Electrophysiological and Morphological Properties of Embryonic Neocortical Grafts Developing in Different Regions of the Host Rat Brain

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SUMMARY

Parallel morphological and electrophysiological studies of embryonic neocortical tissue (primordia of anterior parietal–presumptive sensorimotor–cortex) grafted into different regions of the host adult brain (sensori-motor cortex, caudate-putamen, septum or thalamus) were carried out to investigate to what extent the properties of transplanted embryonic neocortex–an advanced organizational form of neuronal tissue–are affected by homotopic or heterotopic surroundings.

The results point to the importance of the host tissue environment as it influences both the size, the morphological and functional properties of the implanted embryonic cortical plate tissue. The cortical grafts were smaller in size when developed homo-topically in sensorimotor cortex and grew larger in heterotopic environment (caudate-putamen, septum, thalamus). The orientation of neuronal dendrites in the grafts tends to follow that seen in the surroundings. The homotopic grafts showed a better structural integration into the host brain. Differences were seen between intracortical and subcortical transplants in the spontaneous firing rate of neurons and in the ratio of units with various types of spontaneous discharge.

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INTRODUCTION

It is now well established that during the process of brain development cellular elements mutually influence each other and such reciprocal "environmental" effects exist in almost all steps of maturation. However, the question still remains, that, of the properties of a given neuronal circuit, which are inherited and which are elicited by interaction with the surrounding environment?

In vivo experimental data suggest that neuronal circuits require transynaptic stimulation for their definitive formation, as neurons normally die or fail to become fully mature in the absence of their anterograde or retrograde connections. In contrast the production, migration, and the initial steps of differentiation mainly depend on factors determined genetically and are only slightly influenced by the environment (see review /16/).

In order to get a better insight into this question, neuronal grafting techniques have recently been frequently employed with the objective of investigating whether fetal CNS tissue implants, transplanted into different areas of the adult host brain, develop those organizational features which generally resemble the structures seen *in situ*.

Data from literature show that the transplanted precursor cells complete their predetermined program of histogenesis regardless of any discrepancy between the place of origin of the transplant and the site in the host brain, and all the cellular forms typical of the original structure may develop in the transplant /1,3-5, 18,22,31,34/. Other results, however, indicate that the degree of organizational features of the anatomically integrated graft tissue varies depending on the developmental stage, the place of origin and the site of the implantation of the host brain /9,11-13,19,20,24,28, 29,33/.

On the other hand, the question, to what extent does the bioelectrical activity of the graft depend on the surrounding host cortical tissue, still remains to be clarified. Some data show that the graft's cells develop their own organotypic functional characteristics which remain unchanged despite the influence of the new cellular surroundings /2,14,15,25/. Other authors state that grafted cells do not have organotypic characteristics and both homotopical and heterotopical transplants develop bioelectrical activities similar to those seen in the surrounding host brain tissue /6,7,21/. There are also data demonstrating that the transplanted tissue has an influence on the surrounding host brain tissue cells, changing their functional characteristics /8,30/.

The divergence of different data clearly indicates the importance of studying this long-disputed question, i.e., the differences in the development of neocortical grafts as advanced organizational forms of neural tissue both in homotopical and/or heterotopical location in the host brain. Consequently, the purpose of the present study was to carry out morphological studies in parallel with electrophysiological recordings from neocortical grafts implanted into different sites of the host brain: the neocortex, caudate-putamen, septum and thalamus.

MATERIAL AND METHODS

Small tissue pieces (of approximately 1 mm in diameter and 2-3 mm long) were dissected from 16-day embryonic primordia of anterior parietal (presumptive sensorimotor) cortex of Wistar albino rats by a special micro-stereotaxic device developed in our laboratory (Fulop and Pasztor, in preparation) (Figs. 1 and 2) and grafted into the sensorimotor cortex (AP from bregma = 0.7; L from midline = 2.0), caudate-putamen (AP = 0.7; L = 3.0; V below dura mater = 5.0), septum (AP = 0.2; L = 0.5, V = 5.0) or thalamus (AP = -2.3; L = 2.0; V = 6.0) /23/ of the brain of 22 young adult (2-month-old) recipient rats (Fig. 3). Grafts were deposited into the host brain under anesthesia (55 mg Na pentobarbital/kg bw) by pressure-injection with a syringe mounted on a stereotaxic frame.

After 2-3 months of survival each of the 22 animals was examined by the microelectrode technique, but only 18 were found to have surviving transplants. The extracellular single unit activity of the grafts was recorded in order to determine whether the transplants were functionally competent.

Prior to recording, the animals were weakly anesthetized with Na pentobarbital (35 mg/kg bw). To facilitate respiration and to maintain normal body temperature, the animals were incubated and kept on heated pads.

The recording microelectrodes were lowered stereotaxically according to the same coordinates used for

transplantation by a micromanipulator in 2 μm steps through the existing craniotomy hole. The depth of the electrode tip relative to the *pia mater* was measured at each recording level. For extracellular recording glass microelectrodes filled with a saline solution containing 2% pontamine sky blue were used. The dye was injected at the end of recordings for later histological verification of the position of the microelectrode. Single unit activity was monitored on the oscilloscope and recorded on magnetic tape. Data were processed on a multichannel analyzer. First-order periodicity of spontaneous activity was analyzed using interspike interval histograms. All animals used in this study were sacrificed by intracardial perfusion /17/ under deep Na pentobarbital anesthesia and processed by different methods for the histological examination of the grafts and the surrounding host brain tissue.

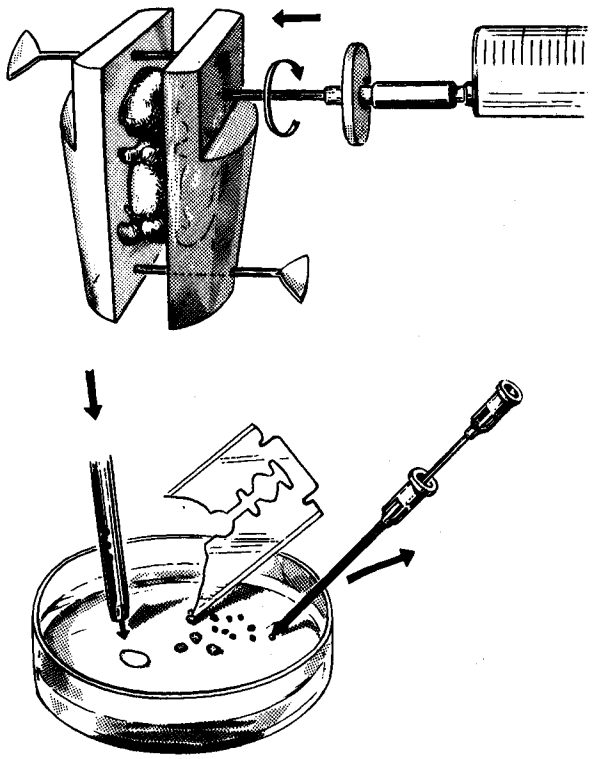

Fig. 1: Schematic drawing summarizing the steps of sampling of embryonic fronto-parietal cortical tissue used for grafting procedure. Slightly anesthetized by ether 16-day-old rat fetuses were transferred into a specially prepared hard plastic chamber, the internal surface of which formed a replica of the embryos of the respective age. This mould served to hold the embryos firmly. Through a previously drilled guide-channel, hitting the required area of the embryonic head, a sharpened hypodermic needle of 1.2 mm internal diameter was driven, punching out an oblong tissue plug. By this method the tissue samples could be obtained very quickly and they consistently contained the required area of the embryonic cortical plate. For implantation brain tissue samples cleaned (under stereo-microscope) of meninges and other surrounding tissues in glucose-saline medium were sucked into a hypodermic needle of about 1 mm diameter, firmly attached to a syringe used for delivery.

For rapid analysis (in eight animals) frozen sections were studied while more detailed studies (in five animals) were done on semi-thin sections. In the latter case the perfused brains were halved in the midsagittal plane and kept overnight in the same fixative solution which was used for the perfusion. Then coronal slabs of 0.5-0.7 mm were cut, washed in phosphate buffer (pH 7.4) and dehydrated through a graded ethanol series followed by propylene oxide and embedded in Durcupan (Fluka).

Serial sections of 1 μm thickness were cut by glass knife on a Reichert ultramicrotome.

Sections mounted on glass slides were stained with 1% toluidine blue containing 1% sodium tetraborate.

Frozen sections of 12 μm thickness were cut on a Reichert rotary microtome and stained with cresyl violet-fast blue.

In five animals brain areas containing grafts were cut out *en bloc* and impregnated with rapid-Golgi procedure /32/. After impregnation, sections of 75, 100 and 125 μm thickness were cut with a tissue chopper, dehydrated through graded ethanol series and flat embedded in Durcupan for light microscopic examination. Having selected the required areas, sections were glued on to plastic holders and recut into 1 μm thick sections. These so-called semi-thin Golgi sections were counterstained with toluidine blue.

RESULTS

The cortical plate of 16-day-old rat embryonic brain, which in this study served as a grafting tissue, is a compact sheet of radially oriented cellular columns composed of neuronal and glial precursors (Fig. 2C). At this age the stages of cellular differentiation are hardly distinguishable, as most of the cells assume a rounded embryonic shape. Only a few of them had

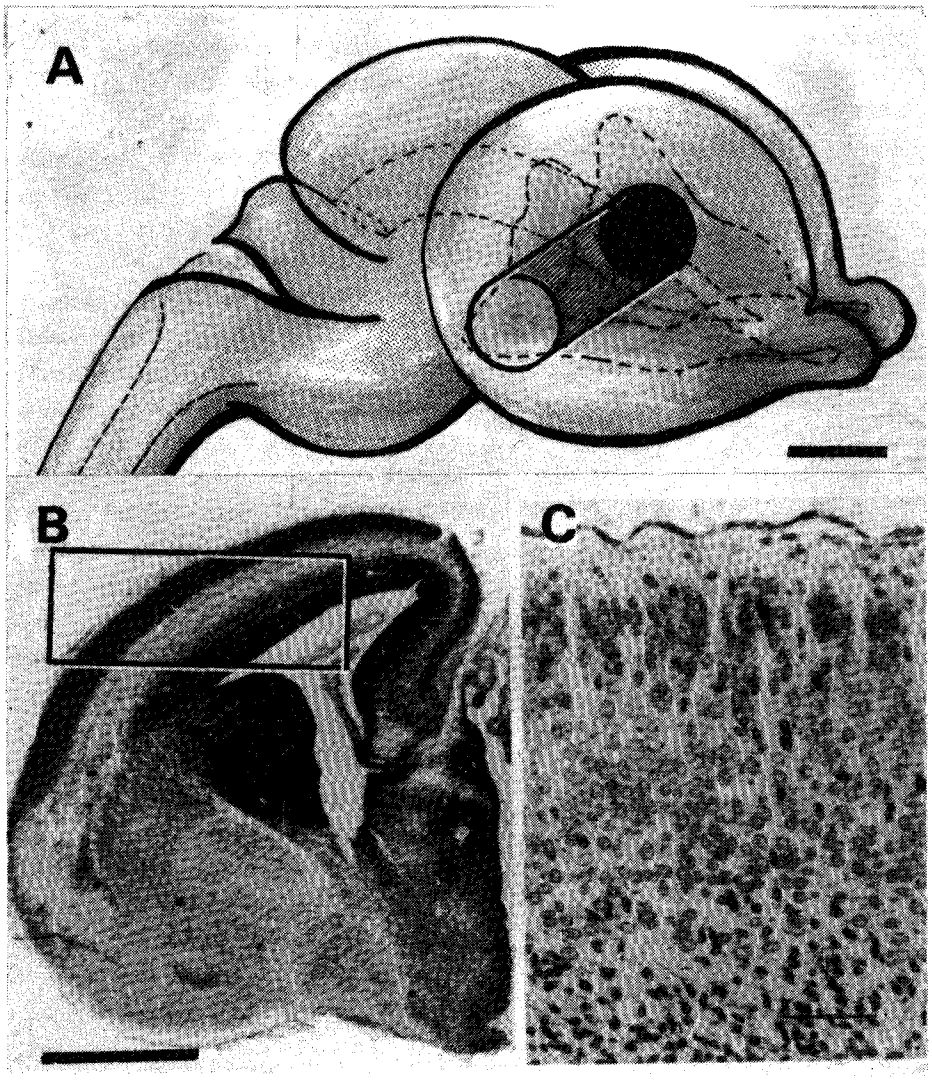

Fig. 2: (A) A schematic drawing of the brain of a 16-day-old rat embryo showing the position of punch used for sampling anterior parietal (presumptive sensorimotor) cortex. (B) A microphotograph of a coronal section of E16 embryonic brain. The framed area corresponds to the extent of sampled and transplanted region. (C) Enlarged field of cortical plate showing the radial orientation of cellular columns consisting of neuro- and glioblasts. Bar scale: A and B - 1 mm; C - 150 μm.

already developed the "growth cone", which is thought to be a morphological sign of the beginning of neuronal differentiation.

The surviving grafts (in 18 animals), analyzed in this study, can be grouped according to their localization: (1) Grafts which were localized within the host sensorimotor cortex (n=6); and (2) Grafts which were localized in subcortical regions, such as the caudate-putamen, septum or thalamus (n=12) (Fig. 3).

The grafts remained relatively small in size (approximately 0.5-1.00 mm^2) when cortical primordia were transplanted homotopically into the host sensorimotor cortex (Fig. 4), whereas they grew quite large (approximately 1-2 mm^2) if they were situated in subcortical structures such as the caudate-putamen, thalamus or septum (Fig. 5).

In the case of homotopic grafting (Fig. 4), the embryonic tissue seemed to be well integrated into the host cortex and had developed a glial scar only weakly marking the border between the host and graft tissue. In contrast, in cases of heterotopic grafts (in the caudate-putamen, septum, thalamus) (Fig. 5), the

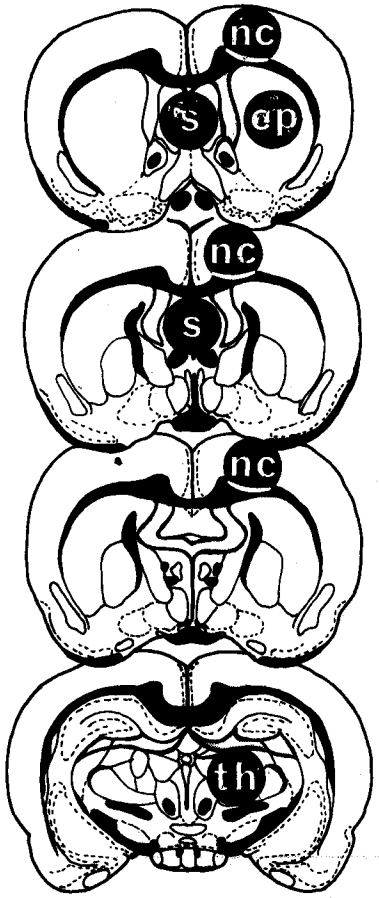

Fig. 3: Serial schematic drawings of coronal sections of adult host forebrain demonstrating variable location of grafts found in this study. nc - neocortex, sp - septum, cp - caudate-putamen, th - thalamus.

cortical tissue often remained isolated from the surrounding host tissue, mostly as a separate cellular mass. In these cases glial cell nuclei formed a more marked borderline at the graft-host interfaces.

In all cases the surviving grafts developed strong and rich vascularization with a typical glial end-feet coat around vessels (Fig. 6).

Closer examination of grafted tissues also showed differences depending on the sites of the grafts. Grafts located within the host neocortex tended to form a layered structure, resembling in Nissl preparations the surrounding host cortex with a mixture of differentiated and undifferentiated cellular elements, among which the pyramidal cells with their radially oriented apical dendrites could be easily distinguished (Fig. 7). The Golgi staining of these grafts also showed typical pyramidal cells, although differences in the stage of their development were found. Fig. 8A and B show a cell similar to the fully differentiated pyramidal neuron, while Fig. 8C and D demonstrate a slightly differentiated pyramidal cell. On semi-thin sections (Fig. 8B and D) prepared from the same impregnated cells seen in pictures 8A and C, respectively, the fine structure of the surrounding neuropile with typical glial and other satellite cell nuclei can be seen.

The cellular assembly found in a graft which developed in the caudate-putamen is presented in Fig. 9. Here the uneven differentiational stages of cells can also be observed. Some are of multipolar shape while others with basal and apical dendrites are similar to pyramidal neurons, although the length of apical dendrites varies and their orientation is also lost. The same was demonstrated also by Golgi impregnation which showed rich dendritic arborization of stellate-like neurons (Fig. 10), forming a complicated network, as well as nontypical pyramidal cells with extremely short apical dendrites. Among these cells there were also many undifferentiated forms.

In the course of electrophysiological study of neurons located in grafts or their surroundings, impulse activity of 168 units was recorded in 18 animals. Of these recordings 114 were found to be long enough (with durations exceeding 300 sec) to make analytical comparison. Fifty-four of these, which undoubtedly belonged to the graft tissue (taken from 12 different animals) are demonstrated in this paper.

According to the types of spontaneous activity and the distribution of interspike intervals, all registered transplant units were divided into three groups. The first, smaller group (a total of 10 cells) represents neurons with well defined bursts of spontaneous activity, intermingled with some single spikes (Groups IA and IIA of Fig. 11 and line A of Table 1). In this case a large number of equal short intervals (1-6 msec)–representing interspike intervals inside the bursts–could be seen, which gave a narrow peak at the left side of the histogram of interspike interval distribution. These intervals appeared relatively stable. However, longer intervals could also be detected between the different bursts, between different single spikes and between the bursts and single spikes as

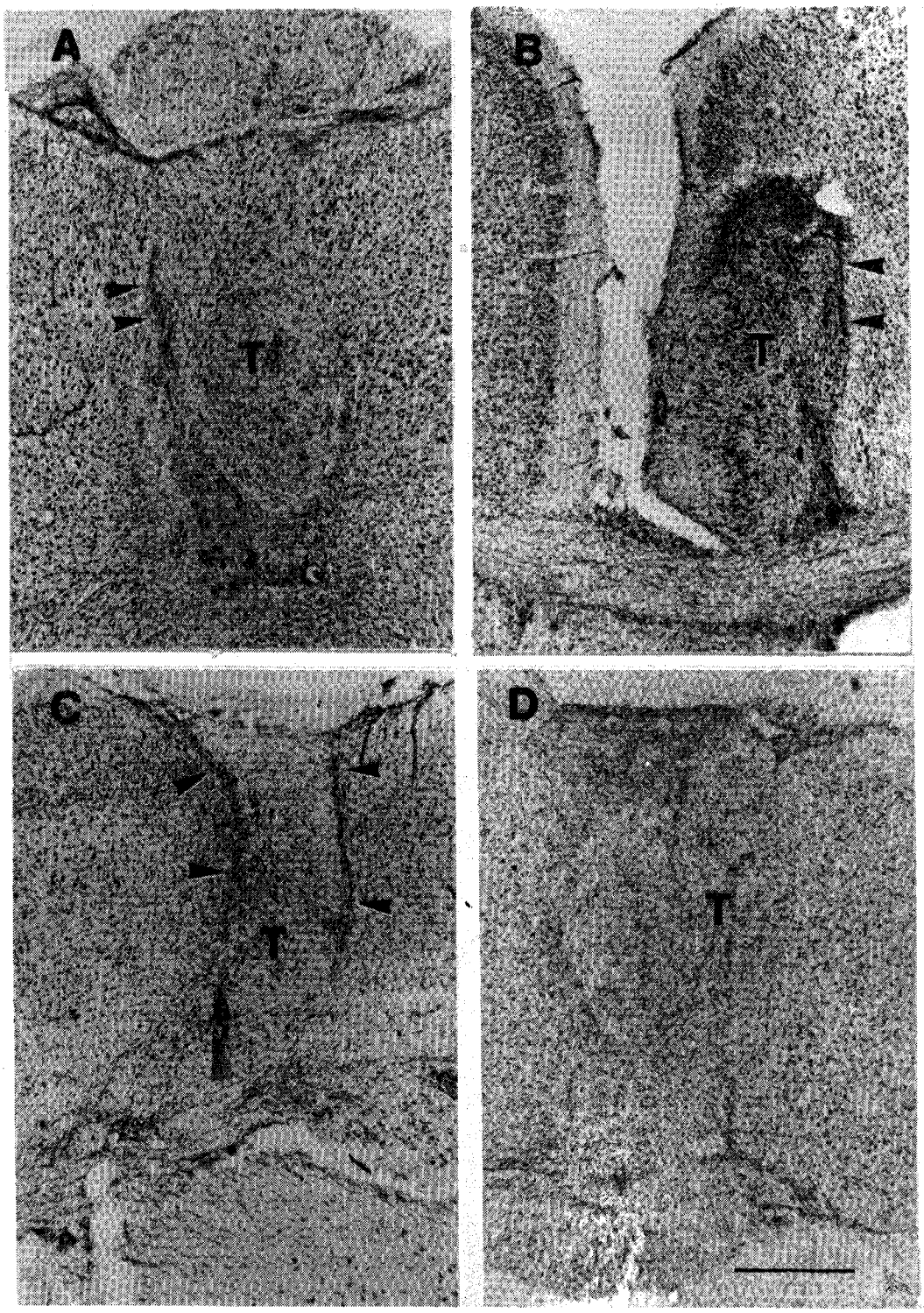

Fig. 4:

Microphotographs of different intracortical cortical grafts (T) after 62 (A and B) and 90 (C and D) days of survival. Despite the glial scares (arrowheads) that can be seen at some areas of the contacting surfaces, all grafts have grown well integrated into the host. Bar scale: 300 μm.

well. The second group (14 cells) contained neurons showing both single spikes and burst activity with different lengths. The number of single spikes, however, in this group was much higher than in the first. In this case in the histogram of interspike intervals not only the left peak can be seen (2-4 msec), but also a larger number of longer intervals, representing a mixed type of activity (Groups IB and IIB of Fig. 11 and line B of Table 1). The third and largest group of neurons (30 cells) contained those having only single spike activities. In this case the left column representing short and equal intervals in the histogram disappeared, but a number of new columns were generated in the middle of the histogram representing the mean values of intervals between separate single spikes (Groups IC and IIC of Fig. 11

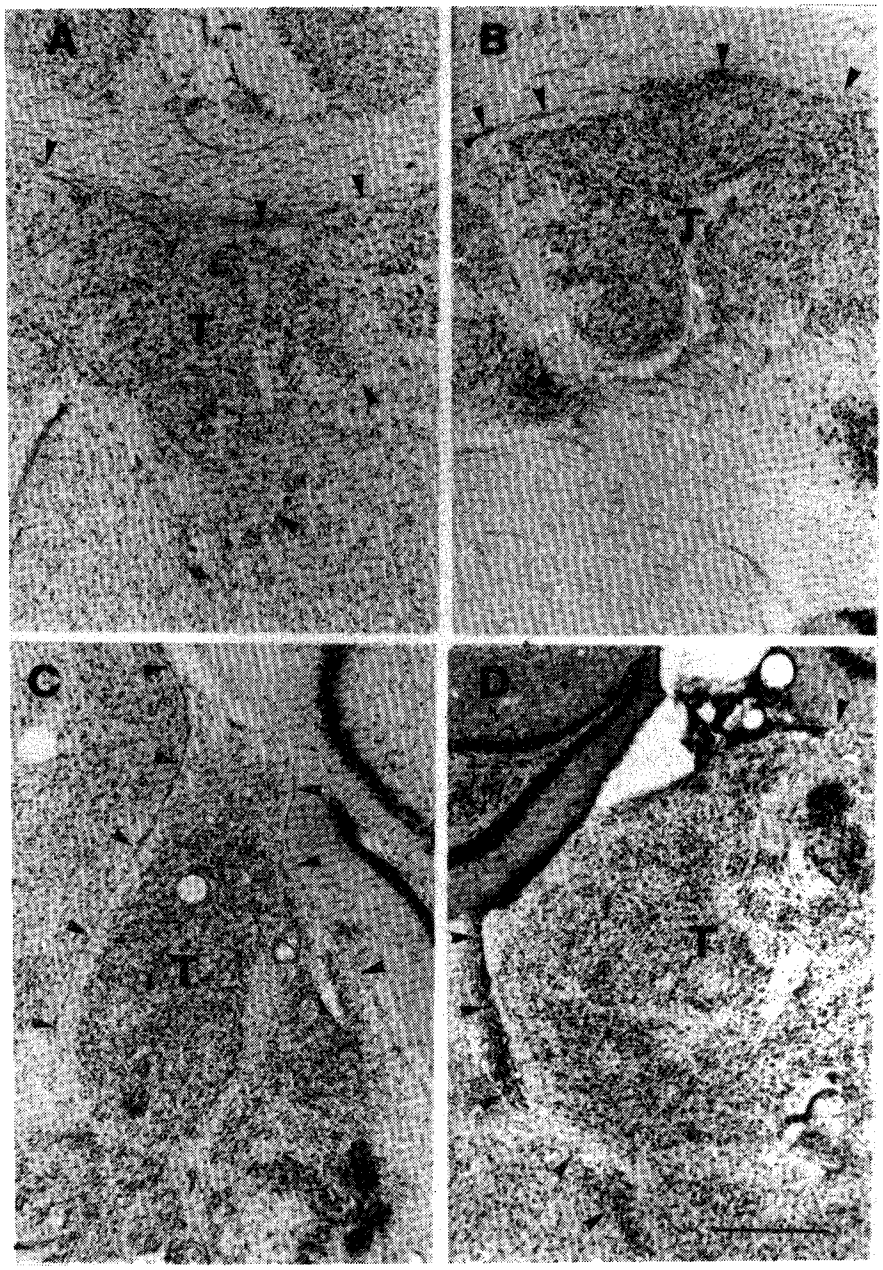

Fig. 5:

Microphotographs of cortical grafts (T) implanted into the septum (A), caudate-putamen (B) and thalamus (C and D). In all cases grafts have grown large and separated from the surrounding host tissue by glial cell accumulation (arrowheads). Note that cellular elements in the grafts show random orientation. Bar scale: 300 μm.

and line C of Table 1). This latter type of distribution of intervals is close to the normal.

According to the localization of the grafts the neurons analyzed could be divided into two groups (see columns 1 and 2 of Table 1 and groups I and II of Fig. 11). The neurons of the first group were localized in grafts lying within the host sensorimotor cortex not deeper than 2500 μm, measured from the cortical surface, while those belonging to the second group were found within the grafts located in the caudate-putamen. The background activity of neurons belonging to the above different groups (I and II) differed in two parameters: the mean discharge rate and the ratio of units with different types of spontaneous activity. The mean spike frequency of neurons belonging to group I (25 cells) was 0.88 ± 0.3 spikes per second. Most of the cells, however, did not discharge with a frequency of more than 1.5 imp/sec

TABLE 1
GROUPING DIFFERENT TYPES OF SPONTANEOUS SINGLE-UNIT ACTIVITY RECORDED IN THE GRAFTS LOCATED IN THE SENSORIMOTOR CORTEX OR IN THE CAUDATE-PUTAMEN

	Location of Grafts		
Types of spontaneous activity	I in neocortex	II in caudate-putamen	Total
A. Burst activity	2	8	10
B. Mixed activity	4	10	14
C. Single spike activity	19	11	30
Total	25	29	54

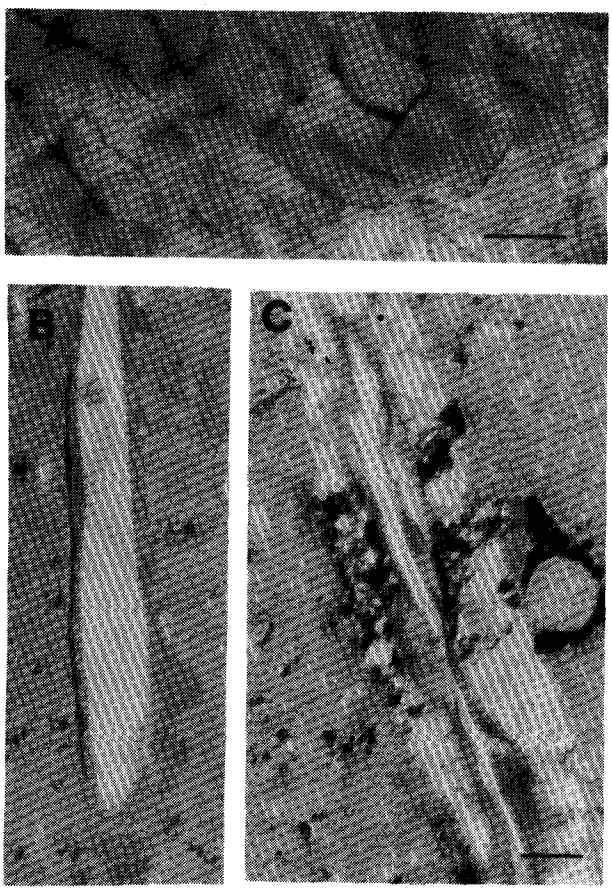

Fig. 6:

Golgi-impregnated sections of a 3-month-old intracortical cortical graft. (A) Rich capillary system as seen in a 100 μ thick section of the surviving graft. (B) and (C) End-feet of astroglial processes form a typical coat around the capillaries. Plastic-embedded semi-thin sections counter-stained with toluidine blue. Bar scale: 100 μm.

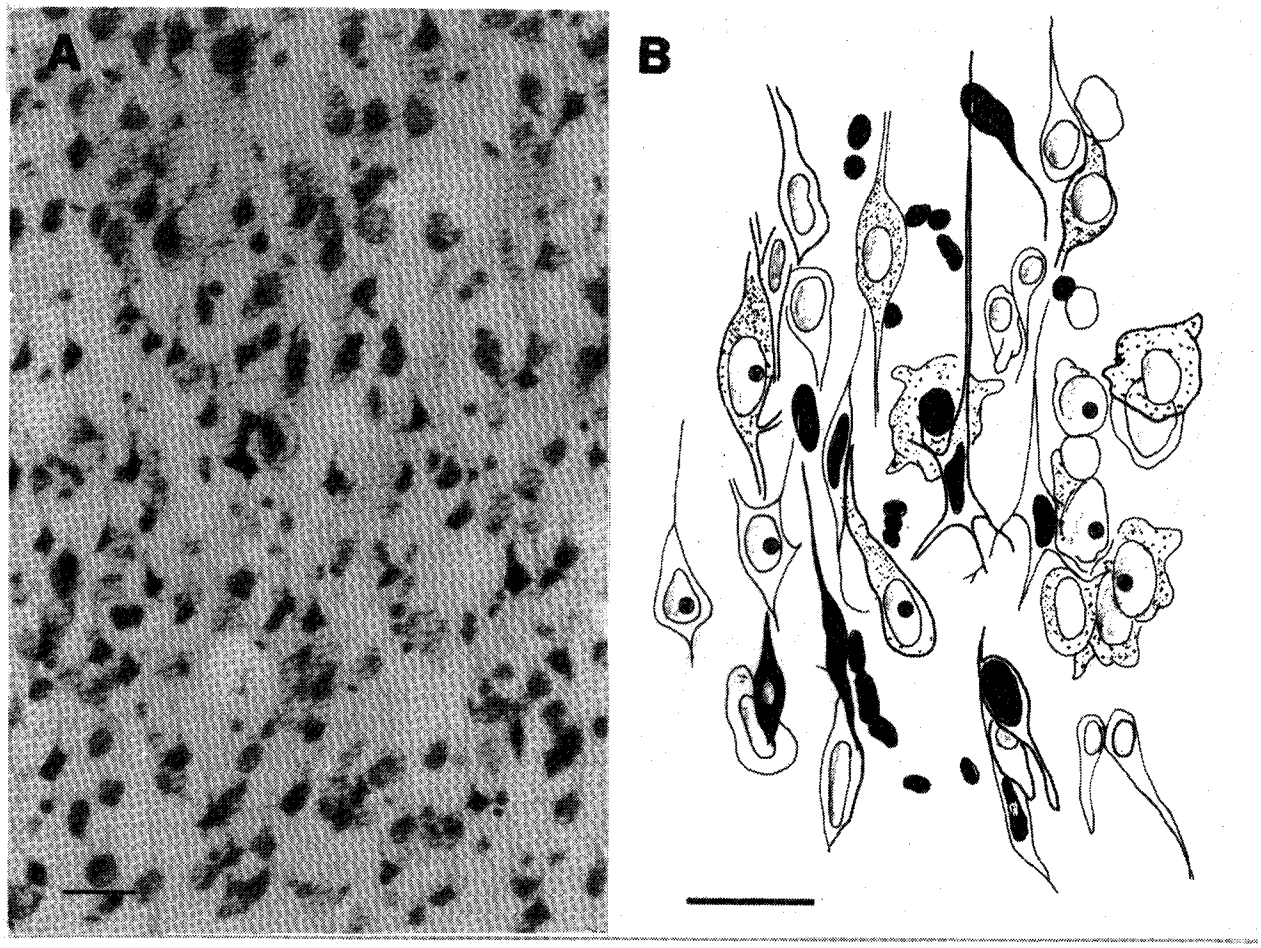

Fig. 7:

(A) A microphotograph of Nissl-stained section prepared from cortical grafts implanted into the neocortex. Neurons of various degrees of differentiation can be seen whilst the radially oriented pyramidal cells are also prominent. (B) The camera lucida drawing emphasizes the radial orientation and the differences in developmental stages of cells found in intracortical grafts. Bar scale: 100 μm.

and only one neuron showed 7 imp/sec (Fig. 12, graph I). In this group 19 of the neurons showed single spikes and only two of them showed bursts. The mean spontaneous firing rate of the neurons of group II was significantly higher than the firing rate of group I (3.0 $\pm$ 0.8 spikes/sec) ($P < 0.01$), while some of the cells showed as many as 13-15 impulses per second (Fig. 12, graph II). The different types of spontaneous activity in this case were represented in closely equal number: 11 neurons showed single spike activity, 8 cells gave bursts, while 10 neurons exhibited mixed activity types.

DISCUSSION

Our data show that the cortical plate of 16-day-old embryonic rat brain grows well in the young adult recipient rat brain independently from the site of implantation (whether cortical or subcortical). However, their size varied, showing correlation with their localization. The graft grew larger in size when it developed in subcortical regions rather than in its homotopic environment.

This observation raises the possibility that the homotopic tissue might have some kind of limiting effect on the proliferation of the graft cells, without affecting the major course of their differentiation. The observed differences in graft development may be explained by the favorable conditions provided by specific regulatory (presumably humoral) factors which may be present in the nervous tissue. Detailed histological and electrophysiological examination shows that some morphological and functional properties of

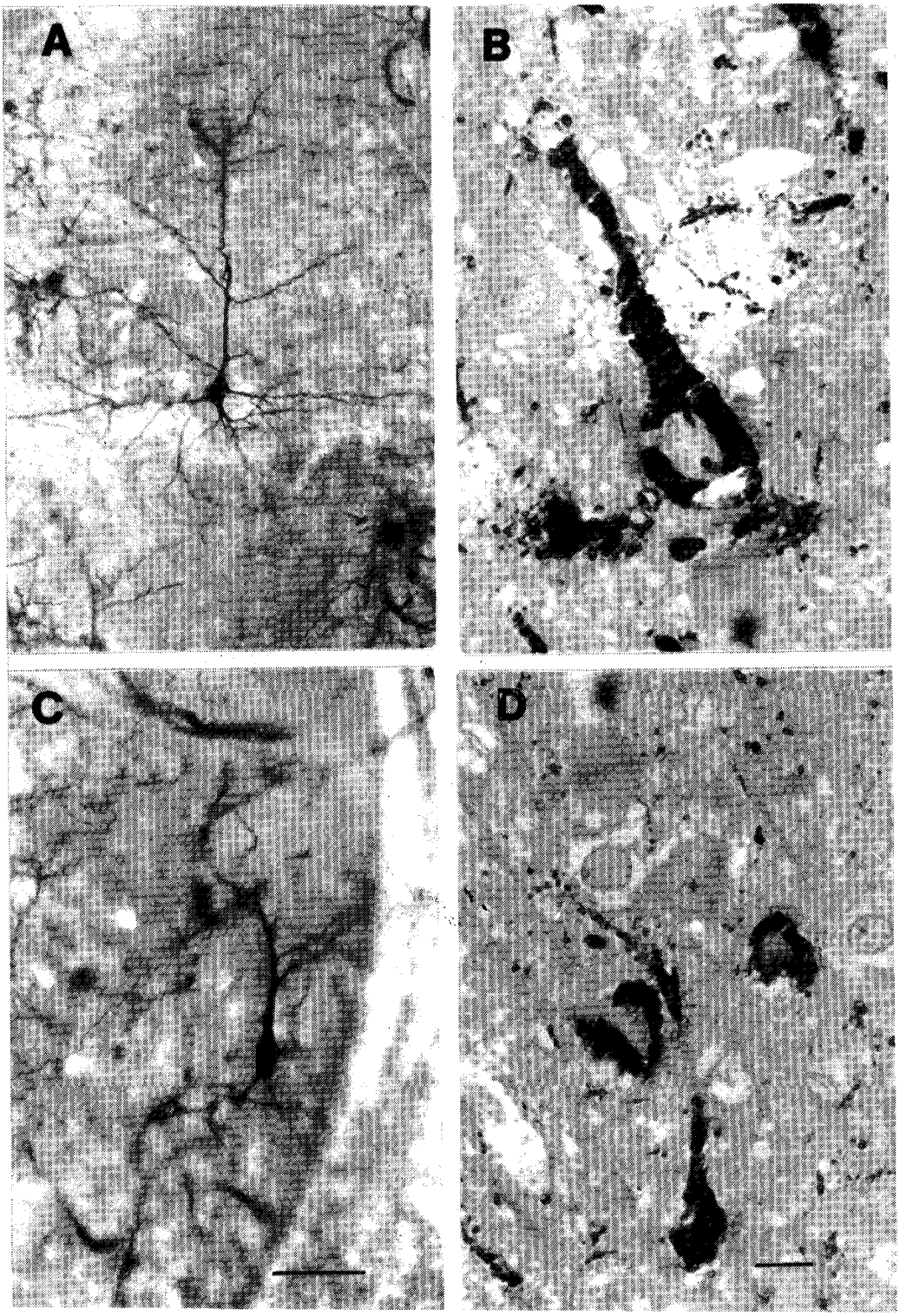

Fig. 8: Golgi staining of the homotopic cortical grafts demonstrating pyramidal cells with developmental differences: Pictures A and B show a cell comparable to the final form of differentiation of this neuron, while C and D demonstrate a weakly differentiated pyramidal cell. Photos B and D show semi-thin sections of the neurons seen in photos A and C, respectively. Bar scale: 100 μm.

grafts also depend on the localization, i.e., on the environment, provided by the host brain tissue.

In both Nissl and Golgi preparations it was clearly demonstrated that the orientation of neuronal dendrites in the grafts tends to follow that seen in the surrounding tissue. If the graft developed in the host neocortex, i.e., in an environment where most of the dendrites show a radial orientation toward the pial surface, the dendrites in the graft were also radially oriented. In the grafted embryonic cortical plate developed in the basal nuclei or in the brain stem parenchyma, where neuronal dendritic processes normally show random orientation, the neurons in the graft tissue also lost their radial orientation and their dendrites developed in various directions.

These findings indicate that the tissue environment influences the structural organization of the grafted tissue: the structures that are genetically closest to the graft have the strongest effect on the differentiation of graft tissue. In particular, the specific afferentation in the intact surrounding tissue may be one of the regulatory factors to influence the direction of dendritic growth in the graft in such a way that they become oriented toward their specific afferent fibers.

Closer histological analysis also showed that all grafts have developed normal vascularization. Semi-thin Golgi sections were rich in capillaries encapsulated by the impregnated end-feet of astroglial cells, known as an anatomical sign of the functioning blood-brain barrier. Assuming that the blood-brain barrier does not allow the passage of regulating molecules produced elsewhere and present in the

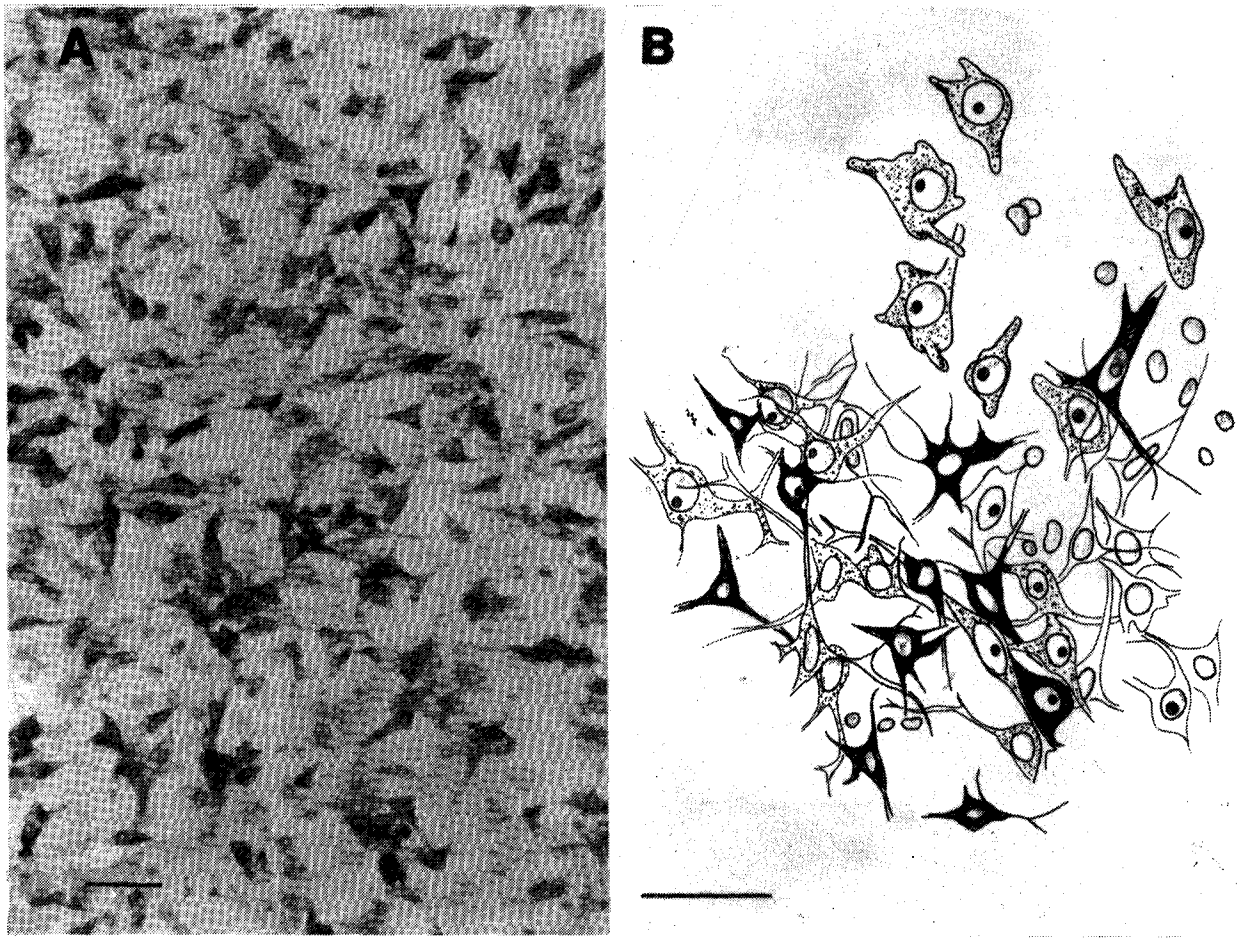

Fig. 9:

A microphotograph (A) and camera-lucida drawing (B) of star-shaped neurons found in a cortical graft which developed in the caudate-putamen. A few pyramidal-like cells are dispersed randomly and their apical dendrites have no determined orientation. Bar scale: 100 μm.

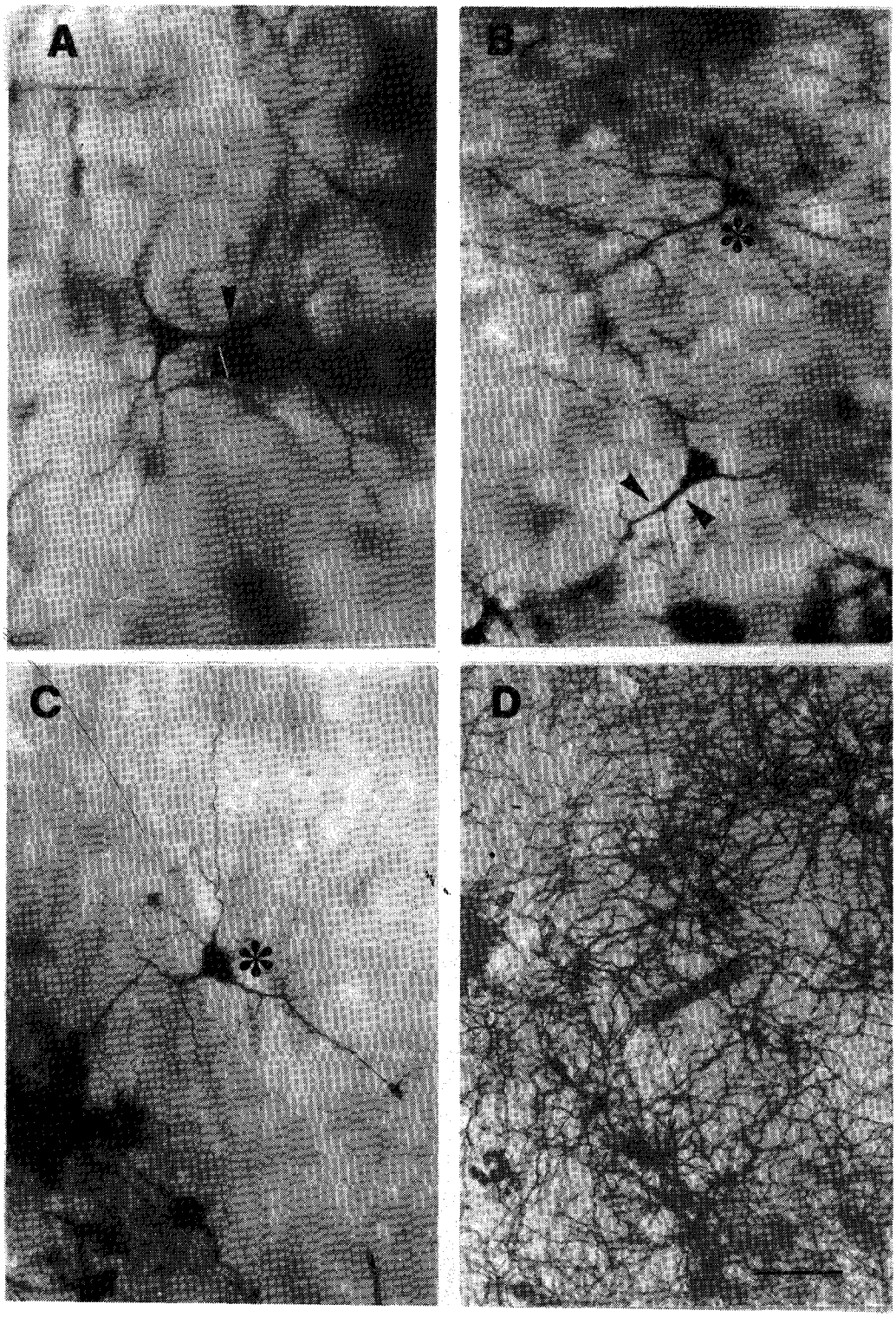

Fig. 10:

Non-typical for the cerebral cortex pyramidal cells with short apical dendrites (arrowheads) in Golgi impregnated cortical grafts implanted into caudate-putamen (A and B). Fibers of star-shaped cells (asterisk) (B and C) form a mesh of fiber network (D). Bar scale: 100 μm.

circulating plasma, these putative regulating chemical factors may well be produced inside the host brain tissue or in the graft itself.

The influence of specific afferentation and of chemical factors on the growth and orientation of dendritic trees has also been shown by other authors /10,26,27,35/. These factors may well explain the random orientation and small size of apical dendrites of pyramidal cells developed in the brain stem environment, to which afferent axons are coming from different directions.

Dependence of bioelectrical activity of the graft neurons on the surrounding host brain environment has also been shown by others /6,7,21/. The heterotopical septal and hippocampal transplants /6/ as well as homotopical neocortical transplants /7,21/ implanted into the neocortex showed characteristics close to those seen in the host neocortex. Similarly, in our experiments the firing rate of cells in the intracortical transplants was lower than in subcortical regions, which showed higher values. Differences were also seen between intracortical and subcortical transplants in the ratio of units with various types of spontaneous discharge. These data may point to functional differences existing between the grafts localized in different sites in the host brain.

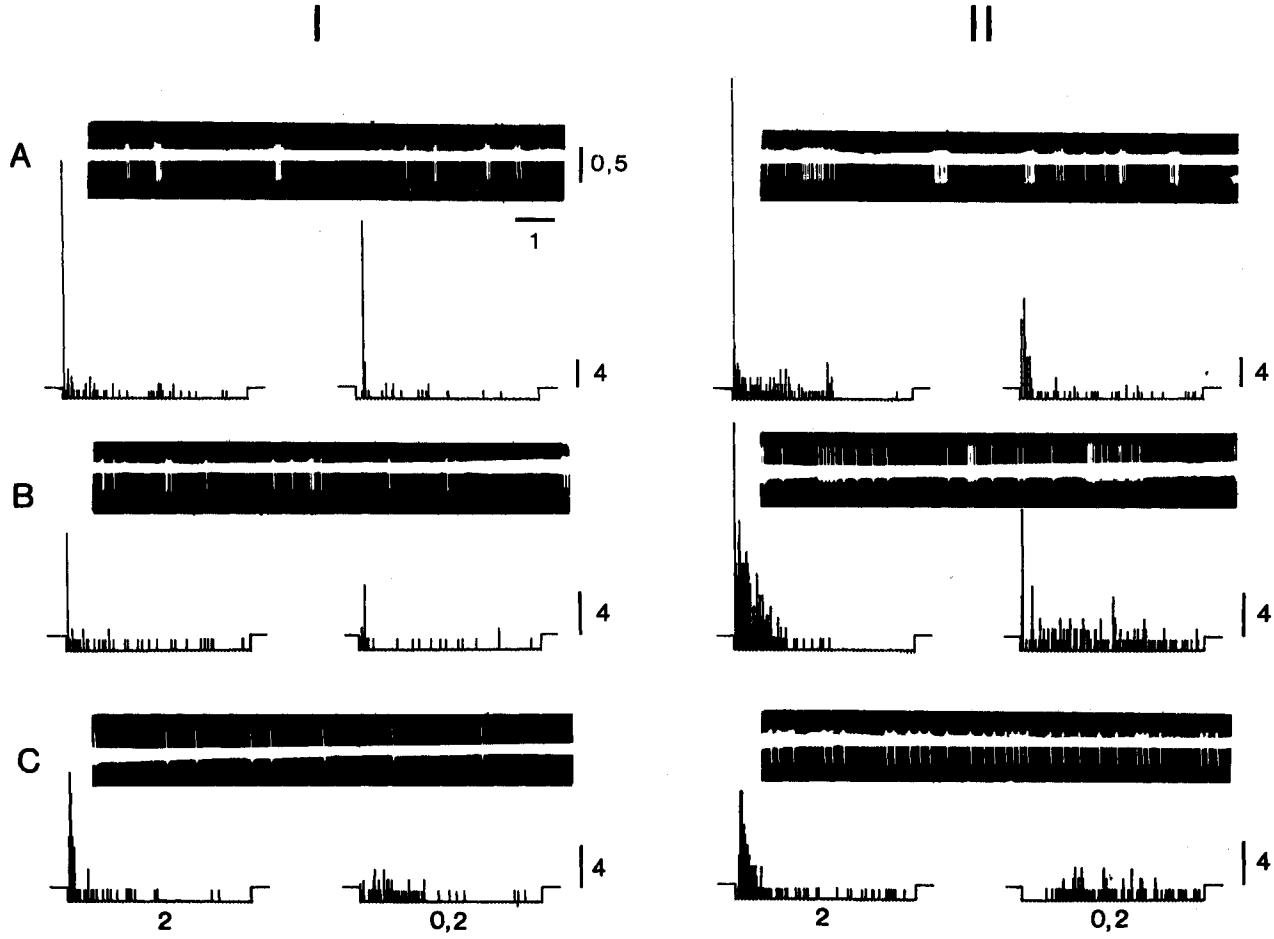

Fig. 11:

Histograms of the interspike-interval distribution of the spontaneous activity and examples of impulse discharge of neurons in grafts localized within the host sensorimotor cortex (group I) and in those localized within the host caudate-putamen (group II). The three types of firing are represented: (A) bursts, (B) mixed, (C) single spike activity. Vertical calibration: spikes, mV. Horizontal calibration: seconds.

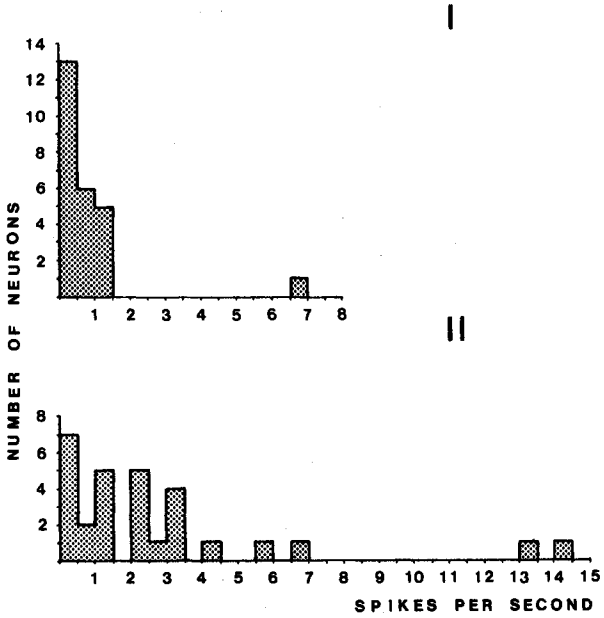

Fig. 12: Distribution of neurons with various mean discharge-rate in cortical tissue grafts implanted into the host sensorimotor cortex (graph I) and the caudate-putamen (graph II).

On the basis of these data, we conclude that the environment influences the formation of both the size, the morphological and functional properties of the implanted embryonic cortical plate. However, the strength of this influence varies depending on the genetic proximity of the graft and the surrounding tissue. Presumably, the closer the genetic programs of the two, the more adequate the influence of the host. In other words, the genetically determined potentialities remain in the transplanted tissue blocks but will only become manifest if all factors provided by the normal environment are present.

The fact that pyramidal cells were found practically in any version of grafting indicates that the development of single cell properties is determined by its genetic program; however, the differences in their shape and size show the strong influence of the environment.

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