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Morphological Assessment of Grafted Rat and Mouse Cortical Neurons: A Light and Electron Microscopic Study

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ABSTRACT

The morphology of cortical neurons grafted into (or near) the rat striatum was studied by means of intracellular Lucifer yellow injections in fixed slices. Rat donor syngeneic cortical tissue (from postnatal day 1 old rats; AO strain) as well as mouse donor xenogeneic cortical tissue (prenatal day 19; C3H/HE strain) were grafted as solid pieces into 8–12 week-old rats (AO strain). Recipients of mouse xenografts were immunosuppressed with a monoclonal antibody against the interleukin-2 receptor. After perfusion and sectioning of the graft-containing areas, individual slices were incubated in the DNA stain 4.6-diamidino-2-phenylindole (DAPI) to visualize the cell nuclei. Grafts could be easily identified by a surrounding rim of astrocytes which outline the border between grafted and host tissue. Grafted cortical neurons were intracellularly filled with Lucifer yellow, DAB-photoconverted, and further processed for light and electron microscopy.

In general, no cortical lamination could be observed in the grafted rat and mouse cortical tissue, but neurons were loosely packed throughout the graft. Two major cell types could be identified in all grafts investigated so far. The majority resembled those described as spiny neurons (85%), which could be further classified into pyramid-like, spiny stellate-like or fusiform spiny neurons, with somata ranging between 15 and 25 μm in diameter. The remaining 15% resembled non-spiny neurons with either a multipolar basket-like or fusiform morphology. Dendrites of spiny and non-spiny neurons, which could extend to distances up to 400 μm, were never seen to cross the astrocytic border, but some main axon and axonal collaterals of spiny neurons were found to leave the graft. On the basis of light microscopic observations no difference was found between mouse and rat grafted cortical neurons. The results of this study show that grafted cortical neurons retain some of the characteristic features of neurons in the intact adult cerebral cortex, although there appears to be a greater preponderance of spiny neurons in grafted tissue. This may reflect an immaturity of the grafted tissue or a response to the striatal environment.

Key words: cortical transplants, intracellular injections, spiny neurons, nonspiny neurons, striatum

The central nervous system (CNS) has traditionally been regarded as a site of immunological privilege (Medawar, '48; Barker and Billingham, '77). However, more recent work has indicated that this concept is in need of revision (Nicholas and Arnason, '89; Brent, '90). Reports emanating from several laboratories have demonstrated that neural allografts (between strains within a species) and xenografts (between species) may be rejected (Mason et al., '86; Nicholas and Arnason, '89; Finsen et al., '90; Lawrence et al., '90). However, prolonged survival of both neocortical allografts and xenografts is achievable with the use of monoclonal antibodies to suppress the host immune response (Honey et al., '90; Wood et al., '92). Thus, it is now possible to address the question of the structural organization and capacity for integration of cortical xenografts in comparison with grafts of syngeneic cortex placed in or near the rat striatum.

Thus far only a few Golgi studies have described the morphology of grafted cortical neurons in a striatal or cortical environment (LeGros Clarke, '40; Das, '75, '78; Das et al., '80; Hallas et al., '80; Jaeger and Lund, '80a,b, '81). The aim of the present study was to investigate the morphology of grafted cortical neurons from rat and mouse donor tissue into the rat caudate-putamen complex by means of intracellular injections into fixed brain slices at

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both the light and electron microscopic level. The aim was to investigate whether the major cell classes characteristic of the intact cortex would be recognized in homotopic and heterotopic grafts or whether their morphology, development, proportions, and laminar distribution are influenced by the process of transplantation and the host environment. Our observations were compared with published literature of transplanted cortical tissue and with findings of the intact cerebral cortex.

MATERIALS AND METHODS

Eleven rats (AO strain, bodyweight 150–250 g) were used for the experiments. Each rat received only a single implant into the right hemisphere near or into the striatum. Rat donor cortical tissue from postnatal day 1 old rats (AO strain, body weight 150–250 g, N = 7) and mouse donor cortical tissue from prenatal E19 day old mouse fetuses (C3H/HE strain; N = 4) were grafted as solid pieces (a volume of 4–5 μl of donor tissue was inserted via a stainless steel cannula) in 8–12 week-old rats (AO strain) at coordinates AP: 0.3 mm; L: 2.5 mm to the bregma; V: 4.5 mm from the brain surface, according to the atlas of Paxinos et al. ('91). Animals with xenografts were immunosuppressed with a monoclonal antibody against the interleukin-2 receptor (Tellides et al., '89; Wood et al., '92) at a dose of 1,500 mg/kg bodyweight for 10 days after the transplantation procedure. After a survival time of 40 days animals with rat (N = 5) and mouse (N = 4) cortical transplants were deeply anasthetized with sodium pentobarbital (60 mg/ml) and perfused through the heart or ascending aorta with 100 mM phosphate-buffered physiological saline containing Heparin (Liquemin, 25,000 IU). This was followed by a mixture of 2.5% paraformaldehyde and 0.5–1% glutaraldehyde in 100 mM phosphate buffer (PB; pH 7.4). For comparison with the younger animals two rats containing rat syngeneic cortical transplants were perfused 7 months postoperatively. For post-fixation the brain was usually left in situ for the next 2 hours. Blocks containing the grafted cortical tissue (at the striatal level) were cut in a coronal plane with a vibratome (Campden Instruments, UK). Thickness of sections ranged from 100 to 200 μm, since injections in thinner slices revealed too little of the dendritic arbor. Slices not immediately required for intracellular injections were stored in 100 mM PB (pH 7.4) in the refrigerator. Under these conditions excellent intracellular fillings were obtained in material left for periods up to 3 weeks.

Grafted neurons were investigated with an intracellular injection approach in fixed slices according to the method described by Buhl and Lübke ('89). Prior to intracellular Lucifer yellow injections, slices were incubated for 5–10 minutes in a 10^{-7} M aqueous solution of the DNA stain 4.6-diamidino-2-phenyl-indole (DAPI, Sigma, UK). This treatment resulted in brightly fluorescent nuclei which provided a visible target for the Lucifer yellow-filled pipette and also showed the outline of the graft by a rim of astrocytes (Fig. 1A–F) surrounded by the host cortical or striatal tissue. After a single brain slice was mounted in the slice chamber, grafted neurons were iontophoretically filled with 5% Lucifer yellow (dissolved in double distilled water) through borosilicate glass micropipettes (1.2 mm o.d. × 0.69 mm i.d.; Campden Instruments, UK) ranging in tip diameter between 0.5 and 1 μm (resistance 90–200 MΩ). Neurons were injected by means of a steady DC current of 1–2 nA. Dye filling of a single cell was judged complete when fine distal dendritic processes appeared brightly fluorescent (usually after 10 minutes of filling). To transform Lucifer yellow into a stable, light microscopically visible reaction product, the Lucifer yellow-filled neurons were photoconverted according to the following protocol (for review, see Lübke, '93). After pre-incubation in diaminobenzidine (DAB Grade II, Sigma; 1.5–2 mg/ml 100 mM [PB] for 30 minutes in the dark), the DAB solution was replaced with a fresh, ice-cold DAB solution. In order to suppress the background staining, potassium cyanide (1 mg/ml) was added to the DAB solution. Cells were then photoconverted with the appropriate Lucifer yellow excitation wavelength until all fluorescence had faded, thus resulting in the formation of homogeneously dark brown, Golgi-like reaction product contained within the perikarya and dendrites of the filled and photoconverted neurons (Figs. 2, 3). Excess DAB was washed out with several rinses in 100 mM PB (pH 7.4). Sections for light microscopy were mounted onto slides and air-dried. To enhance the staining contrast, slices were briefly post-fixed with 1% osmium tetroxide (dissolved in 100 mM PB, pH 7.4 for 2–3 minutes), then dehydrated through an ascending series of ethanol, cleared in Histoclear®, and finally embedded in Eukitt®. For subsequent electron microscopy, neurons were processed according to the protocol of Somogyi et al. ('79). Briefly, the sections were immersed in 1% phosphate-buffered osmium tetroxide (30 minutes) and subsequently dehydrated through an ascending series of ethanol with the inclusion of 1% uranylacetate at the 70% ethanol stage. The sections were then embedded in Epoxy resin (Durcupan ACM, Fluka). Because of the highly osmiophilic nature of the DAB reaction product, DAB-photoconverted neurons appeared intensely black with only moderate background (Figs. 2, 3, 9, 10). Neurons were photographed and drawn with the aid of a camera lucida. Neurons embedded for electron microscopy were photographed, and semithin and ultrathin sections were taken at the level of the DAB-photoconverted neurons, which could be easily identified by their opaque appearance in 1 μm-thick, Toluidine blue-counterstained, semithin sections. When cut profiles of stained neurons were identified, a series of adjacent ultrathin sections was taken. Formvar-coated grids containing the sections were stained with uranyl acetate (10–20 minutes) and lead citrate (8 minutes). Neurons were examined and micrographed in a JEOL 100 electron microscope.

The present study is based on 250 intracellularly filled neurons out of which 80 were drawn with a camera lucida. Neurons in rat (N = 165) and mouse (N = 85) transplants were injected throughout the whole extent of the graft (dorsal to ventral, rostral to caudal; for further details see also Table 1). Only neurons with a "complete" filling (i.e., the dye could be followed to the very tip of distal parts of the dendrites and there were no obvious cut-off profiles) were included in the sample of representative cells.

RESULTS

Light microscopic study

General observations. In all transplants (rat syngeneic grafts, N = 7; mouse xenografts, N = 4 located in the rat cortex or striatum) investigated so far the grafts were either directly located in the striatum (Fig. 1C–E) or at a cortical site near the striatum (Fig. 1A,B,F). Grafts were variable in size and could easily be identified by a surrounding rim of astrocytes from the cortical (Fig. 1A,B,F) and the

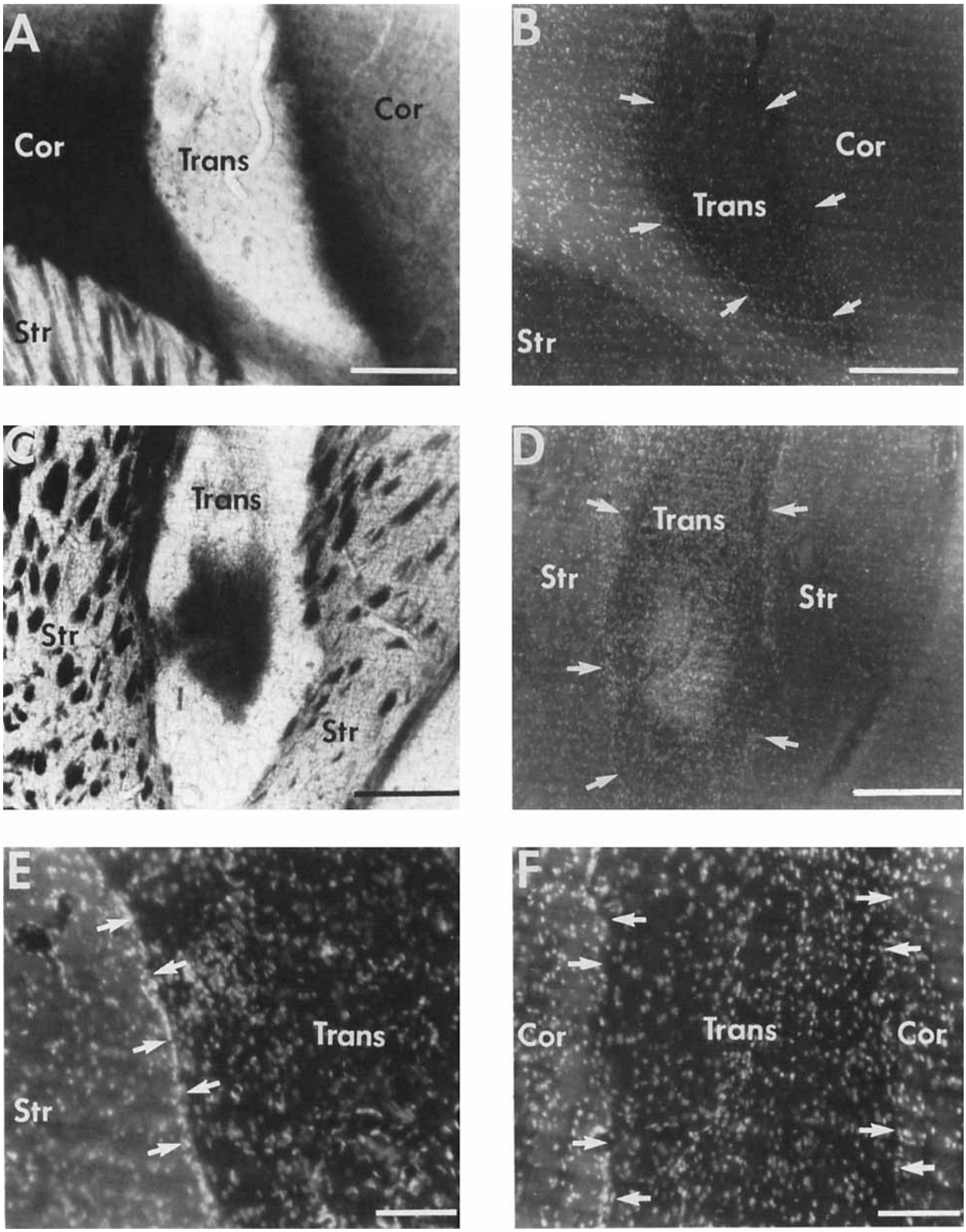

Fig. 1. Representative examples of grafted cortical tissue. **A:** Brightfield images of a mouse xenograft situated in the rat cortex. **B:** Same xenografts as in A after incubation of the slice in the DNA stain DAPI. **C:** Brightfield image of a syngeneic rat cortical graft situated in the rat striatum. **D:** Same graft as in C after incubation in DAPI. **E:** Higher magnification of a mouse xenograft transplanted in the rat striatum. The white arrows outline the astrocytic border between the xenograft and the host rat striatum. **F:** Higher magnification of a syngeneic rat graft transplanted into rat cortex. The white arrows indicate the astrocytic border of the graft and the cortex. Cor, cortex; Str, striatum; Trans, transplant. Scale bars = 500 μm in A–D, 150 μm in E, F.

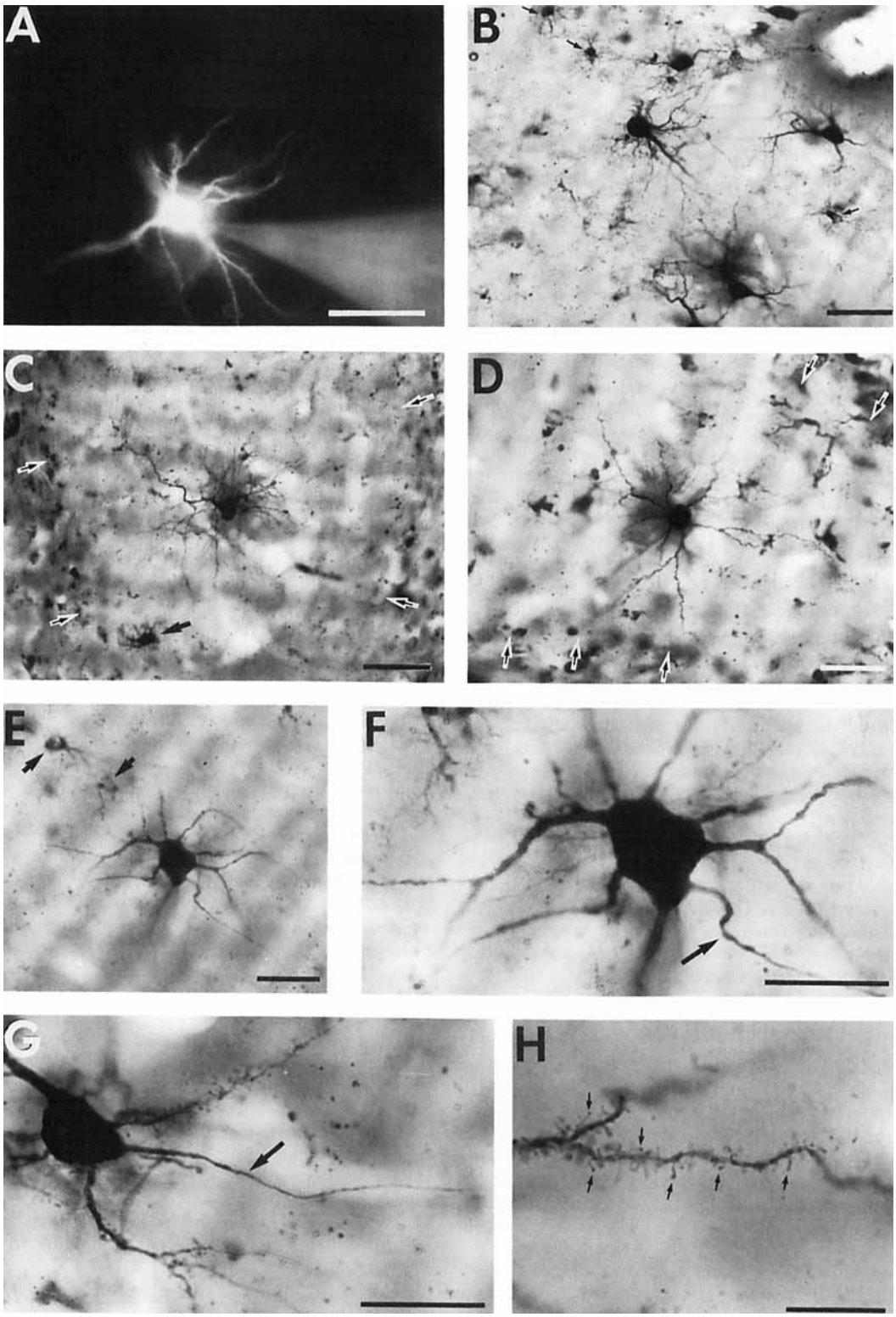

Fig. 2. Representative examples of cortical neurons from a mouse xenograft transplanted in either the rat cortex or striatum. **A:** Fluorescent image of a pyramid-like neuron while filling it with Lucifer yellow through its soma. **B:** Group of four intracellularly injected and photoconverted grafted cortical neurons. The arrows indicate three photoconverted astrocytes. **C:** Typical examples of a pyramid-like neuron. Note the random orientation of the apical dendrite. The white-bordered arrows indicate the border of the xenograft, whereas the black arrow points to a labeled astrocyte. **D:** Representative example of an spiny stellate-like neuron. The white-bordered arrows delineate the border of the xenograft. **E:** Typical example of a non-spiny neuron with a basket-like morphology. The two arrows point to photoconverted astrocytes. **F:** Same cell as in E at higher magnification. Note the asymmetric shape of the soma and the multipolar distribution of the smooth dendrites. The axon which is indicated by an arrow emerges directly from the soma. **G:** Soma region with basal dendrites of a spiny neuron with the axon (arrow) emerging directly from the soma. **H:** Higher magnification of a primary basal dendrite. Note the dense covering with dendritic spines, some of which are indicated with small arrows, along the entire length of the dendrite. Scale bars = 50 μm in A–E, 25 μm in F–H.

TABLE 1. Distribution of Intracellularly Injected Grafted Cortical Neurons

	Total	Rat syngeneic transplants	Mouse xenogeneic transplants
Number of transplants	11	7	4
Number of injected neurons	250	165	85
Pyramid-like neurons	63	25	38
Spiny stellate-like neurons	95	72	23
Fusiform spiny neurons	51	40	11
Non-spiny neurons	41	28	13

striatal (Fig. 1C–E) host brain tissue after incubation in the DNA stain DAPI. On the basis of light microscopical observations no morphological differences were found between the rat and mouse grafted cortical neurons (compare Figs. 2,4–6 with Figs. 3,7,8). In addition, no difference was observed between the syngeneic rat grafts and the mouse xenografts transplanted in the cortex or striatum. In general, no cortical lamination could be observed in both the syngeneic grafts and mouse xenografts, even in animals which after transplantation had survived for extended periods (6 months). Neurons were loosely packed throughout the entire graft (Fig. 1A–F), but no further distinction into different laminae as described for Nissl-stained sections of the intact cerebral cortex could be made in the DAPI-stained cortical grafts.

The present description of neuronal cell types is mainly based upon both somatic morphology and dendritic arborization. Three morphologically distinct types of neurons could be distinguished in both the rat and mouse cortical grafts according to their dendritic configuration and the presence or absence of dendritic spines. The majority of filled rat (N = 137) and mouse (N = 72) grafted cortical neurons resemble those which could be described as pyramid-like, spiny stellate-like, and fusiform spiny neurons (see Table 1). The remainder (N = 41) of the population of neurons found in the rat (N = 28) and mouse (N = 13) grafts resemble non-spiny neurons with either a multipolar or fusiform morphology. In addition, the proportion of pyramid-like neurons seems to be greater in the mouse xenografts than in the syngeneic rat grafts, where spiny neurons tend to be more spiny stellate-like or fusiform (compare Figs. 5 and 7; see also Table 1).

Neuronal types

Pyramid-like neurons. Pyramid-like neurons were defined according to the following criteria: oval- or pyramidal-shaped somata. All neurons within this class possess a prominent (apical) dendrite which is larger and in many cases longer than the other dendrites with several oblique side branches. The basal dendrites are radially distributed around the soma. All of the dendrites bear numerous spines throughout the entire dendritic length. Despite some variation of the morphology of the main (apical) dendrite the pyramid-like neurons described here have similar characteristics to group I cells in neocortical transplants as described by Jaeger and Lund ('81) and the intact cerebral cortex (Ramón y Cajal, '11; Lorente de Nó, '49).

In general, pyramid-like neurons (N = 63) were found throughout the entire graft in both the syngeneic rat grafts and the mouse xenografts (dorso-ventral, rostro-caudal extent). However, in the mouse xenografts we have observed a greater proportion of pyramid-like neurons than in the syngeneic rat grafts (see Table 1). In addition, in both syngeneic grafts and even mouse xenografts they were less numerous in proportion to the other spiny cell types. Representative examples of pyramid-like neurons from syngeneic rat grafts and mouse xenografts are given in Figures 2A,C, 3D, and 4A–C. Neurons have round-to-ovoid somata, ranging in size between 15 and 25 μm. In addition, on some somata fine hair-like processes and/or spine-like protrusions could be identified (not shown). All pyramid-like neurons have a prominent (apical) thick dendrite, which could extend up to 500 μm, giving rise to several oblique side branches of various length (Fig. 2A,C). Some of the prominent (apical) dendrites end in a terminal tuft (Fig. 3D) as described for cortical layer II/III and layer V pyramidal neurons (Valverde, '86; Larkman and Mason, '90; Marin-Padilla, '92) whereas others remained to be untufted as described for layer V (Larkman and Mason, '90; Kasper et al., '93) and for layer VI pyramidal cells (Lübke and Albus, '89). However, the orientation of the apical dendrites in the all grafts investigated so far was found to be random, with no preferred orientation as described for cortical pyramidal neurons (Ramón y Cajal, '11; Lorente de Nó, '49; Feldman, '84; Valverde, '86; Peters and Sethares, '91; Marin-Padilla, '92). In general, three to eight primary basal dendrites emerge directly from the soma extending over relatively long distances up to 300 μm. After a certain distance from the soma (30–50 μm) they give rise to secondary side branches some of which extend up to 150 μm in length, which often branch into short (20–50 μm) tertiary dendrites. Dendritic spines were found throughout the entire extent of the dendrites (Figs. 2H, 3H, 4A–C), whereas dendritic varicosities were mainly found on secondary and tertiary side branches. Dendrites were never seen to cross the astrocytic border, even on neurons which were located near to the border of the graft (not shown here). The axons of pyramid-like neurons, often with a thick initial segment, emerged either directly from the soma (Fig. 4A–C) or from one of the primary dendrites (not shown). They then followed the course of the dendrites giving rise to several ascending or descending collaterals (Fig. 4A–C), some of which were found to leave the graft to enter the surrounding host brain tissue, although no axon could be followed to its terminal site (Fig. 3A–C).

Spiny stellate-like neurons. Spiny stellate-like neurons were defined according to the following criteria: oval- to round-shaped somata. They are distinguished from pyramidal neurons by the absence of the prominent extended apical dendrite characteristic. All neurons have radially oriented primary dendrites which have approximately the same length and thickness and which form a nearly round-shaped dendritic field. All dendrites are covered with numerous spines which could be unevenly distributed along the dendrite. Neurons which fit into this group have similar characteristics as group II neurons in neocortical transplants described by Jaeger and Lund ('81) and for the intact visual cortex (Ramón y Cajal, '11; Lund, '84; Lund and Holbach, '91).

In general, spiny stellate-like neurons (N = 95) were found throughout the entire graft. However, they seem to be more common in syngeneic rat grafts than in mouse xenografts (see Table 1). Representative examples of these neurons are given in Figure 2D, 5A,C, and 7D. They consisted of medium-to-large neurons with somata ranging from 20 to 30 μm, with four to eight primary dendrites that emerged from the cell body in all directions extending up to 300 μm in length (Figs. 2D, 5A–C, 7D). Each of the primary dendrites arborized into two or three secondary branches within 20–50 μm distance of the cell body, and each of these

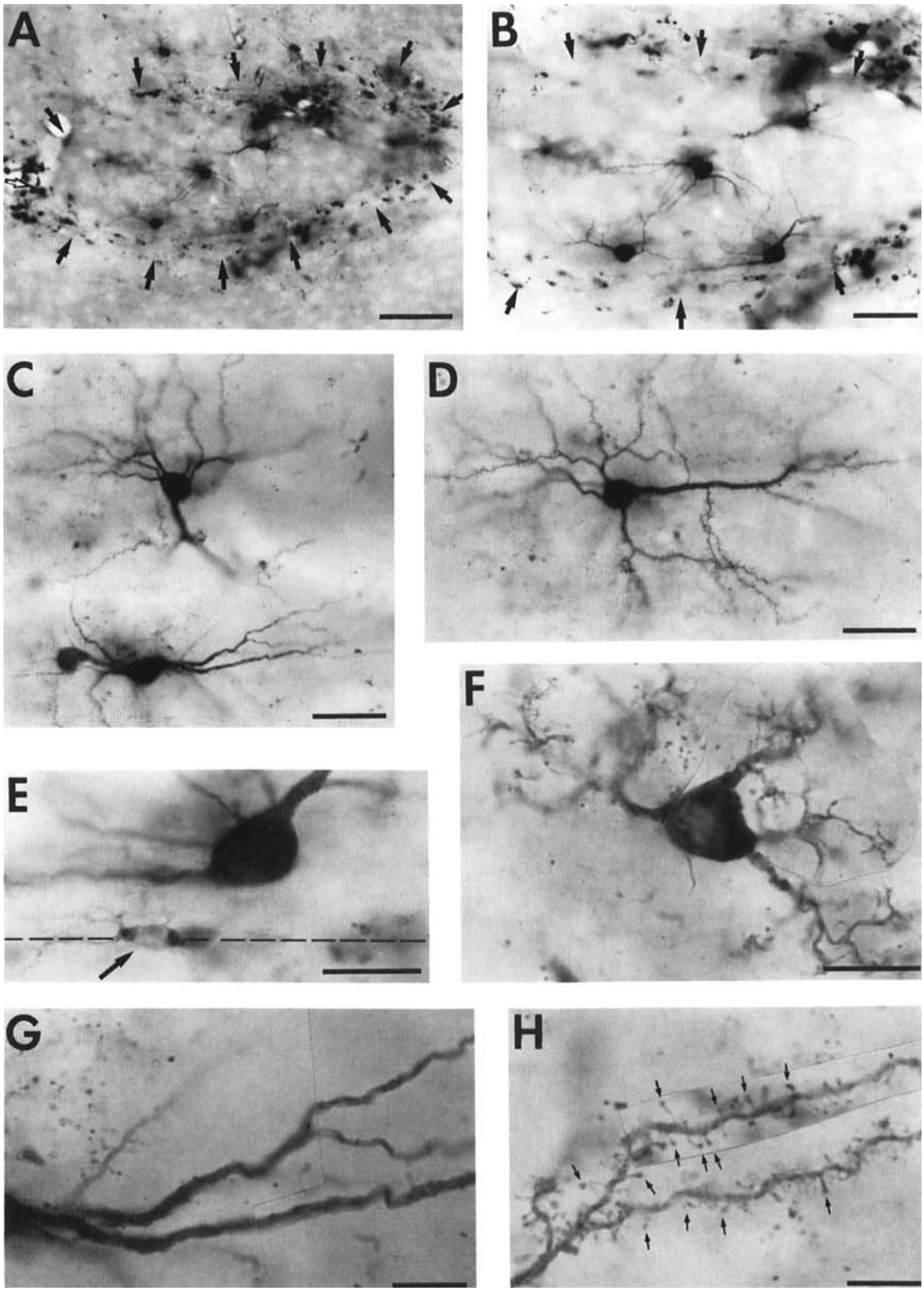

Fig. 3. Representative examples of cortical neurons from syngeneic rat grafts transplanted either in the rat cortex or striatum. **A:** Low-power photograph of a group of five intracellularly injected grafted cortical neurons after photoconversion. The arrows delineate the border of the graft. **B:** The same graft at higher magnification showing the different dendritic arrangement of grafted cortical neurons. Note the two neurons at the bottom part of the picture situated at the border of the graft (indicated by arrows). **C:** Representative example of a more multipolar spiny neuron (top) and a non-spiny neuron (bottom). **D:** Typical example of a pyramid-like neuron with a tufted apical dendrite. **E:** Higher magnification of a neuron located near the astrocytic border of the graft (dotted line). Note the dendrites run parallel to the border but not across it. The arrow indicates a photoconverted astrocyte. **F:** High-power microphotograph of a photoconverted astrocyte showing the extensive arborization. **G:** Same non-spiny neuron as in C at higher magnification. Note the smooth dendrites and the absence of dendritic appendages. **H:** High-power magnification of a primary basal dendrite of a pyramid-like neuron. Note the high density of spines, some of which are indicated by small arrows, which cover the entire length of the dendrites. Scale bars = 250 μm in A, 100 μm in B, 50 μm in C and D, 25 μm in E–H.

mouse donor tissue into rat cortex or striatum

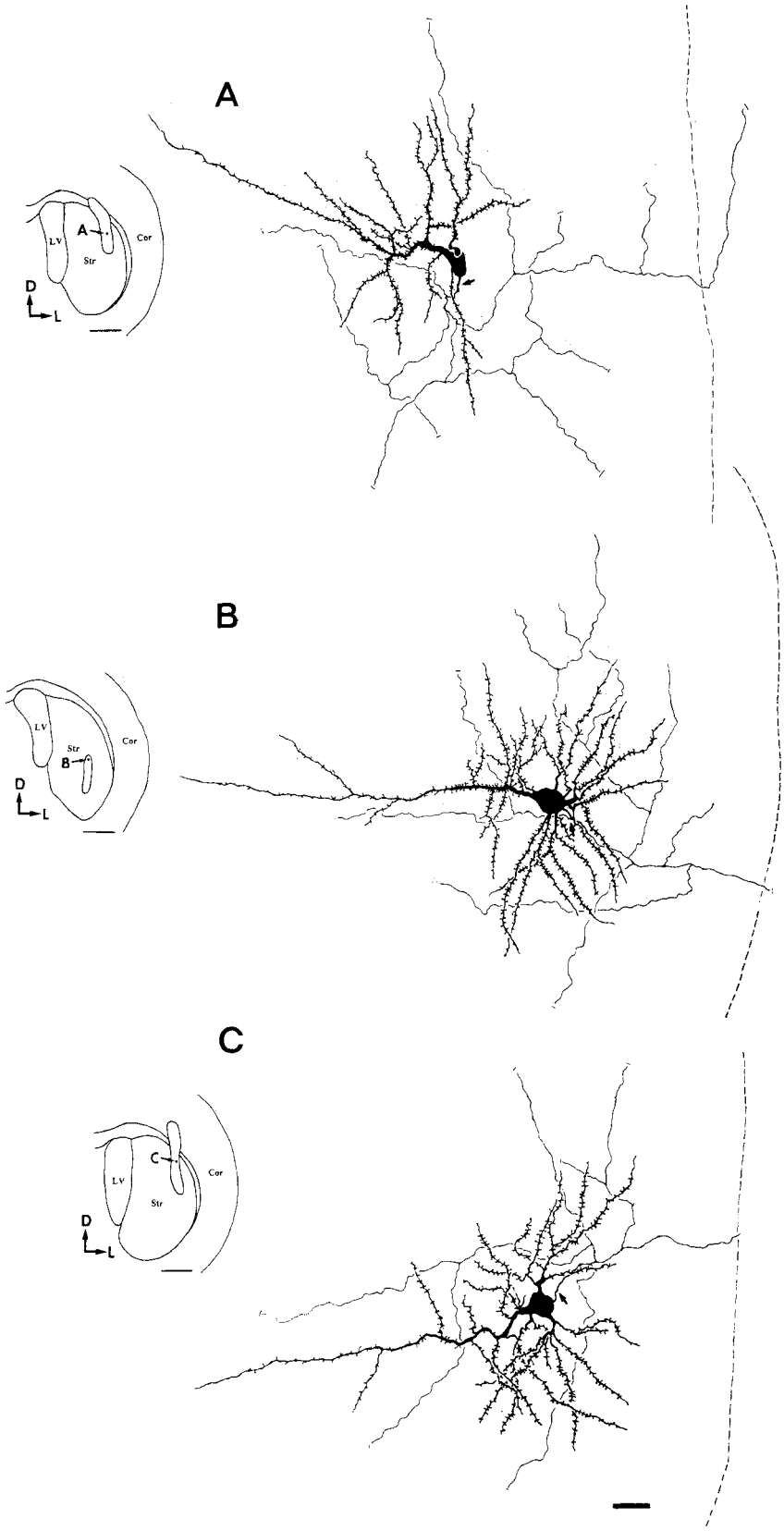

Figure 4

give rise to several short (30–50 μm) and thinner tertiary branches. The dendrites were oriented radially around the cell body so that the entire dendritic field was more or less circular. Dendritic appendages such as dendritic spines cover the entire length of the dendrites, whereas dendritic varicosities were mainly found on secondary and tertiary dendrites (Figs. 5A–C, 7D). Again dendrites were never seen to cross the astrocytic border, but some main axons and axonal collaterals could be seen to leave the graft (Figs. 5A–C, 7D).

Fusiform spiny neurons. Neurons which fit into this group were defined according to the following criteria: round- to oval-shaped somata. All dendrites are approximately equal in thickness, but in contrast to stellate-like neurons dendrites tend to run parallel to one another to constitute a loose bundle that often forms an elongated cylindrical dendritic field. All dendrites are covered with numerous spines. However, we have found a great variability in dendritic field organization in this group of spiny neurons.

This neuronal cell type (N = 51) was also found throughout the entire graft, but was more often found in the syngeneic rat grafts (see Table 1). Representative examples of these neurons are shown in Figure 5B and 7A,C. These neurons were slightly smaller than the spiny stellate-like neurons with soma sizes ranging between 15 and 20 μm. Three to five primary dendrites (up to 350 μm in length) emerged directly from the soma, which after a short distance branch into two or three shorter (up to 120 μm in length) and thinner secondary and tertiary dendrites (20–70 μm). Compared to spiny stellate-like neurons, fusiform neurons had a relatively sparse and distorted dendritic field, although there was no apparent difference in the length of the dendritic field and the spine density along the dendrites (compare Figs. 5A,C, 7D with Figs. 5B, 7A,C). There were also transitional neurons resembling both the spiny stellate-like and fusiform neurons (Fig. 7B), so that it was not always possible to classify cells with certainty. Again the main axon could be seen arising from either the soma or one of the primary dendrites with ascending and descending axonal collaterals, some of which could be followed crossing the astrocytic border to enter the host tissue (Fig. 7A–C).

Non-spiny neurons. Neurons in this group were defined by the following criteria: round-to-ovoid somata variable in size. Neurons have smooth dendrites with only a few or no spine-like appendages. A variety of dendritic field shapes can be found in this group. Neurons can be categorized into stellate-like, bitufted, or bipolar and show similar characteristics as group III cells in neocortical transplants as described by Jaeger and Lund ('81) and Plaschke et al. ('92) and for the intact cerebral cortex (Feldman, '78; Peters and Regidor, '81; Meyer, '83; Kisvarday et al., '93).

The remaining intracellularly injected neurons (N = 41) in both the syngeneic rat grafts and the mouse xenografts (see Table 1) resemble non-spiny neurons with variable morphologies (Figs. 2E,F, 6, 8). Because of the non-laminated structure of the graft the distribution of non-spiny neurons was found to be random as shown for the spiny neurons. Some of them could be described as multipolar with a basket neuron-like morphology (Figs. 2E,F, 6A,C, 8A), whereas others showed a more fusiform morphology (Figs. 6B,C, 8B). These neurons have round (Figs. 6A, 8C) to ovoid (Figs. 6B,C, 8A) somata ranging in size between 15 and 25 μm. In general, three to six primary dendrites up to 350 μm in length emerged directly from the soma in all directions (Figs. 6, 8). Each of the primary dendrites then arborized into two or three secondary side branches within 20–50 μm of the soma, some of which gave rise to short (30–70 μm) and thinner tertiary side branches. In the case of the more fusiform non-spiny neurons, the distribution of dendrites seems to be more sparse and distorted than for the multipolar neurons (compare Fig. 6A,C with Fig. 8B). Only a few dendritic appendages, such as spine-like protrusions or dendritic varicosities, could be found on secondary and tertiary dendrites. The main axon either emerged directly from the cell soma (Figs. 6A,B, 8C) or one of the primary dendrites (Figs. 6A, 8B,C). It then often followed the course of the dendrites giving rise to several ascending and descending collaterals (Figs. 6, 8). However, even on non-spiny neurons located near the border of the grafts, axons and their collaterals were never seen to cross the graft borders. Thus one might suggest that grafted non-spiny neurons may represent local circuit neurons as described in the intact cerebral cortex (Rakic, '75; Somogyi, '77; Feldman, '78; Peters and Regidor, '81; Somogyi et al., '82; Meyer, '83; Lund and Yoshioka, '91).

Electron microscopy

In order to relate the light microscopic findings of somatic and dendritic morphology of intracellularly filled neurons to the ultrastructure and synaptic organization of the grafts individual spiny (three cells) and non-spiny (two cells) neurons from syngeneic rat grafts were further processed for subsequent electron microscopy. Owing to the osmiophilic nature of the DAB reaction product, intracellularly injected neurons could be easily distinguished from adjacent non-filled and DAB-photoconverted neurons by the appearance of a homogeneously distributed opaque reaction product which could be found throughout the entire neuron (Figs. 9A, 10A). In particular, the cytoplasm and dendrites and various cytoplasmic organelles such as mitochondria, Golgi-apparatus, and the endoplasmic reticulum were clearly visible (Fig. 9A). The density as well as the concentration of the label was sometimes reduced in the most distal parts of dendrites (Fig. 10E). However, there was still sufficient DAB reaction product to distinguish between labeled neuronal elements and unlabeled tissue (Figs. 9, 10). In addition, DAB photoconversion made it also much easier to follow dendritic elements to their distal portions and to identify synaptic contacts on the labeled soma and dendrites.

Pyramid-like neurons. A representative example of the ultrastructural features of a single pyramid-like neuron from a rat cortical transplant into the rat striatum is given in Figure 9. Spiny neurons could be easily identified by their large, round-to-ovoid nucleus, their dense heterochromatin, and the unfolded appearance of the nucleus (Fig. 9A) as described by Feldman ('84) and LeVay ('73) for pyramidal cells and Lund ('84) and Saint Marie and Peters ('85) for

Fig. 4. **A–C:** Camera lucida drawings of three representative examples of pyramid-like neurons in mouse xenografts located either in the rat cortex or striatum. The location of the xenograft and the position of the intracellularly injected neuron is given by the adjacent sketch drawing. Note the random orientation of the pyramid-like neuron in the xenograft. The axon is indicated by an arrow. The dotted line represents one border of the xenograft. LV, lateral ventricle; Str, striatum; Cor, cortex. Scale bar = 1 mm in the sketch drawings and 50 μm in the cell drawings.

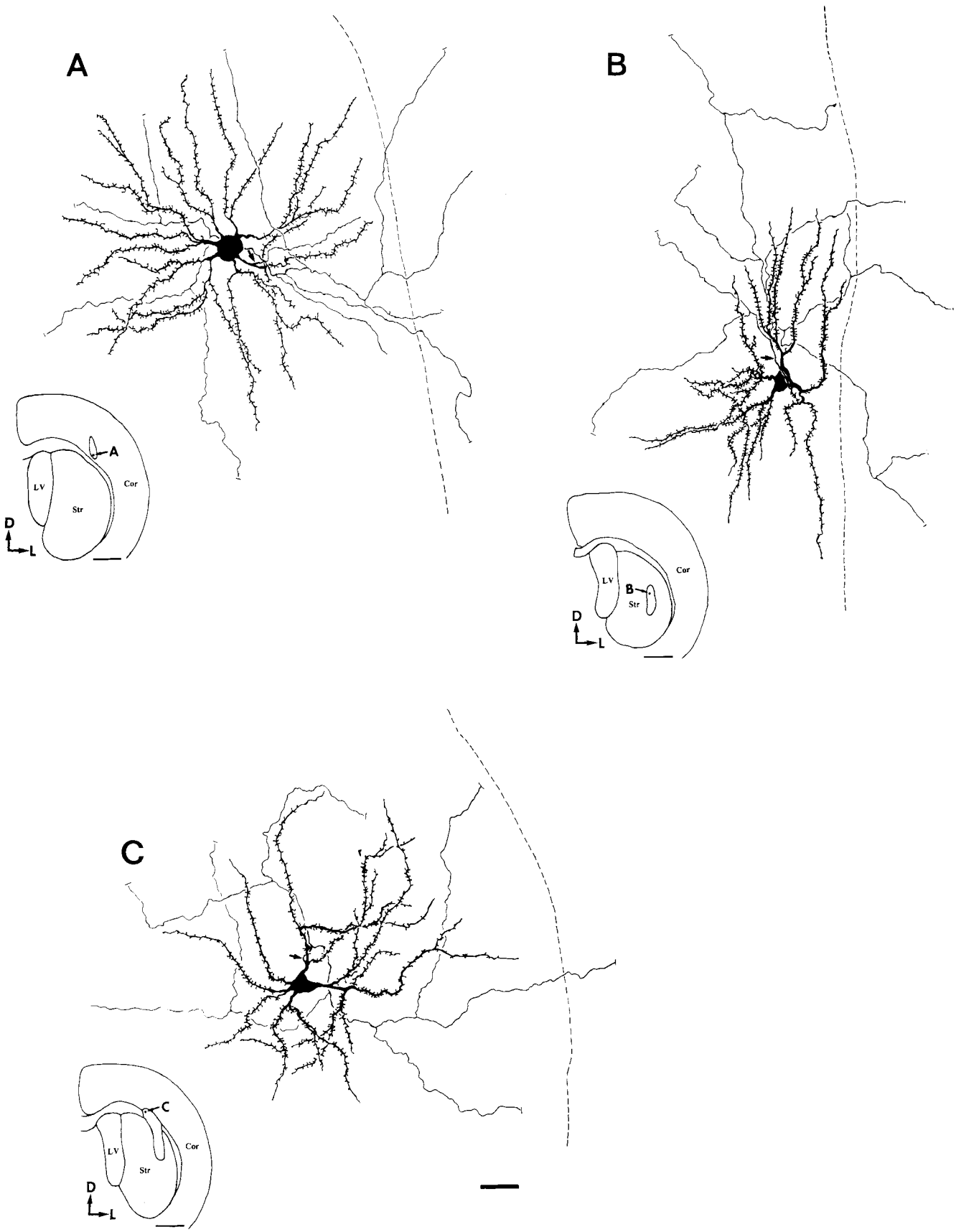

Fig. 5. **A–C:** Camera lucida drawings of three representative examples of spiny stellate-like neurons in mouse xenografts located either in the rat cortex or striatum. Axon is indicated by an arrow. For further explanations see Figure 3. Scale bars = 1 mm in the sketch drawings and 50 μm in the cell drawings.

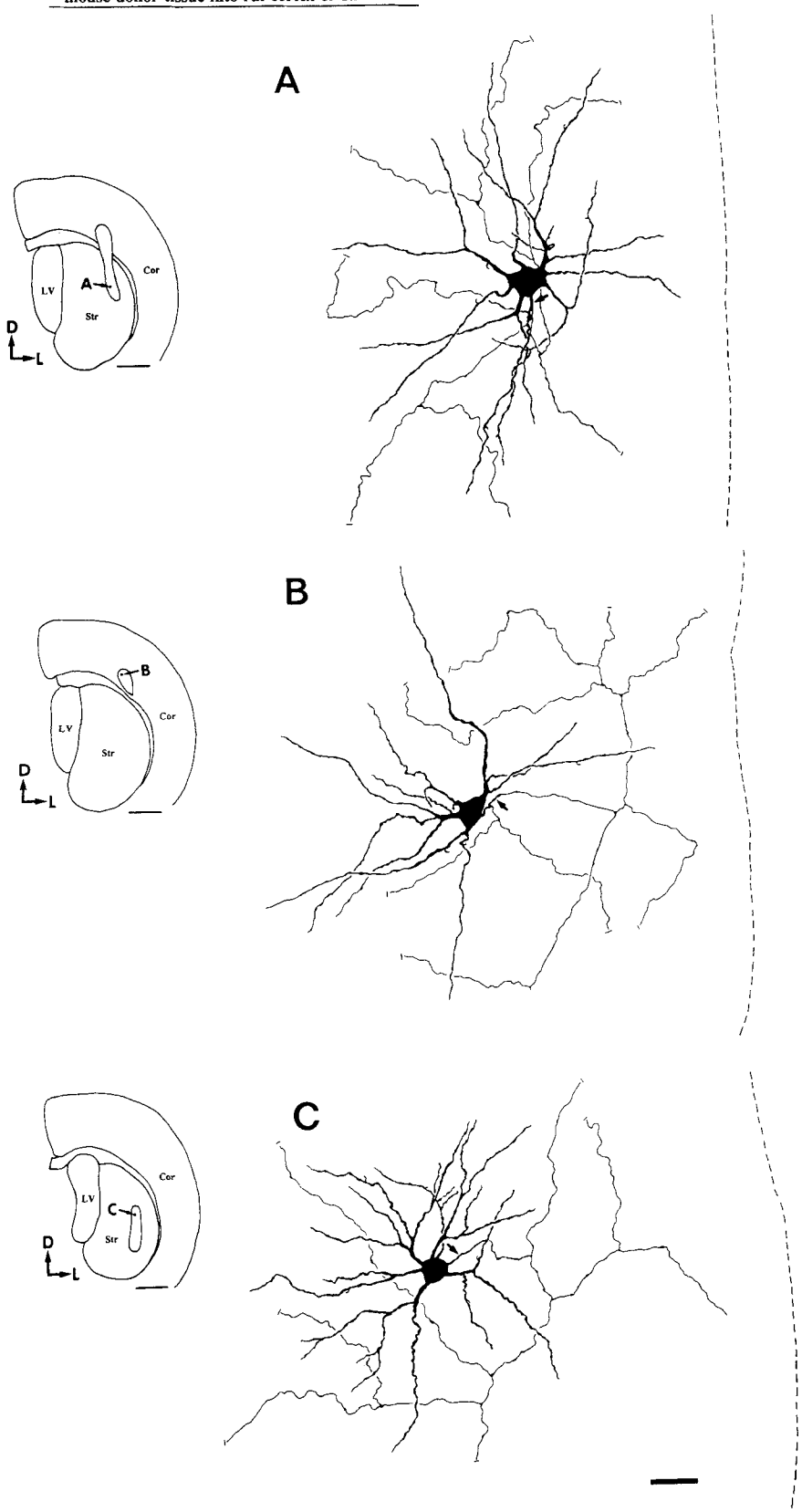

Fig. 6. **A–C:** Camera lucida drawings of three representative examples of non-spiny neurons with a basket-like morphology in mouse xenografts located either in the rat cortex or striatum. Axon is indicated by an arrow. For further explanations see Figure 3. Scale bars = 1 mm in the sketch drawings and 50 μm in the cell drawings.

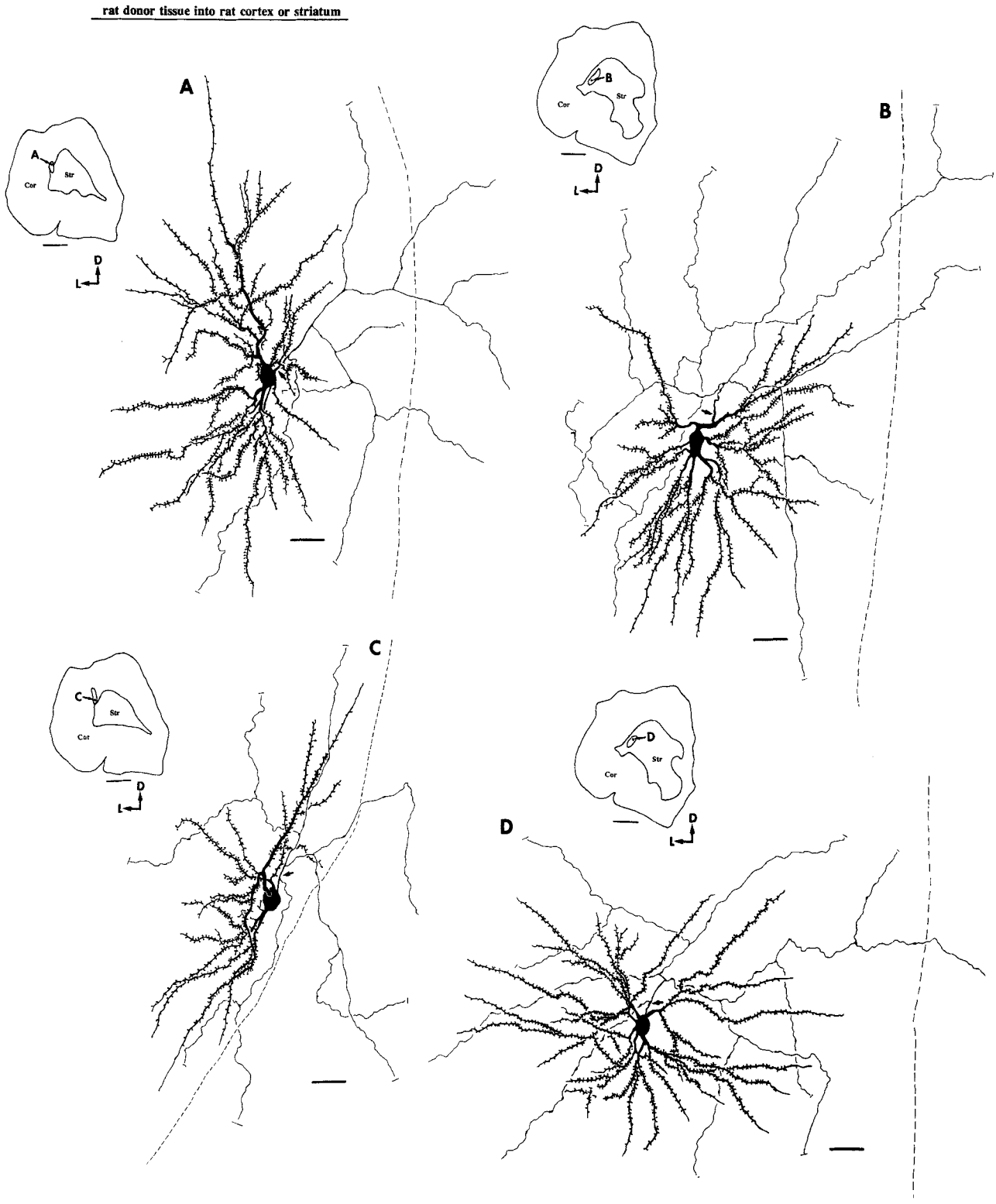

Fig. 7. **A–D:** Camera lucida drawings of four representative examples of spiny neurons of syngeneic rat grafts transplanted either in the rat cortex or striatum. Axon is indicated by an arrow. For further explanation see Figure 3. Scale bar = 1 mm in the sketch drawings and 50 μm in the cell drawings.

rat donor tissue into rat cortex or striatum

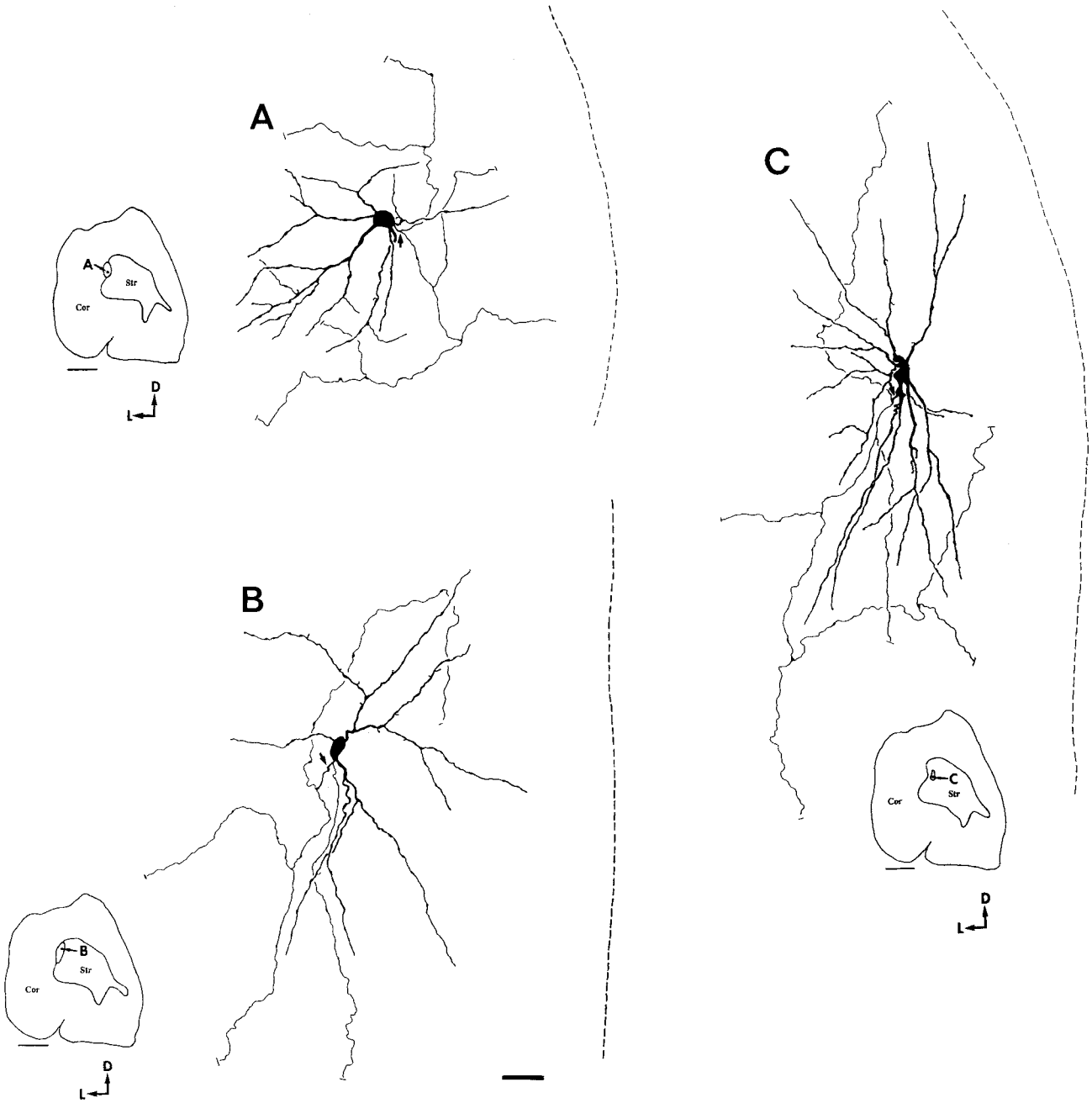

Fig. 8. Camera lucida drawings of three representative examples of non-spiny neurons with a basket-like (neuron **A**) or a more fusiform (neurons **B** and **C**) morphology in syngeneic rat grafts either located in the rat cortex or striatum. Axon is indicated by an arrow. For further explanations see Figure 3. Scale bars = 1 mm in the sketch drawings and 50 μm in the cell drawings.

spiny stellate neurons. All dendrites were covered with numerous spines (Fig. 9B,C) which were contacted by one, sometimes two, synapses with round vesicles (Gray type I). In all neurons investigated so far, we have only seen a few synapses contacting the soma region or making direct synaptic contact to the dendritic shaft (not shown).

Non-spiny neurons. In contrast to spiny neurons non-pyramidal cells can be easily identified by their indented nucleus. One of the two examples of a non-pyramidal, fusiform neuron is given in Figure 10A. No obvious spine-like elements, such as hair-like processes or spine-like protrusions, were found along the dendrites. However, on some dendrites varicose-like swellings could be identified (Fig. 10B). Synaptic contacts were found on the soma region (Fig. 10C) and on proximal (Fig. 10D) as well as on more distal parts (Fig. 10E) of the dendrites and could be of

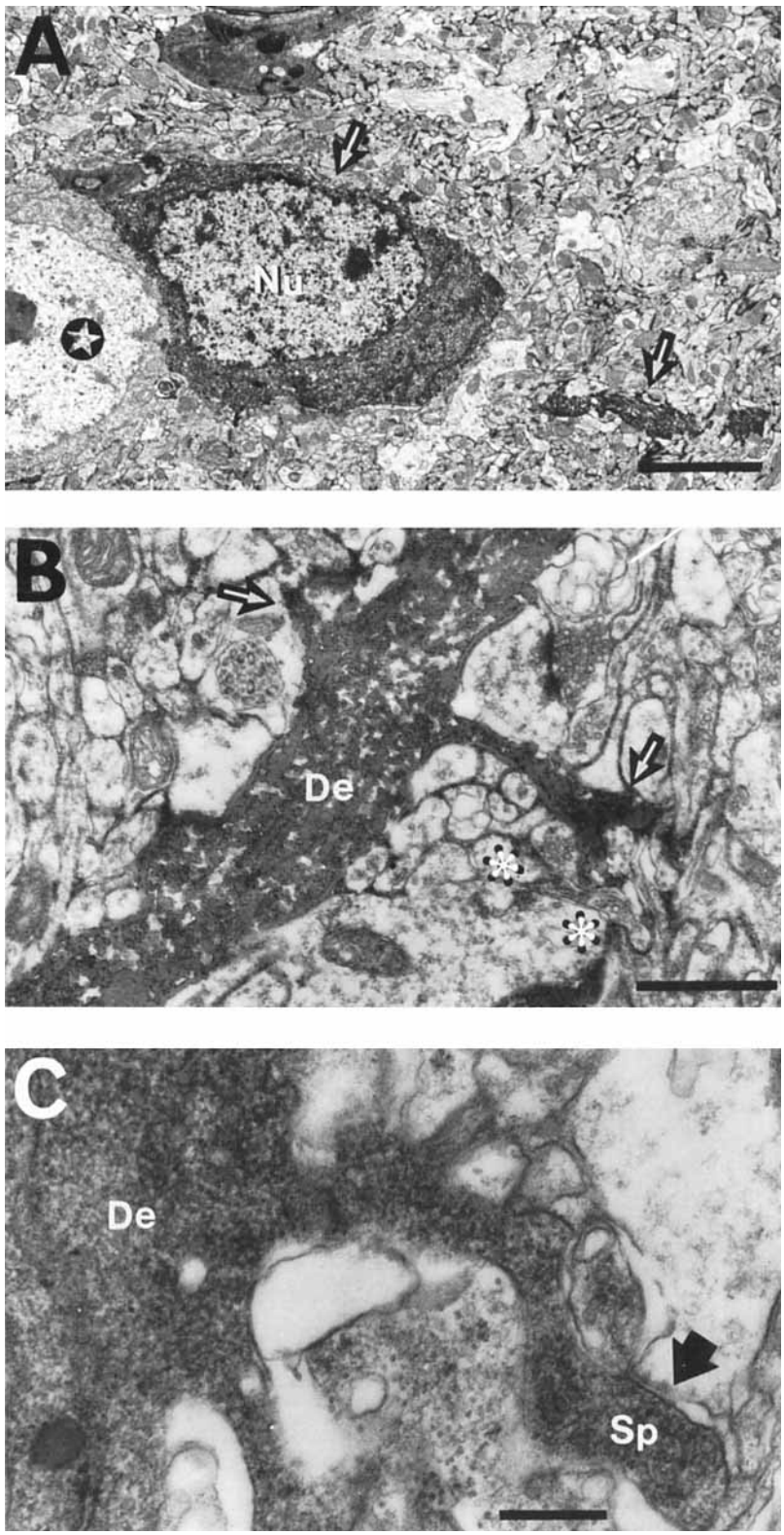

Fig. 9. **A:** Representative example of an intracellularly Lucifer yellow-filled, photoconverted spiny neuron (marked by an arrow) in a rat cortical transplant into rat striatum. Owing to the content of a fine, electron-opaque diaminobenzidine (DAB) reaction product, the cell is darkly contrasted and therefore readily identified from an adjacent non-injected neuron (marked by an asterisk). The other arrow points to part of a primary dendrite. **B:** High-power micrograph of a primary dendrite of a spiny neuron with two clearly visible spines (marked by arrows). Two adjacent synapses are marked by asterisks. **C:** High-power magnification of a single spine (marked by arrow). Nu, nucleus; De, dendrite; Sp, spine. Scale bars = 10 μm in A, 2 μm in B, 1 μm in C.

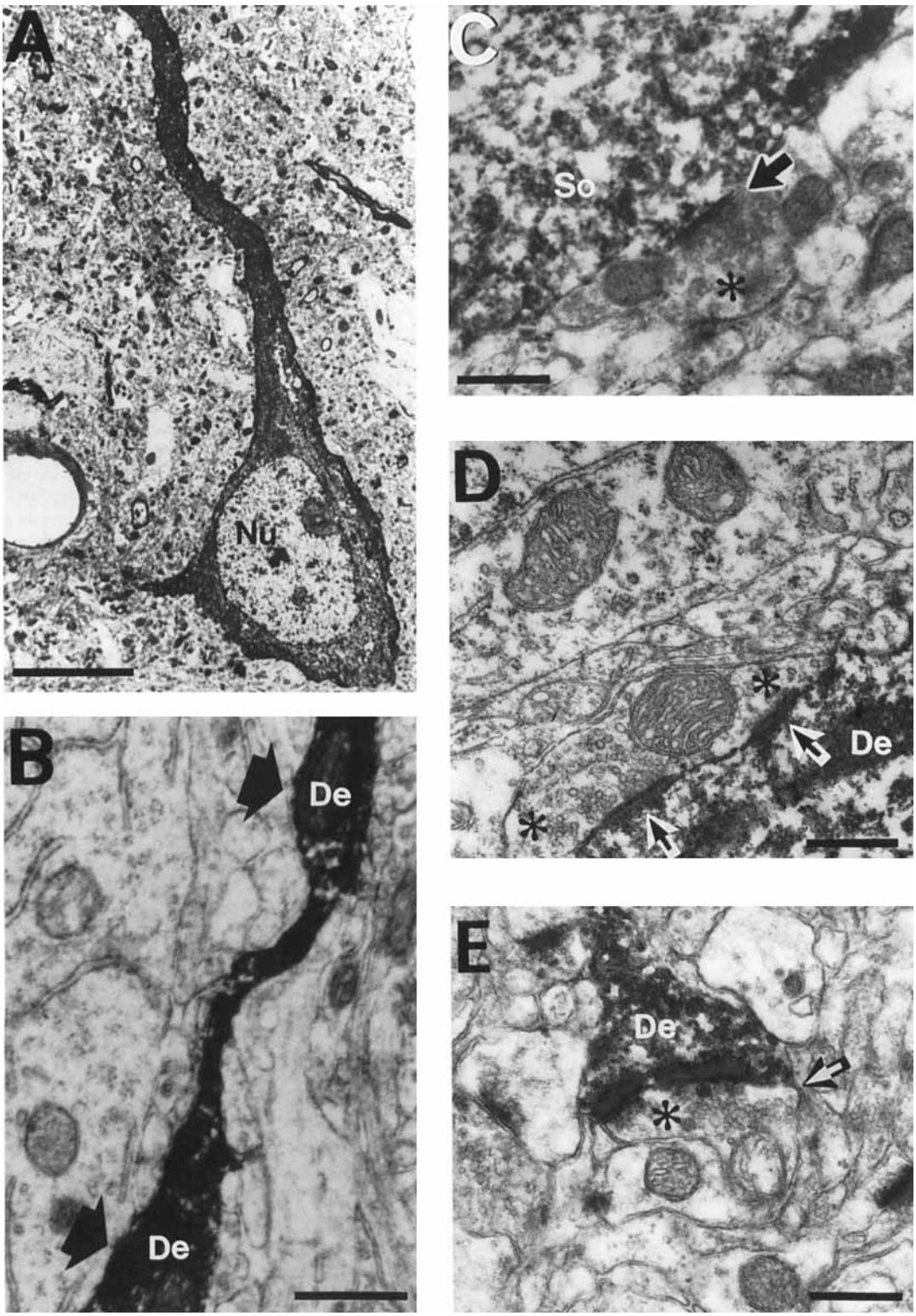

Fig. 10. **A:** Low-power micrograph of an intracellularly Lucifer yellow-filled, photoconverted non-spiny neuron in a rat cortical transplant in the rat striatum. **B:** High-power magnification of a single primary dendrite showing two varicose swellings (marked by arrows) along its course. **C:** Single synaptic contact (marked by an arrow) on the soma region of a non-spiny, non-pyramidal neuron. **D:** Two synaptic contacts (arrows) on the proximal part of a primary dendrite of a non-spiny, non-pyramidal neuron. **E:** Distal portion of a dendrite with a contacting synapse (arrow and asterisk) of a non-spiny, non-pyramidal neuron. Nu, nucleus; De, dendrite. Scale bars = 10 μm in A, 2 μm in B, 1 μm in C and D, 0.5 μm in E.

both the Gray type I and type II. However, the synaptic density along a single dendrite seems to be much lower as found on spiny neurons.

DISCUSSION

Morphological assessment of grafted cortical neurons

Our results clearly show that certain characteristic features of cortical neurons are well preserved in syngeneic rat grafts and the mouse xenografts, even with the use of immunosuppression in case of the mouse donor tissue, whereas other features in comparison with intact cerebral cortical neurons were significantly different. In principal, our results are in agreement with results published by Das ('75, '78), Das et al. ('80) Hallas et al. ('80), and Jaeger and Lund ('80a, '81) for rat cortical transplants. Three classes of neurons could be identified in the syngeneic rat grafts and the mouse xenografts, but the orientation of neurons and dendrites, in particular for pyramid-like and spiny stellate-like neurons, were severely disturbed. In addition, each cell type does not appear to segregate into well-defined laminae, and they could be observed in a random distribution throughout the entire graft. Interestingly, there are some major differences encountered between recognizable cell types in the two types of grafts (syngeneic rat grafts and mouse xenografts) and the intact adult cerebral cortex. Changes include the proportion of the different cell types in the grafts, the random position of the cell bodies, the altered orientation and branching pattern of dendrites, the density of spines along the dendrites, and the projection patterns of the axons.

One of the most striking features in all grafts investigated so far was that pyramid-like cells, which resemble the major cell class in the intact adult cerebral cortex (Ramón y Cajal, '11; Lorente de Nó, '49; Feldman, '84; Valverde, '86; Lübke and Albus, '89; Larkman and Mason, '90; Peters and Sethares, '91; Kasper et al., '93), were found not to be the predominant cell class in either the syngeneic rat graft or the mouse xenograft. In addition, we have found a difference in the proportion of pyramid-like neurons between the mouse xenografts and the syngeneic rat grafts. Surprisingly, more pyramid-like neurons were found in the mouse xenografts than in the syngeneic rat grafts. In contrast to the intact adult cerebral cortex, the majority of spiny neurons resemble those described as spiny stellate-like or spiny, fusiform neurons (Lund, '73, '84; Lund and Holbach, '91). Possible explanations for the absence of a large number of pyramid-like neurons in the transplants could be (a) the transplantation procedure; (b) the deafferentation of projecting pyramid-like neurons from their cortical (interhemispheric via the corpus callosum) and subcortical targets (pontine nuclei, superior colliculus, spinal cord), as described for layer V (Larkman and Mason, '90; Kasper et al., '93) in the rat intact adult visual cortex; and (c) other abnormalities in the dendritic and axonal organization of the transplant. The absence of normal afferents to grafted cortical neurons may, in particular, affect the development of pyramid-like neurons. In addition, in the present study we have found no change in the proportion of pyramid-like cells between the rat grafts of the two different ages. Despite the absence of defined cortical lamination, the findings of the present study concerning pyramid-like neurons support the view of Jaeger and Lund ('81) that although the basic shape and character of pyramid-like neurons is preserved, local conditions may play an important role in shaping the geometry of cell processes. Variable spine density and the arborization of the axons (local and projecting) may be a reflection of transplant circuitry. Nearly all pyramid-like neurons in both the syngeneic rat grafts and the mouse xenografts show an extensive local axonal arborization with the main axon and even some collaterals leaving the graft and entering the host tissue. Unfortunately, the spread of Lucifer yellow into the axon was rather poor, so that its course could not be followed over long distances to look for termination sites. However, intracellular injection of biocytin into grafted striatal neurons in "in vitro" slices have shown an extensive local axonal projection of spiny neurons (Xu et al., '92).

Similar morphological features are exhibited by grafted spiny stellate neurons compared to their counterparts in intact visual cortex. As described for spiny stellate neurons in layer IV of the visual cortex (Lund, '73, '84; LeVay, '73; Jones, '75; Saint Marie and Peters, '85; Lund and Holbach, '91) grafted spiny stellate-like neurons have small somata and a radial distribution of their dendrites. However, the number, branching pattern, spine density, and dendritic length of grafted spiny stellate-like neurons seem to be more extensive compared with their counterparts in the intact adult visual cortex. In particular the distribution and density of spines along the dendrites of spiny stellate-like and fusiform spiny neurons seem to be more like spine densities on immature spiny stellate neurons in the visual cortex as found by Lund and Holbach ('91). A higher density of spines was also reported for spiny neurons in rat striatal grafts transplanted into the rat striatum (Wictorin et al., '92; Xu et al., '92). Whether this reflects the local conditions or the immaturity of the grafts is unclear.

Owing to the absence of defined cortical lamination, spiny stellate-like neurons in both the syngeneic rat grafts and mouse xenografts were intermingled with pyramid-like neurons, spiny fusiform cells, and non-spiny neurons, and were not clearly laminated as has been described for layer IV spiny stellate neurons in the intact adult visual cortex (Lund, '73, '84; LeVay, '73; Saint Marie and Peters, '85; Lund and Holbach, '91). Spiny stellate neurons in layer IV of the visual cortex are the main target neurons for specific afferent thalamic fibers (LeVay, '73; Ribak and Peters, '75). In contrast to the reported intracortical projection of spiny stellate neurons (Lund, '73, '84) in the intact adult visual cortex, some, but not all, of the spiny stellate-like and fusiform spiny neurons were found to project into the host striatum; whether this reflects the local conditions and the deafferentiation of spiny stellate-like neurons from their main thalamic input still remains unclear.

Non-spiny, locally projecting neurons resemble a variety of different cell types in the adult cerebral cortex. They can be described according to their dendritic morphology (Ramón y Cajal, '11; Rakic, '75; Feldman, '78), but more importantly, according to their axonal projection pattern (Rakic, '75; Somogyi, '77; Feldman, '78; Peters and Regidor, '81; Somogyi et al., '82; Meyer, '83; Lund and Yoshioka, '91). All non-spiny neurons are thought to be inhibitory owing to their content of the inhibitory transmitter γ-aminobutyric acid (Rakic, '75; Somogyi, '77; Somogyi et al., '82). The major inhibitory cell type with long-range intracortical connections is the multipolar basket neuron (Somogyi, '77; Somogyi et al., '82; Albus et al., '91; Kisvarday et al., '93). We have found some cells with a basket-like morphology in

both the syngeneic rat grafts and the mouse xenografts intermingled with spiny neurons throughout the entire graft. In addition to neurons with a more basket-like morphology, some fusiform non-spiny cells with elongated dendrites could be found in the graft with a similar dendritic morphology to some neurons in layer III–IV as recently described by Lund and Yoshioka ('91). Interestingly, the proportion of non-spiny neurons to the total population (15% of the total) of grafted cortical neurons seems to mirror the proportion of non-spiny neurons to spiny neurons in the intact cerebral cortex (10–15% of the total). In addition, in some respects the axonal projection pattern seems to be similar to the axonal projection pattern described for locally projecting interneurons in the intact adult visual cortex (Rakic, '75; Somogyi, '77; Feldman, '78; Peters and Regidor, '81; Somogyi et al., '82; Meyer, '83; Lund and Yoshioka, '91; Kisvarday et al., '93). From our results one might suggest that non-spiny neurons seem to be the most preserved cell type in cortical transplants in terms of their dendritic and axonal morphology.

Lamination of cortical transplants

There is still controversy as to whether cortical tissue transplanted into a host environment, in particular the cortex or striatum, develops the same cortical lamination as described in the intact cerebral cortex. So far two studies have provided evidence for the development of lamination in cortical transplants (Das et al., '80; Chang et al., '86). They have, however, transplanted very large pieces of cortex (1–2 mm^2) into the host tissue from a prenatal E15 old donor. At this early time during development the cortical plate has already been generated in the rat, but cortical layers V and VI are generated much later, between E19 and E21 (Miller, '88a; Bayer and Altman, '91). In contrast, and in agreement with our findings, other studies have shown no cortical lamination of cortical transplants implanted in the cortex or in other brain areas, although the donor tissue was taken at similar prenatal ages (E16–E19) and was transplanted into newborn to 2-week-old rats (Jaeger and Lund, '81; Floeter and Jones, '84; Plaschke et al., '92). Interestingly, Floeter and Jones ('84) reported that the grafts did not reconstitute the cortex in the sense of forming missing layers, but that the graft cells tended to remain as discrete clumps within the host brain. Although grafts did not form layers, they did develop a crude internal organization with some aspects of cortical structure, such as whirls or band of cells (Castro et al., '87) or a cell sparse zone similar to the white matter (Das et al., '80; Chang et al., '86; Castro et al., '87). In addition, as reported by Floeter and Jones ('84) both large suspension aggregates as well as solid grafts developed a formation of a cell-sparse area similar to a molecular layer. The appearance of a molecular layer as well as the formation of a rudimentary white matter have also been described by others where pieces of cortex have been transplanted to heterotopic and homotopic locations (Das, '75, '78; Jaeger and Lund, '80a,b). However, the laminated organization described in some studies (Das et al., '80; Chang et al., '86) was not seen in our material, which was serially sectioned and examined section by section. In addition, even in syngeneic rat grafts and mouse xenografts older than 3 months we have found no evidence for cortical lamination. This may, however, reflect differences in the size of the cortical transplants, the age of the transplanted tissue, the method of transplantation, and the constraints of the space in which the transplants grow. The cellular organization of our grafts was more like the loosely packed organization described by Floeter and Jones ('84). The results indicate that certain characteristics of cellular organization of different brain areas may arise from intrinsic properties of cells in those areas.

CONCLUDING REMARKS

The present study has shown that some features of cortical neurons are well preserved in cortical transplants in syngeneic rat grafts and mouse xenografts whereas other features are changed significantly. These changes may be caused by the transplantation procedure, the local condition of the graft, and the host environment. One of the most striking changes is the loss of a cortical lamination and therefore a change in the organization and connectivity of the major cell types in the graft, in particular the projection of the axons of the different cell types. Retrograde and anterograde tracing studies have shown that cortical neurons grafted into neonatal recipients could establish a variety of local and long-range connections with the host tissue, projecting to cortical (interhemispheric via the corpus callosum) and subcortical targets such as the mesencephalic periaqueductal gray, red nucleus, intermediate gray of the superior colliculus, pontine nuclei, and a variety of thalamic nuclei (Jaeger and Lund, '80b; Oblinger and Das, '82; Floeter and Jones, '84; Castro et al., '87; Sorensen et al., '92). It would therefore be interesting to know which of the recognizable cell types found in the transplants project to the host brain, and which only make local connections within the graft. Since a "complete" filling of the axon is rather poor in a fixed slice preparations we plan to use a living slice approach. This would enable us to record simultaneously from single neurons and to correlate their intrinsic electrophysiological properties with the morphology by injecting the cells with biocytin. Experiments addressing this question are in progress.

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