in neonatal rats exist in a more depolarized or inactivated state than in the adult. The reason(s) for this are not yet clear. It is possible that this results intrinsically, from immature membrane properties of the neonatal neurons. Recent in vitro studies have demonstrated developmental changes in the density of voltage-activated ion channels in cortical pyramidal neurons[23], and in voltage- and ligand-activated conductances in noradrenergic locus coeruleus neurons[41]. In both studies, a developmentally dependent decrease in the duration of the action potential was also noted, similar to that found for nigral dopaminergic neurons in the present study. It is well-known that pharmacological blockade of potassium channels leads to increased spike durations in many neurons[22], and others have demon-

strated developmental changes in the functional properties of potassium channels[19], and in the density of sodium channels[40] in myelinated nerve fibers. Thus, the appearance of a relatively depolarized state of neonatal neurons may arise from a reduced density or function of voltage-dependent potassium or sodium channels compared to that obtained in mature neurons.

Another factor may be that descending inhibitory striatonigral and/or pallidonigral pathways are not yet fully functional in the early neonatal period. Many striatonigral and pallidonigral neurons utilize GABA as a neurotransmitter[13], and several biochemical markers suggest that GABAergic systems are not fully functional in early neonatal periods in many parts of the rat central nervous system, an observation confirmed by recent in vitro electrophysiological studies[26,33]. Although judging from the poststimulus inhibitions and synaptic potentials described in this report, the striatonigral pathways are present and are electrophysiologically competent in early postnatal life, they are not yet functional in the absence of external stimulation, as discussed above[26].

Finally, an altered functioning of somadendritic dopamine autoreceptors early in the neonatal period could play a role in the polarization level of nigrostriatal neurons, since activation of these receptors has been shown to hyperpolarize dopaminergic neurons by increasing a potassium conductance[20], and nigral dopaminergic neurons from 4-week-old rats are less sensitive to the inhibitory effects of systemic apomorphine than adults[29].

In summary, nigral dopaminergic neurons are active in neonatal rats at least as early as the day of birth, and show several signs of existing in a depolarized state relative to nigral dopaminergic neurons of mature rats in situ. At this time, the dopaminergic axons are capable of conducting impulses to terminal zones in the neostriatum, and have already arborized to a considerable extent. Between the third and fourth weeks of age, most of the physiological properties of these neurons have reached, or are close to those of dopaminergic nigral neurons in mature rats. Because of the close similarities of the electrophysiological properties of nigrostriatal neurons from neonatal rats and those of fetal dopaminergic neurons grafted to the neostriatum, it is likely that the electrophysiological, morphological and pharmacological properties of grafted dopaminergic neurons are better compared to those of dopaminergic neurons from neonates rather than adults.

Acknowledgements. We thank Judith S. Rankin for excellent technical assistance. Many thanks to Dr. Anne Mayer and the Institute of Animal Behavior for supplying us with rat pups. This research was supported by MH 45286 and a Rutgers University Research Council Grant. J.M.T. is a Henry Rutgers Research Fellow.

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