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ELECTROPHYSIOLOGICAL CHARACTERISTICS OF CELLS WITHIN MESENCEPHALON SUSPENSION GRAFTS

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Abstract—Both spontaneous and evoked extracellular electrophysiological activity of neurons within fetal mesencephalon suspension grafts to the dopamine-depleted striatum of rats were examined. In some cases, extracellular recording was combined with intracellular labeling to identify recorded neurons. Grafted rats displaying a complete cessation of ipsilateral rotations following amphetamine administration were examined at post-implantation time intervals of two, four, five, eight and nine months.

Four separate classes of neurons were distinguished within the transplanted striatum based on electrophysiological properties. The first of these groups, the type I cells, appeared to be non-grafted striatal neurons. When spontaneously active, these striatal-like cells fired bursts of action potentials separated by periods of decreased activity. Evoked responses in these cells were characteristic of striatal cells. Type I cells which were intracellularly labeled were found outside the grafts and displayed the characteristic morphology of the medium spiny neuron of the neostriatum. The other three cell classes displayed electrophysiological properties similar to neurons recorded *in situ* within the reticular formation, substantia nigra pars compacta and substantia nigra pars reticulata. Neurons from these three groups which were labeled with an intracellular marker were found to lie within the suspension grafts. The spontaneous activity of the pars compacta dopaminergic-like neurons was predominantly irregular, with some cells also firing in a regular or pacemaker-like pattern. Infrequently, irregular firing dopaminergic-like neurons displayed episodes of doublet bursting. Many of the grafted neurons responded to electrical stimulation of prefrontal cortex and striatum, indicating that the graft was receiving functional inputs from host neurons.

Comparison of the firing rate and pattern of grafted neurons to *in situ* mesencephalic neurons as a function of time following grafting suggested that the grafted neurons and/or the neuronal circuitry is slowly developing within the host environment. A prolonged time-course for the maturation of the graft may be reflected in the time required to achieve improvements in some behavioral deficits following transplantation. However, the relatively rapid recovery of drug-induced rotational asymmetry following grafting suggests that this form of recovery may not require mature functioning of the grafted neurons.

Parkinson's disease is a progressive and debilitating disorder of voluntary movement that occurs with the degeneration of dopaminergic neurons in the substantia nigra pars compacta.[26] The current therapy of choice for the relief of parkinsonian symptoms is the administration of the dopamine precursor L-DOPA.[41] However, drug therapy does not alter the course of the underlying disease, and often produces side effects which include dyskinesias and psychoses.

In animal models of Parkinson's disease, the transplantation of neuronal tissue containing dopamine-producing cells has been explored as a possible alternative approach to the treatment of parkinsonian symptoms. Fetal tissue containing dopamine neurons has been shown to survive implantation into the striatum of rats with a prior lesion of the nigrostriatal pathway using the catecholamine neurotoxin 6-hydroxydopamine (6-OHDA).[4,38] Such grafted neurons extend catecholamine-containing fibers into the host brain[5] and exhibit robust dopamine synthesis and metabolism.[42] Depending on the size and placement of the grafts, rats display significant recovery of sensory and motor deficits.[9–11,14] However, some behavioral abnormalities remain in these grafted animals even after extended survival and growth of the implant within the lesioned brain.[13] Among other possibilities, such impairments may reflect abnormal physiological development of the grafted cells within the new environment, an absence or reduced density of connections between the host and graft tissues, or the establishment of inappropriate circuitry within the graft or between graft and host neurons.

To date, there is little known of the electrophysiological properties of neurons within dopamine-rich grafts, or of the electrophysiological interactions between grafted and host neurons. In an early study, Wuerthele *et al.*[54] examined the electrophysiological properties of fetal neurons within solid mesencephalon grafts placed in the lateral ventricle of rats lesioned with 6-OHDA. The ventricular placement appeared to prohibit the growth of host fibers into the graft as well as to limit the outgrowth of fibers from the graft into the host brain. When examined five to

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Abbreviations: DAB, 3,3′-diaminobenzidene; HRP, horseradish peroxidase; 6-OHDA, 6-hydroxydopamine; PSTH, peristimulus time histogram; TH, tyrosine hydroxylase.

18 weeks following transplantation, spontaneously active neurons were found within the ventricular grafts which displayed the wide action potentials and slow, irregular firing rates which characterize some dopamine neurons within the substantia nigra.[18] Thus, even in isolation from normal afferents and efferents, dopaminergic neurons within a graft appear to develop some of the physiological properties typical of *in situ* dopamine neurons. That the development of such properties may be crucial for behavioral recovery following transplantation has been suggested by the work of Arbuthnott *et al.*[1] These investigators examined the electrophysiological activity of neurons within solid mesencephalon grafts in rats which were assessed for drug-induced rotational behavior following grafting. On histological examination, surviving dopamine-containing neurons were found within the grafts of all of the implanted rats. However, spontaneously active neurons with properties similar to *in situ* dopamine neurons were only found within rats that exhibited recovery from motor asymmetry following grafting.

In the present study, we examined the electrophysiological activity of grafted mesencephalic neurons within 6-OHDA lesioned rats that had recovered from rotational asymmetry following transplantation. The extracellular electrophysiological properties of cells within a mesencephalon suspension graft were characterized and compared with those typical of cells within the adult mesencephalon. Suspension grafts, rather than the solid tissue implants used in previous studies, were chosen for investigation because these grafts can be placed directly within the striatum. Such intraparenchymal placements have been shown to enhance graft–host and host–graft interactions.[4,6] The functioning of connections between host and graft neurons was also explored by examining responses of grafted neurons to electrical stimulation of cortex and neostriatum. Finally, possible developmental changes in the electrophysiological properties of grafted neurons were investigated by examining grafts at various time intervals following transplantation.

EXPERIMENTAL PROCEDURES

Lesions and grafting

Female Sprague–Dawley rats (180–220 g) were anesthetized with a mixture of ketamine, rompun and acepromazine and placed in a stereotaxic frame. Rats received an injection of 6-OHDA (12 μg in 2 μl saline supplemented with 0.2 mg/ml ascorbic acid) into the left medial forebrain bundle (AP −4.4 mm from bregma, L 1.1 mm, V 7.5 mm from dura) according to the atlas of Paxinos and Watson.[37] Rats exhibiting at least seven ipsilateral turns per minute to amphetamine (5 mg/kg, s.c.,) when tested seven to 14 days post-lesion, were selected for transplantation.[42] Two locations within the denervated striatum (AP +0.7 mm, L 2 mm, V 4–5 mm; AP +1.5 mm, L 3 mm, V 4–5 mm) were implanted with 5 μl each of cell suspensions prepared from ventral mesencephalon of 13- to 15-day embryonic rats crow–rump length = 11–14 mm, as described previously.[5] Rats were returned to home cages and allowed to recover for one month. Implanted rats were tested with amphetamine approximately once every one to three months for periods of up to nine months.

Improvements in rotational asymmetry are directly correlated with the number of surviving grafted neurons.[35] Thus, to increase our chances of recording from neurons within the grafts, recordings were obtained only from those rats which exhibited either complete rotational compensation (cessation of ipsilateral rotations) or overcompensation in rotational behavior (rotations to the contralateral side) following amphetamine administration. Thirteen transplanted rats exhibiting such compensation were examined electrophysiologically at post-implantation time intervals of two, four, five, eight and nine months.

Electrical stimulation and recording

Rats meeting the behavioral criteria were anesthetized with urethane (1.3 g/kg, i.p.) and placed in a stereotaxic apparatus. Points of contact between the animal and the stereotaxic frame were covered with xylocaine ointment (5%). The skin overlying the skull was removed and some cerebrospinal fluid was drained through a puncture in the atlanto-occipital membrane. The holes in the skull through which the suspension grafts had been injected were re-exposed and the dura was removed. Four additional holes were drilled to position one bipolar enamel-coated stainless steel stimulating electrode (tip separation approx. 250 μm) in prefrontal cortical white matter (AP 3.5 mm, L 1.5 mm, V 2.0 mm angled 20° anterior to the coronal plane) and three additional electrodes within the striatum in a triangular pattern 1–2 mm from the grafts (V 4–5 mm). Electrodes were secured to the skull with cyanoacrylate glue and dental cement. The animal was suspended by clamps on the C2 vertebra and the tail to reduce respiratory pulsations. Body temperature was maintained at 37 ± 1°C with a heating pad, and the electrocardiogram was monitored throughout the experiment.

Extracellular single unit recordings were obtained using glass microelectrodes filled with either 4% horseradish peroxidase (HRP) in 0.5 M potassium methyl sulfate or a 3% solution of the biotin-lysine complex biocytin in 0.5 M potassium acetate (tips approx. 0.2–0.5 μm with *in vivo* impedance of 30–90 MΩ). Recordings obtained from spontaneously active cells in the striatal region containing the graft were amplified through a WPI M-707 preamplifier, displayed on a Tektronix RM 565 oscilloscope (d.c.–0.1 MHz band pass) and collected on audio tape. Digitized records of the activity were obtained using a Nicolet 2090 digital oscilloscope connected to a PDP-11/03. Depth and position of each recorded cell was noted. Following recording of spontaneous activity within the striatum, responses to stimulation of cortex and neostriatum were tested. Stimuli consisted of monophasic rectangular pulses of 0.2 ms duration, delivered at a rate of 0.6 Hz. Cortical stimuli were typically 1–2 mA while lower stimulus intensities of 50–500 μA were used for striatal electrodes to minimize the possibility of direct activation of grafted cells. Following the extracellular characterization of neuronal activity, intracellular impalement was attempted solely for intracellular labeling and identification of the recorded neuron. Cells were injected with hyperpolarizing current (less than 1.5 nA) immediately upon impalement to stabilize the neuron. Stable cells, as determined by membrane potentials greater than 40 mV and/or action potential amplitudes greater than 45 mV, were injected iontophoretically through the bridge circuitry of the WPI preamplifier with either HRP or biocytin at 1–3 nA for 5–10 min or until the recording deteriorated. Experiments were typically terminated and animals perfused within 1 h of injection to minimize morphological deterioration of the labeled cell. Spontaneous activity was also collected as described above from cells within the intact substantia nigra pars compacta, substantia

nigra pars reticulata and reticular formation (AP 2.1 anterior to lambda, L 2.2, V 6.5–8.0) of untreated, control rats for comparison with activity from grafted striata.

Tyrosine hydroxylase, horseradish peroxidase and biocytin histochemistry

At the end of the recording session, animals were deeply anesthetized with urethane and perfused transcardially with a wash of oxygenated rat Ringer solution (pH 7.2) followed by 1% paraformaldehyde and 2% glutaraldehyde in 0.15 M phosphate buffer at room temperature. Brains were removed, placed in phosphate buffer overnight, and then sectioned at 60 μm on a vibratome. Free-floating sections were reacted for HRP using a modified glucose oxidase method.[28] These sections were then passed through an ascending glycerine series (20–100%) over 1 h, mounted in 100% glycerine and coverslipped. Sections were examined with a light microscope for the presence of cells containing HRP reaction product. Labeled cells were photographed and sections containing these cells were demounted to phosphate buffer and further processed for tyrosine hydroxylase (TH) immunoreactivity. The protocol of Armstrong *et al.*[2] was followed for TH labeling using a dilution of 1:600 for the primary antibody to TH (Eugenetec) with 3,3′-diaminobenzidene (DAB) as a chromagen. Double reacted sections (HRP and TH) were passed through an alcohol series, cleared in xylene and mounted with permount. The dense intracellular HRP reaction product appeared to obscure TH labeling of the same cell. Therefore, biocytin was used in some experiments as an intracellular marker.[3] Biocytin was exposed in alternate free-floating sections using either avidin–fluorescein flourescence or avidin–HRP with DAB as a chromagen.[25] The sections were then passed through glycerine as described above and coverslipped. Sections containing labeled cells, and those unreacted sections adjacent to the cell, were further processed for TH immunoreactivity using the peroxidase–antiperoxidase method[46] with DAB as a chromagen. Microelectrode and stimulating electrode tracks were reconstructed from stained sections with the aid of the noted cell coordinates. Camera lucida drawings of intracellularly labeled neurons were made at 60× with a Leitz Ortholux II microscope equipped with a drawing tube.

Data analyses

Solid mesencephalon grafts within the striatum have been reported to contain neurons which display extracellular action potential waveforms and spontaneous firing rates typical of *in situ* nigral neurons.[1,54] In the present study, several extracellular parameters which have been shown to distinguish between mesencephalic neurons *in situ*[7,18,24] were obtained for each neuron encountered in the striatal area containing the suspension graft, and then compared against properties of neurons commonly found within the neostriatum and mesencephalon as an aid in classifying the cells. These parameters included: (i) the shape of the action potential waveform; (ii) the width of the action potential, as measured from the onset of the initial component of the waveform to the point at which the late component of the waveform crossed baseline; (iii) spontaneous firing rate and (iv) the spontaneous firing pattern. Spontaneous firing patterns were statistically analysed with (v) autocorrelation histograms. Certain features of firing rate autocorrelations have been shown to be distinctive "signatures" for different cell types within the substantia nigra, reticular formation and striatum.[31,52,53] Evoked responses were characterized with peristimulus time histograms (PSTHs). In those cases in which intracellular labeling was achieved, the electrophysiological responses could be associated with the location and/or morphological characteristics of the recorded neuron.

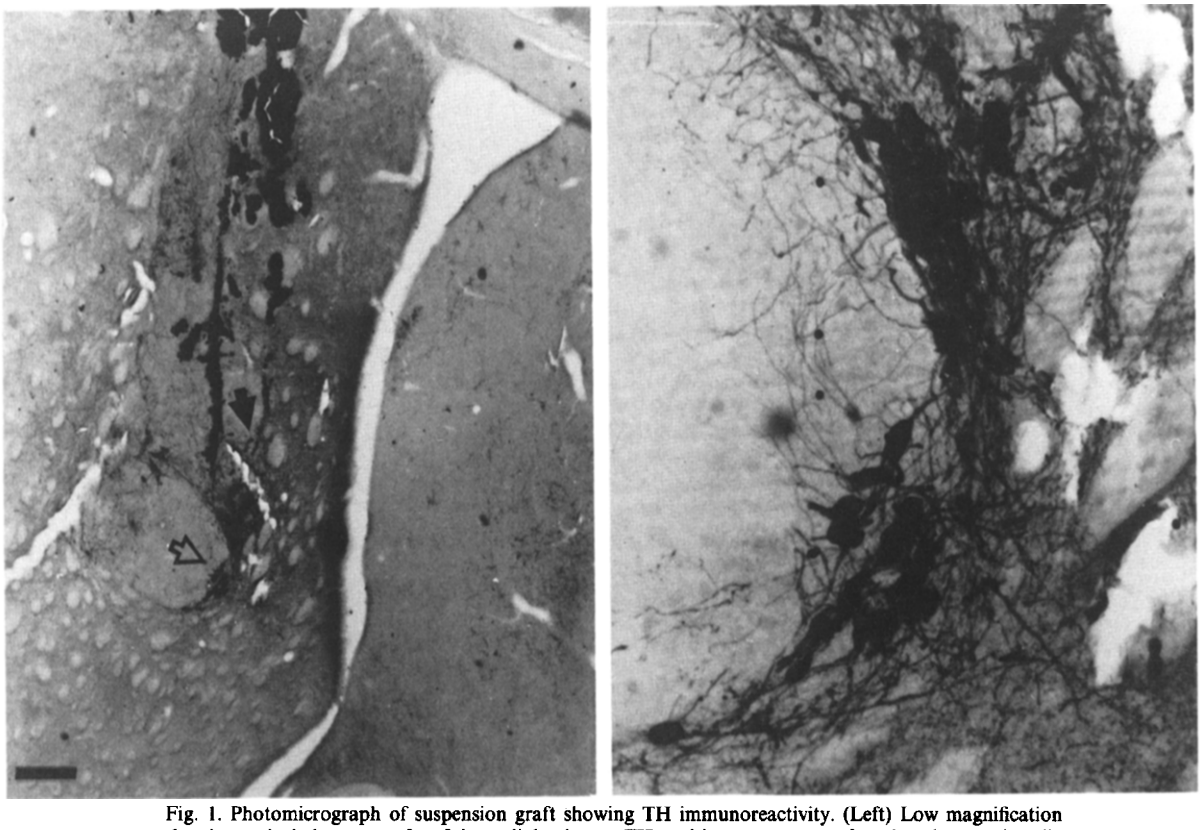
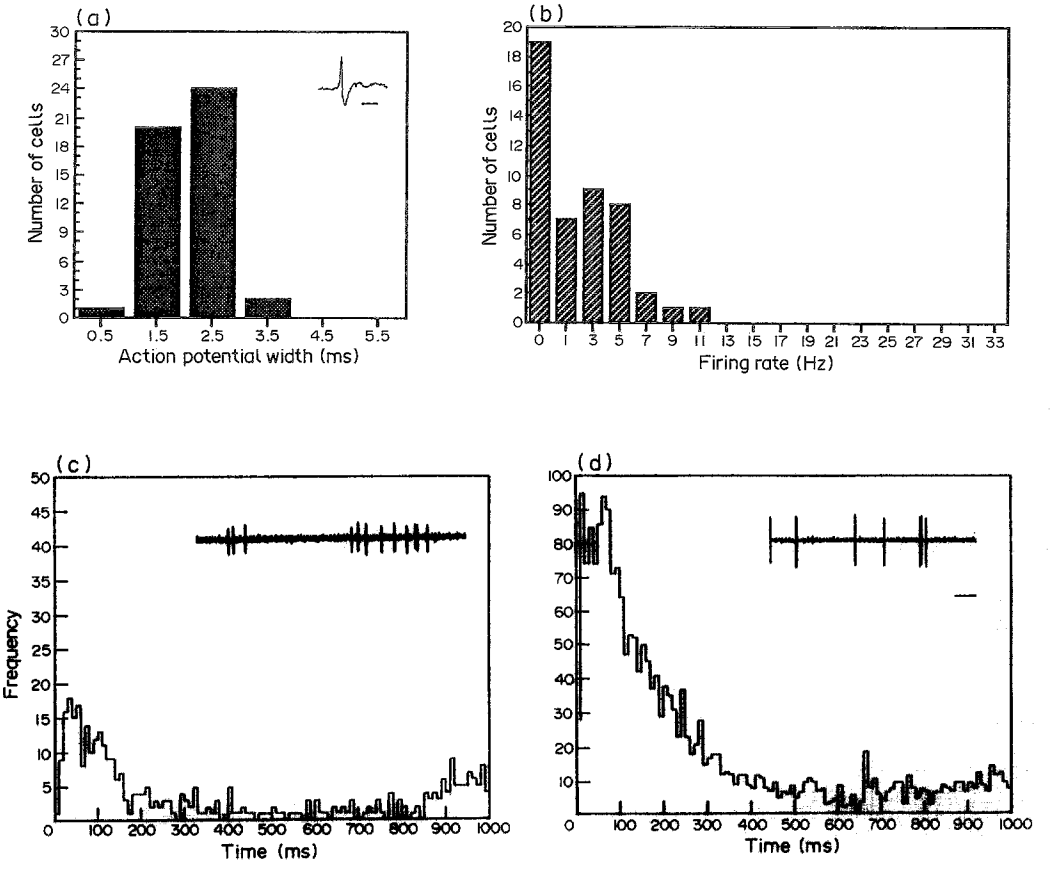


Fig. 1. Photomicrograph of suspension graft showing TH immunoreactivity. (Left) Low magnification showing typical placement of graft in medial striatum. TH-positive neurons were found to cluster primarily around the edges of the graft (open arrow). Several electrode tracks are visible. (Right) Higher magnification of the neurons indicated by open arrow. Scale bar = 200 μm (left); 40 μm (right).

RESULTS

Behavior and anatomy

All of the implanted rats used for electrophysiological analyses displayed complete compensation of amphetamine-induced rotational behavior (4.9 ± 1.0 rotations/min contralateral to graft vs 10.0 ± 0.4 rotations/min ipsilateral to lesion prior to grafting). Upon histological examination, TH-positive neurons were found within the grafts of all of these rats. These TH-positive neurons were grouped in clusters, often around the periphery of the graft. A typical example of a graft at five months following transplantation is shown in Fig. 1. By the earliest evaluated time period



(e)

Stimulation site	Excitation	Inhibition	No response
Cortex (n=21)	100%	0	0
Striatum (n=9)	56%	33%	11%

Fig. 2. Electrophysiological properties of type I neurons. (a) Distribution of action potential widths. Inset shows a typical biphasic action potential for a neuron in this group. Scale bar = 1 ms. (b) Distribution of firing rates for type I cells. (c) Example of an autocorrelogram obtained from a type I neuron. Note the increased frequency of firing immediately after a spike, followed by a period of extended quiescence. (d) Autocorrelogram obtained from a neuron within an intact, non-grafted, striatum. The insets in both c and d show the firing patterns of each cell displayed in the autocorrelograms. Bin width 10 ms, scale bar = 50 ms for both c and d. (e) Responses of type I neurons to cortical and striatal stimulation. Number of cells tested is shown in parentheses. Single cells that were tested with both cortical and striatal stimuli are listed twice in the table. Thus, the total number of cells tested is less than the total number shown.

of two months post-implantation, TH-immunoreactive fibers from neurons within the grafts were seen to extend into the striatal parenchyma.

Electrophysiology

Extracellular recordings were obtained from 103 cells in and around the grafts from 13 behaviorally compensated rats. Analyses of the spontaneous firing patterns of these cells provided the primary criteria for classifying the recorded neurons into four different cell groups, types I–IV. The shape and duration of the action potential waveforms, as well as the spontaneous firing rates, were used as secondary criteria for distinguishing neurons with similar firing patterns. For example, neurons firing in a regular manner were classified as type II if the cell exhibited a narrow biphasic action potential waveform, or type IV if the action potential was wide and triphasic. PSTHs were found to distinguish type I cells from the other cell classes as described below.

Type I *cells* (n = 47)

The majority of cells in this category had a biphasic action potential waveform (87%). The average width of the action potential, which did not differ from two to nine months post-implantation, was 2.3 ± 0.7 ms (Fig. 2a). Many type I neurons were not spontaneously active and were identified either through transient activity elicited by the approach of the recording electrode or by responses to cortical stimuli (see below). Neurons exhibiting spontaneous activity fired at rates less than 12 Hz (Fig. 2b) in a slow irregular pattern, often punctuated by bursts (Fig. 2c). There was an indication that cells with higher firing rates were prevalent within younger grafts. Between two and five months after grafting, 63% of type I neurons (19 cells from five rats) fired at frequencies greater than 4 Hz. By eight to nine months post-implantation, 75% of type I neurons (28 cells in six rats) fired less than 4 Hz (Fisher Exact Test, $P < 0.06$). Autocorrelation histograms of the firing patterns for type I cells were characterized by an increase in the frequency of firing immediately after a spike, followed by an extended period of decreased activity (Fig. 2c). The initial trough of the autocorrelation averaged 60 ms (range = 9–150 ms). Similar autocorrelation properties are observed for striatal neurons recorded from intact rats (Fig. 2d), as has been described previously.[52]

The responses of type I cells to stimulation of cortical white matter also distinguished this class. The responses of 21 type I cells to cortical stimuli are tabulated in Fig. 2e. Individual cells which were tested for responses to both cortical and striatal stimuli ($n = 4$) are listed twice in the table. Typically, type I cells responded to cortical stimulation with either a single or multispike response ("excitation"), often followed by a period of decreased and then increased firing as commonly seen for striatal cells in intact rats.[39,40,43] A characteristic PSTH from a type I cell, elicited by cortical stimulation, is shown in Fig. 3a. The latency to the initial spike following the cortical stimulus averaged 5.8 ± 2.5 ms. Responses to striatal stimulation were mixed, with excitation predominating (56% of cells). Inhibition of type I activity, as indexed by a decrease in spontaneous firing, was only observed following low intensity (<0.5 mA) stimulation of striatum (three out of nine cells).

Intracellularly labeled cells ($n = 4$) with type I electrophysiological characteristics were always found in striatal tissues outside the graft. None displayed TH immunoreactivity. All of these neurons were characterized by medium-sized somata (17–20 μm) which were round or ovoid, and dendritic processes that were covered with spines. An example of one of these neurons, intracellularly labeled with biocytin, is shown in Fig. 4. The morphology of these type I neurons appeared similar to the common medium spiny cell within the striatum.[29,51]

Type II *cells* (n = 7)

The action potential of type II cells was generally biphasic (89%) with an average width of 1.9 ± 0.9 ms (Fig. 5a). These cells were most prominently characterized by moderate to fast regular firing between 8 and 32 spikes/s (median = 11 spikes/s, mean ± S.E.M. = 15.1 ± 9.2, Fig. 5b). Due to the small number of these neurons recorded, changes in the action potential widths or firing of type II cells as a function

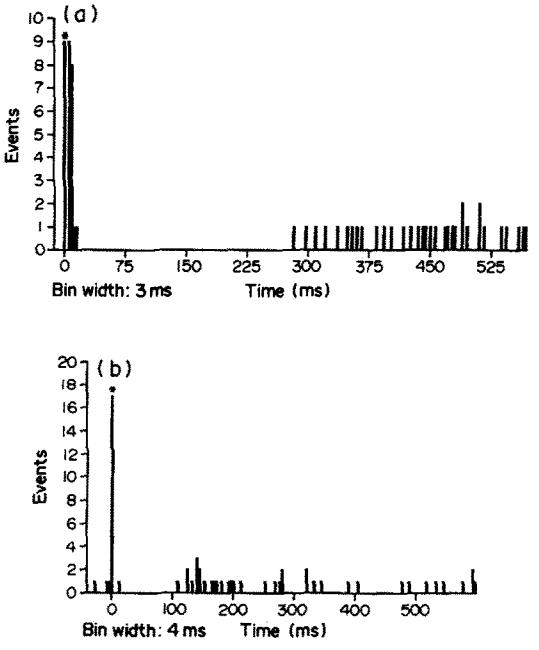


Fig. 3. Typical PSTHs for recorded neurons. (a) PSTH from a type I neuron displaying the characteristic spike–inhibition–rebound sequence which followed cortical stimulation. (b) PSTH from a type IV neuron displaying the decrease in activity following cortical stimulation which was typical of all grafted neurons. Asterisk in both a and b indicates the cortical stimulus.

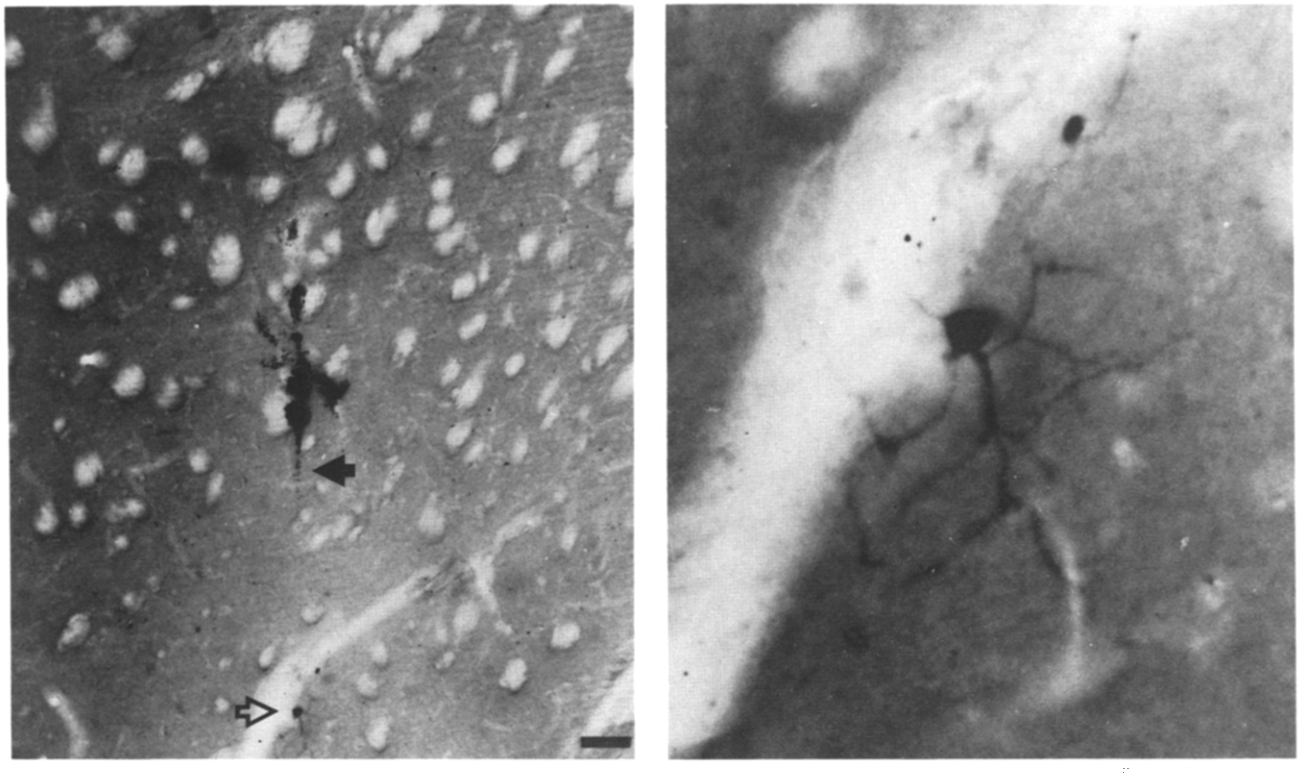


Fig. 4. Photomicrograph of a type I neuron intracellularly labeled with biocytin. This cell was located ventral to the suspension graft (open arrow). An electrode track is visible (arrow). Higher magnification of labeled cell is shown on the right. Note the spines on the dendrites, which are characteristic of the common spiny striatal neuron. Scale bar = 50 μm (left); 10 μm (right).

of graft age could not be determined. Autocorrelation histograms of type II firing patterns revealed the tendency of these cells to fire immediately following a relatively constant refractory period (repetitive firing, Fig. 5c). A brief initial trough in the autocorrelation was typical (average = 55 ms, range = 10–105 ms). Such properties are similar to those described for *in situ* non-dopaminergic pars reticulata neurons[53] (Fig. 5d). Neurons with these properties which were labeled with an intracellular marker ($n = 3$) were found to be located within the graft and were not TH-immunoreactive. Cell somata were triangular or polygonal in shape and of medium size (15–18 μm), as has been observed for pars reticulata neurons intracellularly labeled with HRP.[22]

Stimulation of cortical white matter elicited a decrease in spontaneous firing in two out of the four type II cells tested (Fig. 5e). One of the four cells, examined with both cortical and striatal stimuli, displayed a short latency (8 ms) orthodromic spike to cortical activation but was unresponsive to striatal stimulation.

Type III *cells* (n = 11)

The action potential of type III cells was often triphasic (64%), with an average width of 3.2 ± 1.2 ms (Fig. 6a). These cells exhibited a range of firing rates similar to that of type II cells (mean ± S.E.M. = 10.6 ± 4.6 Hz, Fig. 6b), but were distinguished by an irregular and slightly clustered pattern of action potentials (Fig. 6c). The initial trough in the autocorrelation was brief (mean = 24 ms; range = 10–48 ms). These characteristics are reminiscent of those observed for neurons in the reticular formation[31] (Fig. 6d). Cells with type III characteristics were not successfully injected with an intracellular marker. Reconstruction of electrode tracks established that one of these neurons was within the graft. The electrode tracks associated with the remaining neurons could not be reliably identified.

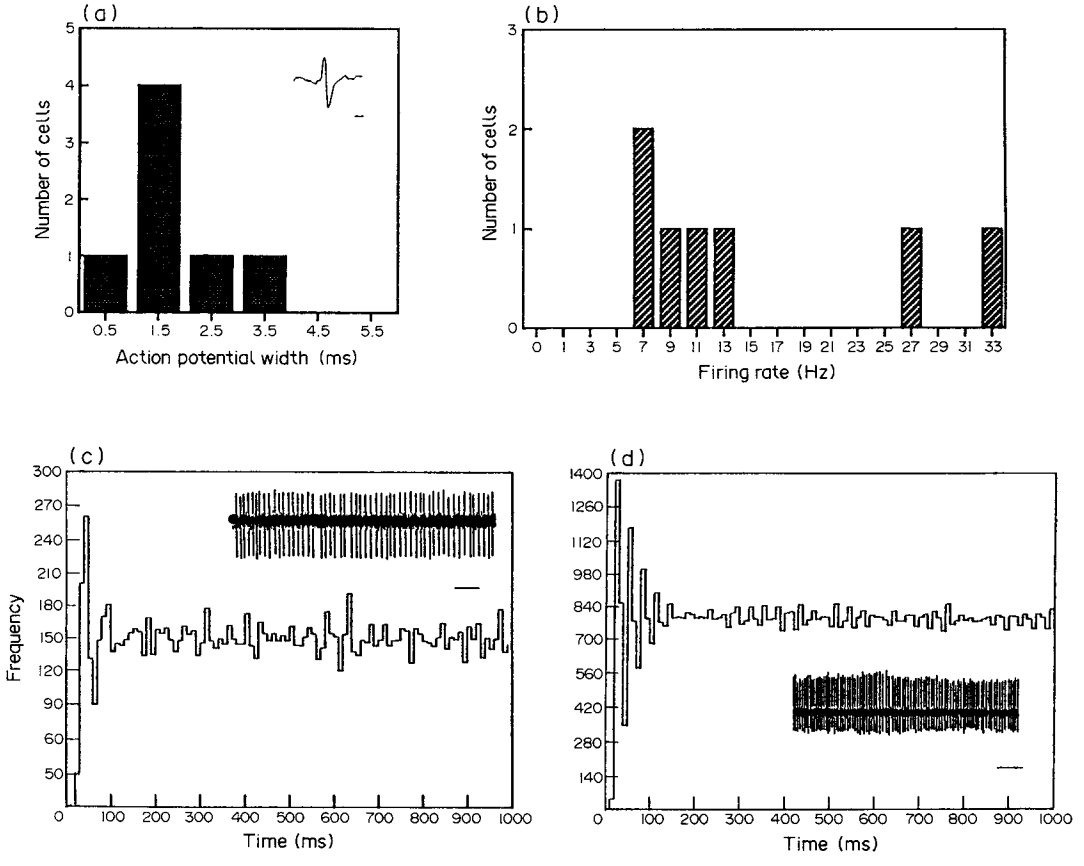
Type III cells displayed a trend towards increasing firing rates at longer post-implant time periods. All cells in eight- to nine-month grafts (four cells from two rats) fired greater than 9 Hz, while 71% of those in four- to five-month grafts (seven cells from two rats) fired at frequencies less than 9 Hz (Fisher Exact Test; $P < 0.06$). Type III action potentials tended to be wider in younger grafts. Over half of the cells recorded in grafts four to five months post-implantation displayed action potentials wider than 4 ms. By eight to nine months after grafting, type III action potential waveforms were always less than 4 ms in width.

Stimulation elicited responses in five out of the six type III cells tested. The two cells which responded to stimulation of cortical white matter displayed an inhibition of spontaneous activity (Fig. 6e).

Stimulation of host striatum also inhibited type III activity in two of these cells. The decreases in firing began approximately 2–18 ms following stimulation, as estimated from the PSTHs. One cell which was tested with cortical and striatal stimuli exhibited a decrease in activity following cortical stimulation but a short latency orthodromic spike (5 ms) to striatal stimulation.

Type IV *cells* (n = 21)

This final category of cells was distinguished from the other cell classes in several ways. Firstly, the action potential of type IV cells was always greater than 2 ms in width (mean ± S.E.M. = 3.7 ± 1.5 ms) and frequently (62%) had a triphasic waveform (Fig. 7a). Occasionally, an inflection on the initial rising phase of the waveform was observed. Secondly, the spontaneous activity from type IV cells was very distinctive. These cells fired in either a slow, irregular pattern (67%) or in a regular or pacemaker-like manner (33%). Two of the 21 cells which fired in an irregular pattern exhibited doublet bursts with an interspike interval of 74.3 ± 9.0 ms. Regardless of firing mode, the spontaneous firing rate of type IV



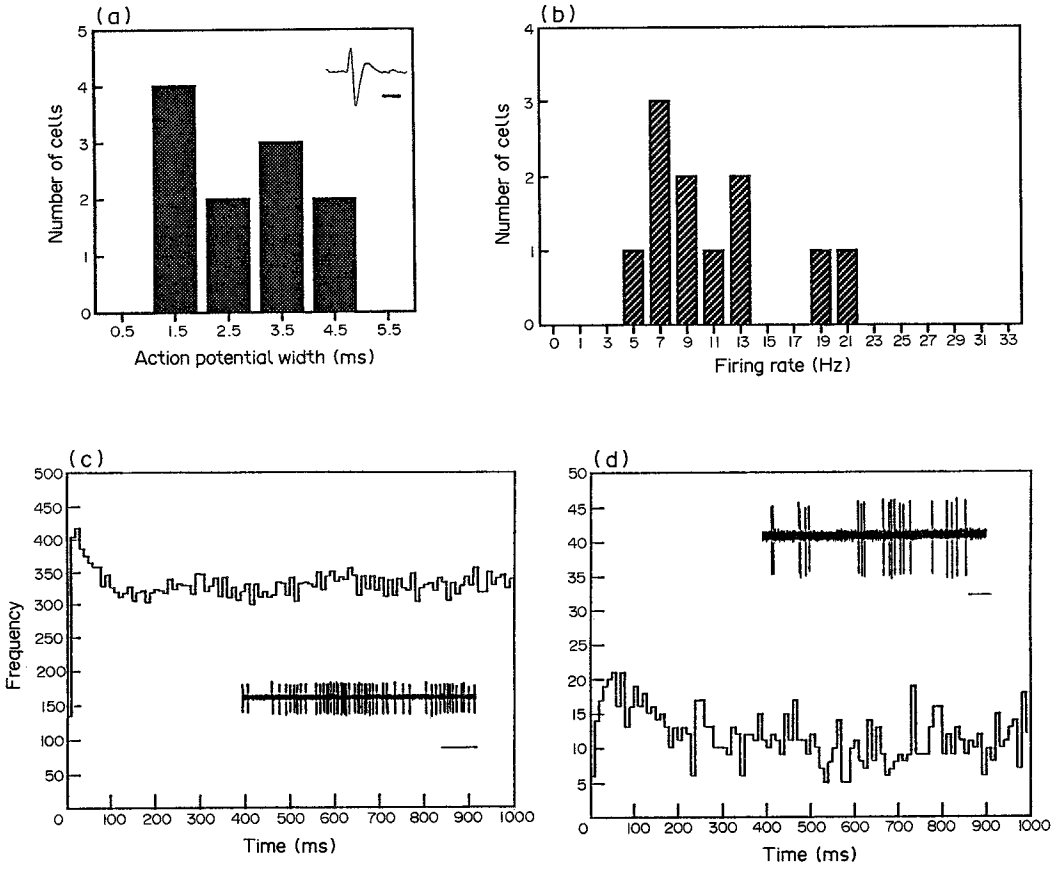
(e)

Stimulation site	Excitation	Inhibition	No response
Cortex (n=4)	25%	50%	25%
Striatum (n=1)	0	0	100%

Fig. 5. Electrophysiological properties of type II neurons. (a) Distribution of action potential widths. Inset shows an example of a biphasic action potential recorded from a neuron in this group. Scale bar = 1 ms. (b) Distribution of firing rates for type II cells. (c) Example of an autocorrelation obtained from a type II neuron. Inset shows the fast, regular firing pattern of this cell. (d) Autocorrelogram of a histologically identified neuron within the substantia nigra pars reticulata. Note the faster rate, but similar firing pattern of this cell, as compared with the cell shown in c. bin width 10 ms, scale bar = 50 ms for both c and d. (e) Responses of type II neurons to cortical and striatal stimulation.

cells was always less than 9 Hz (mean ± S.E.M. = 2.4 ± 2.7 spikes/s; Fig. 7b). Autocorrelation histograms revealed another distinguishing characteristic of this class (Fig. 7c). In comparison with the other cell groups, the initial troughs in type IV autocorrelation histograms were long (mean = 171.5 ms; range = 46–400 ms). Taken together, the extracellular electrophysiological properties seen for type IV cells are similar to those described for dopaminergic, substantia nigra pars compacta neurons.[15,18,19,24,53] For comparison, autocorrelation histograms of a type IV cell and an antidromically identified nigrostriatal dopamine neuron are shown in Fig. 7c, d.

Slower firing type IV neurons appeared predominant in younger grafts. The majority of neurons (64%) encountered within the grafts two to five months post-implantation (11 cells from three rats) fired at frequencies less than 1.5 Hz, while 90% of the type IV neurons recorded eight to nine months after grafting (10 cells from five rats) fired at frequencies



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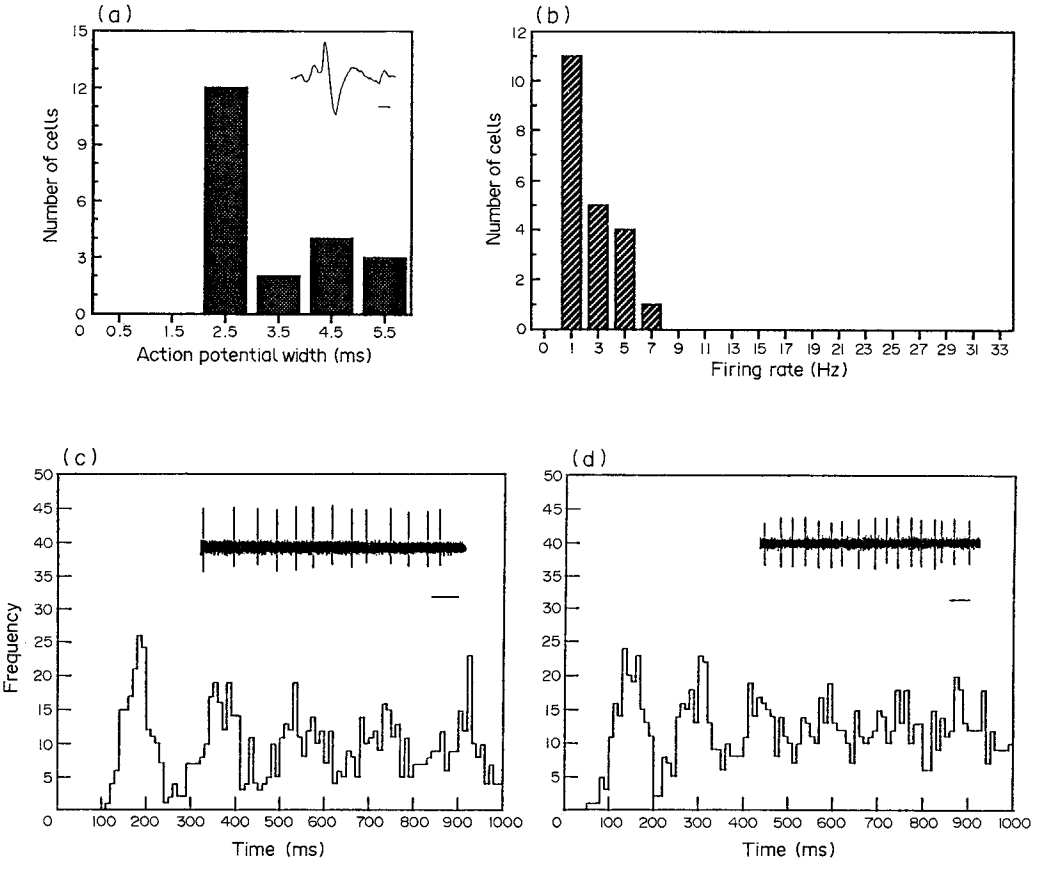
Stimulation site	Excitation	Inhibition	No response
Cortex (n=4)	0	50%	50%
Striatum (n=3)	33%	67%	0

Fig. 6. Electrophysiological properties of type III neurons. (a) Distribution of action potential widths. Inset shows a typical triphasic action potential recorded from a neuron in this group. Scale bar = 1 ms. (b) Distribution of firing rates for type III cells. (c) Example of an autocorrelogram obtained a type III neuron. There is an increased frequency of firing for a short period after a spike which is then followed by a random probability of firing. (d) Autocorrelogram obtained from a histologically identified neuron within the mesencephalic reticular formation. Insets for both c and d show the firing patterns of the cells displayed in the autocorrelations. Bin width 10 ms, scale bar = 50 ms for both c and d. (e) Responses of type III neurons to cortical and striatal stimulation.

greater than 1.5 Hz (Fisher Exact Test, $P < 0.03$). The average width of type IV action potentials in eight- to nine-month grafts (4.0 ± 1.7 ms) was not significantly greater than those in two- to five-month grafts (2.4 ± 0.1 ms).

A tabulation of cortical and striatal evoked responses from a total of 10 type IV neurons is shown in Fig. 7e. Cells which were tested for responses to both cortical and striatal stimuli are listed twice in the table. As seen for the other cell classes, electrical stimulation elicited responses in the majority (90%) of type IV cells tested. And, as observed for type II and type III cells, the predominant response of type IV cells to either cortical or striatal stimulation was inhibition of spontaneous firing. A typical PSTH for a type IV neuron is shown in Fig. 3b. Five of the 10 type IV cells were tested for responses to both cortical and striatal stimulation and were found to respond in a similar manner to both stimuli. Two of the cells were inhibited by the stimuli, two increased activity



(e)

Stimulation site	Excitation	Inhibition	No response
Cortex (n=9)	33%	56%	11%
Striatum (n=6)	33%	50%	17%

Fig. 7. Electrophysiological properties of type IV neurons. (a) Distribution of action potential widths. Inset shows an example of a triphasic action potential recorded from a neuron in this group. Note the inflection on the initial rising phase of the waveform. Scale bar = 1 ms. (b) Distribution of firing rates for type IV cells. (c) Example of an autocorrelogram obtained from a type IV neuron. Note the delay after a spike before the occurrence of another spike (long initial trough). The inset illustrates the repetitive firing pattern of this cell. (d) Autocorrelogram obtained from an antidromically identified cell within the substantia nigra pars compacta. Bin width 10 ms, scale bar = 50 ms for both c and d. (e) Responses of type IV neurons to cortical and striatal stimulation.

following stimulation and one was non-responsive. The latency to cortical excitation was often unusually long (22 ms). Only one cell was found to respond to cortical stimulation with a short latency orthodromic spike (7 ms). The two cells which increased firing following striatal stimulation both displayed a latency of 5.5 ms to the initial spike.

Three cells with type IV electrophysiological properties were successfully recovered following intracellular injection. These cells were located within the grafts, often near cell clusters which were TH-immunoreactive. The injected cells displayed cell somata which were fusiform or ovoid and of medium size (18–21 μm). Typically, two to three dendrites were seen to emanate from the cell body. One cell intracellularly injected with biocytin, shown in Fig. 8, displayed TH immunoreactivity when examined in an adjacent section. Dendritic processes from this neuron appeared to be varicose, as indicated by the dark arrows in Fig. 8. The axon was found to arise from one of the primary dendrites. These morphological properties of type IV neurons are similar to those described for identified nigrostriatal dopamine neurons intracellularly labeled with HRP.[49]

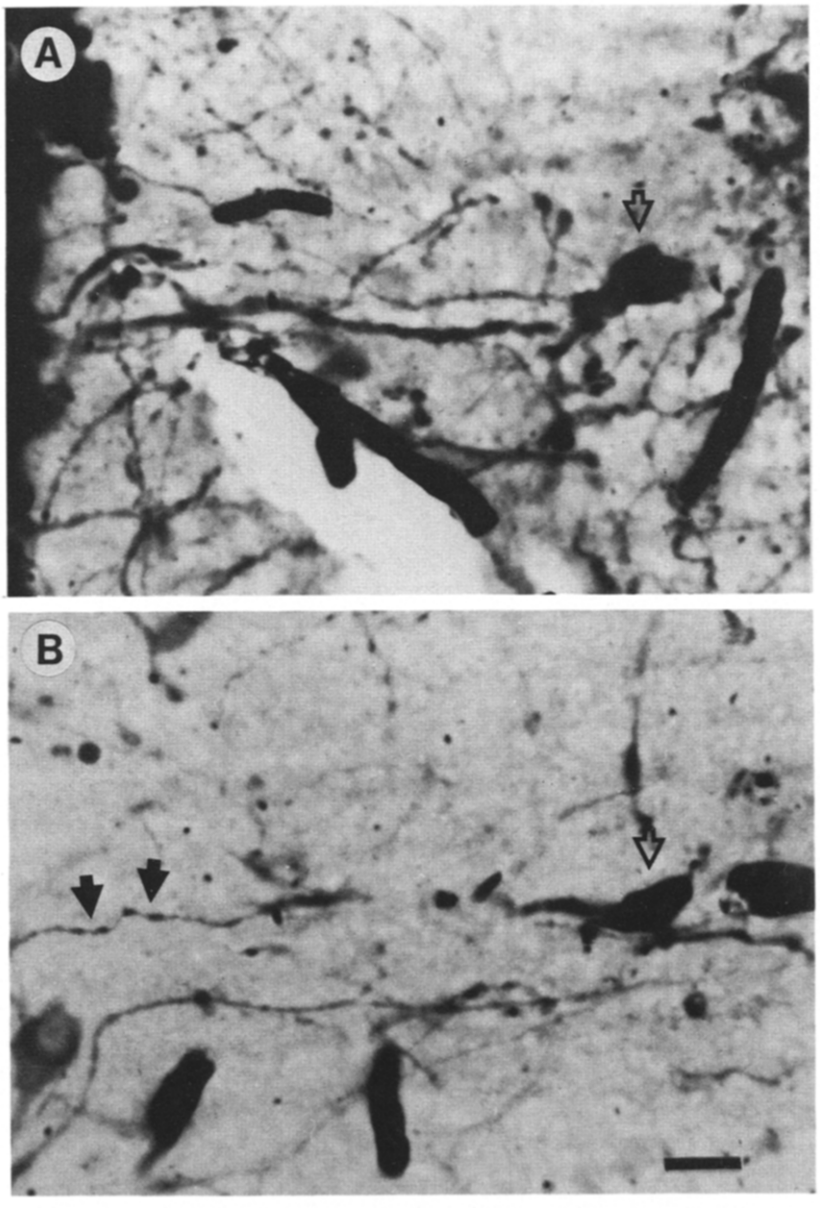


Fig. 8. Photomicrograph of biocytin labeled cell with type IV electrophysiological characteristics. (A) High magnification of TH-immunoreactive neuron indicated by dark arrow in Fig. 2. (B) Adjacent section showing same cell filled with biocytin (open arrow). Dark arrows indicate dendritic process of labeled cell which appears varicose. This section was processed for TH following the biocytin reaction and revealed the cell adjacent to the biocytin-labeled cell as TH-immunoreactive. Scale bar = 20 μm for both A and B.

Unclassified cells (n = 17)

The remaining neurons could not be readily placed in any of the above groups. In most cases (82%), cells could not be categorized because insufficient spontaneous activity was recorded. Two cells with adequate spontaneous records displayed properties common to several of the classes. One neuron which was distinctly different from any of the four cell classes fired only in bursts.

DISCUSSION

Several different neuronal cell types are present within the grafts

Four distinct classes of neurons could be identified in the grafted rat striatum based on the evaluation of several electrophysiological properties recorded for each cell. Notably, there was no single electrophysiological parameter alone which could distinguish among the different neuronal groups. A combined analysis based on the spontaneous firing pattern, firing rate and action potential shape and width was necessary to classify the cells. The first of the four classes, the type I cells, exhibited spontaneous activity and evoked responses similar to those of neurons commonly encountered within the striatum.[34,39,40,43] This electrophysiological characterization was supported by the location and morphology of the intracellularly labeled type I neurons. These neurons were found in striatal tissue outside the grafts and closely resembled medium spiny I neurons, the most common type of neostriatal cell.[29,36,51] Thus, in all likelihood, type I cells represent non-grafted, host neostriatal medium spiny neurons.

The spontaneous firing rate of type I cells appeared to decrease as the graft aged within the host striatum. It has been reported that striatal neurons initially increase in firing following a 6-OHDA lesion, and spontaneously return to a low level of firing at longer post-lesion intervals.[44] Similar spontaneous recovery of activity may have been observed in the present study. However, an alternative basis for recovery is suggested by the work of Stromberg *et al.*[47] These investigators found that striatal neurons located adjacent to a mesencephalon graft (< 1 mm) fired more slowly than those outside the graft reinnervation field in the host striatum (> 2 mm), suggesting that catecholamine (or other) outgrowths from the graft influenced the firing properties of striatal neurons. Since we routinely sampled cells within a 1 mm zone about the original placement of the suspension grafts, the observed changes in type I firing with graft age may similarly reflect growth and increasing influence of grafted fibers within the host striatum.

The extracellular properties of cells in the other three cell groups, types II–IV, resembled those of cells commonly found within the adult rat mesencephalon. Type II cells were similar to the non-dopaminergic neurons within the substantia nigra pars reticulata, while type IV cells had properties resembling the dopaminergic neurons within the substantia nigra pars compacta. The electrophysiological characteristics of the type III neurons resembled those of cells within the mesencephalic reticular formation dorsal to substantia nigra.

Neurons with wide action potentials and slow firing rates, similar to our type IV neurons, have previously been identified within solid mesencephalic grafts placed in a lateral ventricle[54] or in a cortical cavity.[1] Cells with these characteristics have been found to be responsive to pharmacological agents in a manner similar to *in situ* pars compacta dopaminergic neurons;[54] local pressure application of dopamine agonists decreased the activity of grafted dopamine-like neurons while dopamine antagonists increased the activity of these cells. Cells similar to our type II pars reticulata-like neurons have also been found within mesencephalic grafts.[54] It is not surprising that several different mesencephalic neuron populations would be present within these grafts, since the fetal tissue dissection does not solely isolate the dopamine cells of the pars compacta area of the substantia nigra. In fact, the present classification of different cell types found within mesencephalic grafts is probably not a complete assessment of all cell types present within such grafts. Some of our grafts were found to contain serotonin-immunoreactive neurons, as has been reported by others.[12] In addition, since the intent of the study was not to obtain a representative sampling of neurons within the transplants, but rather to characterize the neurons encountered, the number of cells in each group may not necessarily reflect the proportion of each cell type present within the grafts.

Cells within the grafts may have a prolonged developmental time-course

Although there were marked similarities to *in situ* neurons, interesting differences were observed between the spontaneous electrophysiological properties of the grafted type II–IV neurons and their putative *in situ* counterparts. Firstly, the grafted neurons often exhibited a wider action potential than is typical for *in situ* mesencephalic neurons. The broad waveforms did not appear to be associated with damage to the neurons, since in many cases the shape and size of the action potentials remained stable for periods of up to 1 h. Secondly, the average firing rates of the grafted neurons, especially at early post-grafting time intervals, were lower than those seen for their *in situ* counterparts.[7,17,19,48] Both of these properties have been reported to be characteristic of neurons within the developing brain. In early postnatal rats, action potentials from neurons within the locus coeruleus and in the substantia nigra are wider than those seen in the adult,[32,48] and may be associated with immature membrane properties. Observations that neurons within the locus coeruleus

of the neonatal rat increase in firing in conjunction with the development of afferent connections,[32] and the firing rate of nigrostriatal neurons increases steadily from birth to the fourth postnatal week[48] suggest that slow firing may reflect a lack of connectivity. Although our samples were small, we also found a trend towards increasing firing rates for type III and IV neurons as the graft aged within the host brain, suggesting a possible increase in host innervation of the grafted tissue. Interestingly, during the same post-implantation time periods in which cell firing rates were observed to increase, the average width of the action potentials recorded for the grafted neurons remained abnormally wide. These combined observations suggest that the development of grafted mesencephalon cells is delayed within the striatum. Whether these apparent developmental changes reflect alterations in intrinsic membrane properties of the grafted neurons, increased formation of connections within the graft and/or between the host and graft, or some other possibility remains to be clarified. That some components of the grafts may be immature is supported by ultrastructural studies of mesencephalic implants. Jaeger[27] has reported that some dopamine-containing neurons within nigral grafts express morphological features characteristic of immature dopamine neurons at post-transplant periods of up to seven months.

Cells within the grafts show a striking similarity to their in situ *counterparts*

Even though some differences were observed between the spontaneous electrophysiological activity from type II–IV neurons and their *in situ* counterparts, the similarities of the grafted neurons to adult mesencephalic neurons were very striking. For example, type IV dopamine-like neurons were found to fire in both irregular and burst firing modes, patterns which are observed for dopamine neurons within the substantia nigra pars compacta.[15,18,20,24] It was interesting to note that the few incidences of bursts observed for the type IV neurons always comprised two action potentials. Grace and Bunney[20] have reported that doublet bursts may be a precursor for longer burst episodes (3–10 spikes/burst) exhibited by dopaminergic neurons within the substantia nigra. As there were few bursting dopamine-like neurons recorded in the present study, the sole occurrence of double spike bursting may simply reflect the restricted sample size of the type IV neuron population recorded. Notably, however, Tepper *et al.*[48] have found that during the first postnatal week of development, bursts occur very infrequently and only consist of two spikes. Between the second and fourth weeks following birth, the occurrence of bursting increases and the bursts often display longer spike episodes. Thus, the bursting pattern observed in the present study may reflect a relatively immature status of some grafted neurons.

Functional connections between host and graft neurons may be "normalizing" activity within the graft

While the mechanisms which regulate the activity of dopamine neurons *in situ* are unclear, at least some of the firing modes may be dependent on afferent inputs. Gariano and Groves[16] report that dopamine neurons firing in an irregular manner within the ventral tegmental area can be induced to fire in bursts by stimulating frontal cortical areas. We also found an irregular firing type IV dopamine neuron within the graft which was triggered into burst firing with stimulation of prefrontal cortical white matter. Such bursting has not been observed in the slice preparation, where the substantia nigra is isolated from its normal afferents.[30,45] It is thus possible that host innervation of the graft may play an important role in establishing "normal" patterns of activity for the implanted mesencephalic neurons. Correlating the pattern of spontaneous activity observed for grafted neurons with responses of the grafted neurons to stimuli would help clarify this issue. The finding that over 50% of the grafted cells tested in the present study were responsive to stimulation of cortical or striatal tissue supports the possibility that host inputs may be regulating graft activity.

We further noted that not only were a large proportion of grafted cells responsive to stimulation of cortical and striatal inputs, but that they responded in a manner similar to *in situ* mesencephalic neurons. Specifically, cortical and striatal stimulation predominantly elicited a decrease of spontaneous firing of the grafted cells, as is typically observed following such stimuli for neurons within the substantia nigra and the mesencephalic reticular formation.[7,8,17,33,48] While the GABAergic output neurons of the striatum contribute an inhibitory input *in situ*,[8,21] the pathways mediating host-elicited responses in the graft are unclear. Since glutamate, the excitatory neurotransmitter utilized by cortical afferents, excites neurons within the substantia nigra and reticular formation,[8] it seems likely that a decrease in graft activity following cortical stimulation is mediated through a multisynaptic pathway. Possible routes for a cortically evoked decrease in activity include a pathway mediated through collaterals of the inhibitory spiny neurons within the striatum,[23] or one mediated through inhibitory neurons within the graft itself. Alternatively, activation of cortical axons in white matter or within the striatum may result in a decrease in the spontaneous activity of grafted neurons through a reduction in tonic cortical input (disfacilitation). Such a disfacilitation has been shown to occur within the striatum following cortical stimulation.[50] Additional studies are necessary before these or other pathways may be implicated in the observed host-elicited responses. Regardless of the manner in which this inhibitory neurocircuitry is established, these findings indicate that connections are formed between host and graft neurons which are functionally similar to those found *in situ*.

The degree to which normalization of activity occurs within the host–graft system over long post-implantation periods, and the level of development necessary to achieve improvements in specific behavioral tasks needs further examination. In the present study, all grafted animals exhibited complete reversal of drug-induced rotational asymmetry, yet electrophysiological properties of many grafted cells appeared less developed than adult mesencephalon neurons *in situ*. Thus, recovery of rotational behavior may not require mature functioning or connectivity of grafted neurons. In contrast, Dunnett *et al.*[13] have reported that tasks such as paw reaching are significantly impaired up to four months following grafting. Since we observed further developmental trends in the electrophysiological properties of grafted neurons between five and nine months post-implantation, it is possible that recovery of certain behaviors may become evident at relatively long post-implantation times with maturation of the graft and its interconnections.

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